13th World Congress on Inflammation

London

July 8-12, 2017

Hilton London Metropole Hotel
London, UK

PROGRAM AND ABSTRACT BOOK

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13th World Congress on Inflammation is in your pocket!

All the scientific and social content of the meeting is at your fingertips with the mobile app. Mobile app is right with you with its extensive media content from abstract book to meeting photographs!

HOW CAN I USE THE MOBILE APPLICATION?

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SCIENTIFIC SECRETARIES

Prof. Graham Wallace
Dr. Peter Bunyard
scientific@wci2017.org

PROFESSIONAL CONGRESS ORGANIZER

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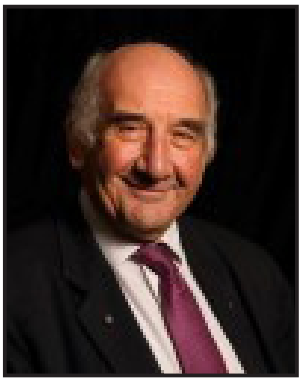
Welcome to London 2017!



Prof. Peter Barnes FRS
Congress President

London is a fitting choice for this 13th International Congress. This is one of the best connected cities in Europe, boasting a proud history of scientific innovation and discoveries in inflammation. Many of the world's most famous innovative thinkers have lived and worked in London including Peter Medawar, James Black and Paul Nurse and many famous inflammation researchers including Rod Flower, John Vane, and other IAIS lifetime awardees.

The biennial Congress is the pinnacle for the growing number of Societies within the IAIS, and others who attend our Congress, and represents a key opportunity to share knowledge across borders and professional disciplines as we work towards a common goal of improving the understanding and control of inflammatory diseases. Thank you very much for your participation and contributions to this vibrant scientific event.

The Congress Scientific programme will present the best in inflammation science from distinguished researchers from across the globe. Covering key topics from across the spectrum of inflammatory mechanisms and therapeutic areas with a specific focus on inflammation in ageing and cancer. The Congress is the best place to build skills and knowledge through hearing the latest topics in the field.

The Congress venue, the Hilton London Metropole Hotel is located close to Paddington station and major shopping and entertainment areas. It is the perfect site to explore many of London's famous landmarks when there is space in between busy congress schedules. Travel around London is very easy with the extensive underground system and the famous red buses and black taxis. I hope that you will take this opportunity to explore the many treasures that this city has to offer.

Ian Adcock
Chair

Dear Friends and Colleagues,

On behalf of the British Inflammation Research Association and the International Association of Inflammation Societies, welcome to London for the 13th World Congress on Inflammation! Following a terrific event in Boston in 2017, we are very pleased to bring this meeting to London.

The World Congress on Inflammation is always a lively event in which we cross-fertilize ideas from diverse topics, and the 13th WCoI promises to be continue this tradition. We have received 272 submitted abstracts, and we have registered participants from 41 countries. Thank you all very much for your participation and bringing your unique perspective to this event, as this is what makes WCoI such an exceptional event.

I am very excited for the scientific program that we have prepared for you. Our lineup of plenary speakers features very notable researchers that are leaders in their fields. This is really an incredible opportunity to hear from such distinguished speakers all in one place.

I would also like to thank all of our IAIS member societies and executive committee members for their help with the preparation of this meeting. They have arranged very exciting symposia sessions on a number of important topics within the scope of inflammation research. This promises to be a very stimulating event for all of us, regardless of your particular area of expertise.

For those of you who have attended an IAIS event before, I would like to welcome you back. To our newcomers, I hope that this congress serves as a springboard for your involvement with this organization. I encourage you to become an active member of your national inflammation research association, and I look forward to contributions here in London and beyond.

LOCAL ORGANIZING COMMITTEE

Prof. Ian Adcock, *Imperial College London, UK*
Dr. Matt Barnes, *Heptares Therapeutics, Welwyn Garden City, UK*
Dr. Peter Bunyard, *RedX Pharma, Liverpool, UK*
Dr. Diane Cooper, *Queen Mary University of London, UK*
Dr. Julie Keeble, *Kings College London, UK*
Prof. Paul Kirkham, *University of Wolverhampton, UK*
Prof. Michael Seed, *University of East London, UK*
Prof. Graham Wallace, *University of Birmingham, UK*
Prof. Paul Winyard, *University of Exeter, UK*

IAIS EXECUTIVE STEERING COMMITTEE

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Prof. Graham Wallace, *Vice-President*
Dr. Nathalie Vergnolle, *Secretary*
Dr. Nick Wilson, *Treasurer*
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Prof. Tetsuya Taga
Prof. Mauro Teixeira
Dr. Joel E Tocker
Prof. Wim van den Berg
Dr. Nathalie Vergnolle
Adjunct Prof. John L Wallace
Dr. Nick Wilson
Dr. Mike Yeadon

Registration Desk

The Congress Registration desk will be located West Wing Entrance Foyer.

Registration Desk Hours

Saturday July 8th, 2017	09:00 - 20:00
Sunday July 9th, 2017	08:00 - 18:30
Monday July 10th, 2017	09:00 - 18:30
Tuesday July 11th, 2017	09:00 - 20:00
Wednesday July 12th, 2017	08:00 - 13:30

Language

The official language of the congress is English. There will not be any simultaneous translation of this event.

Certificate of Attendance

Certificates of Attendance can be collected from the Congress Registration Desk.

Name Badges

Please wear your name badge at all times during the congress. Badges are color coded as follows:

Committee Member
Faculty
Participant
Exhibitor
Staff

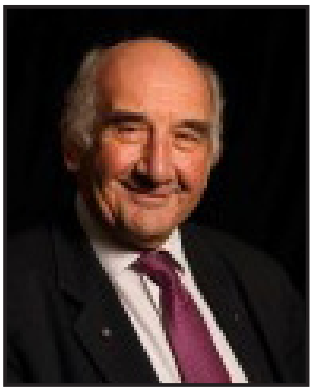
Slide Check

Speakers will check their slides in the room in which their presentations will be delivered. You are kindly requested to check in during the morning before your presentation.

E-Posters

E-Poster machines will be displayed in the Exhibition Area.

Poster presenters are asked to be in front of their posters at their designated presentation time. Poster presentations will take place during lunch breaks and poster sessions.



Peter Barnes FRS

Peter Barnes is Professor of Thoracic Medicine and Head of Respiratory Medicine at the National Heart and Lung Institute and Honorary Consultant Physician at Royal Brompton Hospital, London.

He qualified at Cambridge and Oxford Universities (first class honours) and was appointed to his present post in 1987. He has published over 1000 peer-review papers on asthma, COPD and related topics and has edited over 40 books (h-index = 153). He is also amongst the top 50 most highly cited researchers in the world and has been the most highly cited clinical scientist in the UK and the most highly cited respiratory researcher in the world over the last 20 years. He was elected a Fellow of the Royal Society in 2007, the first respiratory researcher for over 150 years. He has been a member of the Scientific Committee of global guidelines on asthma (GINA) and COPD (GOLD). He also serves on the Editorial Board of over 30 journals and is currently an Associate Editor of Chest, Journal of COPD Foundation, Respiratory Editor of PLoS Medicine and Editor in Chief of Up-to-Date Pulmonary Diseases. He has given several prestigious lectures, including the Amberson Lecture at the American Thoracic Society, the Sadoul Lecture at the European Respiratory Society and the Croonian Lecture at the Royal College of Physicians. He has received honorary MD degrees from the Universities of Ferrara (Italy), Athens (Greece), Tampere (Finland) and Leuven (Belgium). He is an Emeritus NIHR Senior Investigator,a Master Fellow of the American College of Chest Physicians and a member of the Academia Europaea. He was President of the European Respiratory Society 2013/14. He co-founded an Imperial spin-out company RespiVert, which was acquired by Johnson & Johnson and has developed novel inhaled treatments for COPD and severe asthma.

His research is focused on cellular and molecular mechanisms of asthma and COPD, understanding and developing therapies and research into biomarkers for these diseases. He is involved in multidisciplinary translational research which integrates basic science with clinical studies, thereby providing novel insights into common airway diseases.

Adrian Hayday, FRS, F. Med. Sci

Adrian Hayday was trained in biochemistry at Cambridge, and obtained a PhD in molecular virology from London University. He began studying immunology in 1982 at MIT, where he and his colleagues first described the wholly unanticipated T cell receptor gamma chain genes. Since then Professor Hayday has used many parameters to establish that gamma-delta T cells are clearly distinct from conventional T cells. Those parameters include the cells' responses to different products of a novel gene family expressed by body surface epithelia, that Professor Hayday and his colleagues identified. He showed that this unique biology allows gamma-delta T cells to make rapid responses to tissue dysreguation, rather than to specific pathogens, and thereby to monitor tissue integrity. This may explain Professor Hayday's observation that gamma-delta T cell deficiency is associated with a profound susceptibility to skin carcinogens, findings that were instrumental in promoting his and others' interest in the cells' clinical application, and the foundation of GammaDelta Therapeutics. In related clinical activities, Professor Hayday directed a team characterising the human immune response to vaccination; and described the unique properties of human autoantibodies. Additionally, he is the lead-investigator of a Wellcome Trust-supported, multi-centre, high-throughput phenotyping screen identifying novel genetic regulators of the immune system.

Professor Hayday has authored over 200 papers, of which he is first, last, or corresponding author on over 120, and of which 150 are original research contributions. He has received many awards, including the William Clyde deVane Medal, Yale College's highest honour for scholarship and teaching, an honorary fellowship of King's College London, the King's College Business Award, 2008, and the Business of Science Leadership Award, 2017. He was elected to lead the British Society of Immunology (2005-09) and has organized many scientific meetings including the 2014 Gordon Conference in Immunochemistry and Immunobiology, and the scientific programme for the 2012 European Congress of Immunology. He is an elected fellow of the Academy of Medical Sciences and of the Royal Society.

Janet M Lord

Janet Lord is Professor of Immune Cell Biology and director of the Institute for Inflammation and Ageing at Birmingham University Medical School and is also director of the MRC-Arthritis Research UK Centre for Musculoskeletal Ageing Research. Her primary research focus is in the effect of ageing upon immune function and how this limits the ability of older adults to resolve inflammation and predisposes them to chronic inflammatory disease such as Rheumatoid arthritis. She also researches the link between chronic systemic inflammation and physical frailty. In this context Professor Lord has a particular interest in the role played by stress (physical and psychological) and the altered HPA axis in modulating immunity and frailty in old age and following an injury such as hip fracture, or a major injury such as burn. She has recently worked on the role depression plays in worsening the outcomes for hip fracture patients.

In 2013 she was awarded the Lord Cohen of Birkenhead medal for her outstanding research in human ageing by the British Society for Research in to Ageing and in 2014 was awarded the Glenn Award for biological mechanisms in Ageing by the US Glenn Foundation. She was elected a Fellow of the Academy of Medical Sciences in 2015. She has published over 185 original papers and reviews and has an h-index of 44.

Mihai G. Netea

Mihai Netea was born and studied medicine in Cluj-Napoca, Romania. He completed his PhD at the Radboud University Nijmegen, The Netherlands, on studies investigating the cytokine network in sepsis. After working as a post-doc at the University of Colorado, he returned to Nijmegen where he finished his clinical training as an infectious diseases specialist, and where he currently heads the division of Experimental Medicine, Department of Internal Medicine, Nijmegen University Nijmegen Medical Center. He is mainly interested in understanding the factors influencing variability of human immune responses, the biology of sepsis and immunoparalysis, and the study of the memory traits of innate immunity.

Luke O'Neill FRS

Professor Luke O'Neill was appointed to the Chair of Biochemistry at Trinity College Dublin in 2008, where he leads the Inflammation Research Group. He has a PhD in Pharmacology from the University of London and carried out Post-Doctoral research at Cambridge U.K. on the pro-inflammatory cytokine IL-1 and innate immune signaling. His research is in the area of the molecular basis to inflammatory diseases, with a particular interest in Toll-like receptors, inflammasomes and metabolic control of inflammatory cell signaling. He has won numerous awards for his research, notably the Royal Irish Academy Medal for Biochemistry, the Royal Dublin Society/ Irish Times Boyle medal for Scientific Excellence and in 2014 the European Federation of Immunology Societies Medal. He was elected a member of EMBO in 2005. In 2016 he was named by Thompson Reuters as one of the world's most influential scientists, being in the top 1% in both Immunology and Pharmacology/Toxicology. He is a European Research Council Advanced Grant Holder and is co-founder of 2 Spin-out companies from his lab - Opsona Therapeutics, a drug development company working in the area of Toll-like receptors, and Inflazome, which is developing inhibitors of inflammasomes for inflammatory diseases. He was elected a Fellow of the Royal Society in 2016.

Fiona Powrie FRS

Fiona Powrie is the Director of the Kennedy Institute of Rheumatology and Principal Investigator in the Translational Gastroenterology Unit, University of Oxford.

Her research interests include characterisation of the interaction between the intestinal microbiota and the host immune system and how this mutualistic relationship breaks down in inflammatory bowel disease.

Fiona's work has identified the functional role of regulatory T cells in intestinal homeostasis and shed light on their development and mechanism of action. She has also shown that both adaptive and innate immune mechanisms contribute to intestinal inflammation and identified the IL-23 pathway as a pivotal player in the pathogenesis of chronic intestinal inflammation.

Her current work seeks to translate findings from model systems into the clinic in inflammatory bowel disease patients.

Fiona Powrie received the Ita Askonas Award from the European Federation of Immunological Societies for her contribution to immunology in Europe and the Louis-Jeantet Prize for Medicine 2012.

She was elected a Fellow of the Royal Society in 2011, EMBO in 2013 and the Academy of Medical Sciences in 2014.

Michal Schwartz

Schwartz is a Professor of Neuroimmunology, incumbent of The Maurice and Ilse Katz Professorial Chair in Neuroimmunology, at the Weizmann Institute of Science, Rehovot, Israel.

Schwartz received her BSc degree with a major in chemistry, cum laude, from the Hebrew University, Jerusalem, Israel, and her PhD in Immunology from the Weizmann Institute. She performed postdoctoral research in Neuroscience at the University of Michigan, Ann Arbor, studying nerve regeneration.

Schwartz's research is focused on the role of innate and adaptive immunity in central nervous system (CNS) plasticity in health and disease, and on developing methodologies to manipulate the immune system for the benefit of the CNS under acute injuries, chronic neurodegenerative conditions, mental dysfunction, and brain aging. Schwartz pioneered the pivotal role of the systemic immune system in healthy brain function and repair. Schwartz basically redefined the relationships between the brain and the immune system in health and disease. She was the pioneer suggesting that both monocytes and T cells are needed for repair of injured CNS tissues (Nature Medicine, 1998; Nature Medicine. 1999; PLOS Medicine, 2009). She coined the concept of "protective autoimmunity", as a physiological response that protects the brain. Schwartz further demonstrated that T cells are needed for healthy brain functional plasticity (Nature Neuroscience, 2006). Subsequently, she identified the brain's choroid plexus epithelium, which forms the blood-CSF-barrier, as an active physiological immunological interface between the brain and the circulation, and as an entry gate for leukocytes, needed for brain homeostasis and repair.as the "permissive" immunological interface between the brain and the circulation (Immunity, 2013; Brain 2013). This led to her over the last 2 years to discover that brain aging and neurodegenerative diseases are associated with dysfunction of this interface (Science, 2014; J. Neuroscience, 2015; Nature communication, 2015), and that unleashing the immune system can combat Alzheimer's disease (Nature Communications, 2015; Nature Medicine, 2016).

Schwartz's work is highly cited (H index 97; Google Scholar). She has received a number of prestigious awards for her research, including the 2002 Friedenwald Award from ARVO (Association for Research in Vision and Ophthalmology), for her outstanding contribution to vision research and ophthalmology. She was appointed by the American Spinal Cord Injury Association to the Distinguished G. Heiner Sell Memorial Lectureship in 2002 for outstanding achievement in the field of spinal cord injury. She was one of the recipients of the NARSAD (The Mental Health Research Association) Distinguished Investigative Award (2007), she received twice Advanced European Research Commission award (2008, 2017), a honorary doctorate from Ben-Gurion University (2009), and a Brain research award for her pioneering work (2009). In 2015 she received the Blumberg Prize for excellence in medical science. In 2016 her book: "NEUROIMMUNITY: How Brain Science Will Revolutionize the Way We Live and Age", by MICHAL SCHWARTZ with Anat London, Yale University Press (https://proseawards.com/winners/), received Accolade from the annual PROSE Awards. Recently, Schwartz was profiled by Britannica Book of the year 2016, covering selected individuals and events that impacted the course of human history. Schwartz is the elected incoming president of the International Society of for Neuroimmunology (2016-2018). Lately, she received the 2017 Rappaport Prize for Excellence in the Field of Biomedical Research (awarded to an established Israeli biomedical researcher). She has mentored numerous graduated students some of them are already holding leadership in neuroimmunology.

Fiona Watt FRS

Fiona Watt obtained her first degree from Cambridge University and her DPhil, in cell biology, from the University of Oxford. She was a postdoc at MIT, where she first began studying differentiation and tissue organisation in mammalian epidermis. She established her first research group at the Kennedy Institute for Rheumatology and then spent 20 years at the CRUK London Research Institute (now part of the Francis Crick Institute). She helped to establish the CRUK Cambridge Research Institute and the Wellcome Trust Centre for Stem Cell Research and in 2012 she moved to King's College London to found the Centre for Stem Cells and Regenerative Medicine.

Saturday, July 8th, 2017				
	Balmoral	*Sandringham*	*Meeting Rooms 1-6*	*Monarch Suite*
18:00-19:00	**Opening Ceremony**			
19:00-20:00				**Welcome Reception**

Sunday, July 9th, 2017				
	Balmoral	*Sandringham*	*Meeting Rooms 1-6*	*Monarch Suite*
09:30-10:30	Plenary Lecture ***Ageing & Inflammation*** Janet Lord			
10:30-11:30				**Coffee Break**
11:00-12:00	Plenary Lecture ***Neuroinflammation*** Michal Schwartz			
12:00-13:00				**Lunch & Posters**
13:00-14:30	Symposia Session – BIRAs ***Inflammageing***	Symposia Session - GREMI ***Cell death & inflammation***	Symposia Session – Australia ***Novel inflammatory pathways and targets***	
14:30-15:45	**Oral Abstract Communications**	**Oral Abstract Communications**	**Oral Abstract Communications**	
15:45-16:15				**Coffee Break**
16:15-17:45	Symposia Session – Brazil ***Neuroinflammation***	Symposia Session – Canada ***The microbiome as a drug target***	Symposia Session – ISBD & BUS ***Rare Inflammatory Diseases: what can we learn?***	
17:45-18:45				**Posters**

Monday, July 10th, 2017				
	Balmoral	*Sandringham*	*Meeting Rooms 1-6*	*Monarch Suite*
09:30-10:30	Plenary Lecture ***The regulation of inflammation by epithelia and local T cells*** Adrian Hayday FRS			
10:30-11:00				**Coffee Break**
11:00-12:00	Plenary Lecture ***Innate Immune memory*** Mihai Netea			
12:00-13:00				**Lunch & Posters**
13:00-14:30	Symposia Session - Brazil ***Resolution and Repair***	Symposia Session – Japan ***Comprehensive perspectives of systemic and organ specific inflammatory responses***	Oral Abstract Communications	
14:30-15:45	**Oral Abstract Communications**	**Oral Abstract Communications**	**Oral Abstract Communications**	
15:45-16:15				**Coffee Break**
16:15-17:45	Sponsored Symposia – RSPCA ***3Rs in inflammation research***	Symposia Session – Italian ***Society of Pharmacology Targets of Inflammation***	Symposia Session ***Young Investigators Oral Presentations***	
17:45-18:45				**Posters**

Tuesday, July 11th, 2017				
	Balmoral	***Sandringham***	***Meeting Rooms 1-6***	***Monarch Suite***
09:30-10:30	Plenary Lecture ***Microbiome & Immunity*** Fiona Powrie FRS			
10:30-11:30				**Coffee Break**
11:00-12:00	Plenary Lecture ***Intestinal fibrosis in Inflammatory*** Bowel Disease Tom Holvoet			
12:00-13:00				**Lunch & Posters**
13:00-14:30	Symposia Session – USA ***Autophagy and Immune-regulation***	Symposia Session – Novel ***Therapies Redox therapeutics for inflammatory disease***	**Oral Abstract Communications**	
14:30-15:45	**Oral Abstract Communications**	**Oral Abstract Communications**	**Oral Abstract Communications**	
15:45-16:15				**Coffee Break**
16:15-16:30	**Young Investigator & Lifetime Achievement Awards**			
16:30-17:15	**Lifetime Achievement Lecture**			
17:15-18:00	Plenary Lecture ***Inflammation & Metabolism*** Luke O'Neill FRS			
18:00-19:00				**Posters**

Wednesday, July 12th, 2017				
	Balmoral	***Sandringham***	***Meeting Rooms 1-6***	***Monarch Suite***
08:30-09:30	**Women in Science Award & Lecture**			
09:30-10:30	Plenary Lecture ***Cancer & Inflammation*** Fiona Watt FRS			
10:30-11:00				**Coffee Break**
11:00-12:30	Sponsored Symposia – GSK-Epinova ***Epigenetics and inflammation***	Symposia Session – Russia ***Integrative Inflammatory Mechanisms***	**Oral Abstract Communications**	
12:30-13:15	President's Lecture & Closing Address ***Inflammageing & future therapies*** Peter Barnes FRS			

Sunday, July 9th		
09:30-10:30	**Balmoral**	Plenary Lecture **Ageing & Inflammation** *Janet LORD (Birmingham University)*
	Monarch Suite	**Coffee Break**
11:00-12:00	**Balmoral**	Plenary Lecture **Neuroinflammation** *Michal SCHWARTZ (Weizman Institute of Science)*
	Monarch Suite	**Lunch & Posters**
13:00-14:30	**Balmoral**	Symposia Session – BIRAs **Inflammageing** **This symposia brought to you by:** YEADON CONSULTING LTD **SASP, splicing regulation and rescue of senescence in primary human cells.** *Prof. Lorna HARRIES, University of Exeter* **Inhibiting inflammation to enhance human immunity in vivo.** *Prof. Arne AKBAR, UCL* **Neutrophil dysfunction in ageing & chronic inflammatory disease** *Dr. Elizabeth SAPEY, University of Birmingham* **Targeting inflammation to combat cellular ageing: Mitochondria as possible intracellular allies?** *Dr. Joao PASSOS, University of Newcastle.*
	Sandringham	Symposia Session – GREMI **Cell death & inflammation** **Roscovitine as new anti-inflammatory therapy promoting neutrophil apoptosis** *Adriano ROSSI, University of Edinburgh, UK* **C1q, cell death and inflammation** *Marina BOTTO, Imperial College London, UK* **Proteinase 3 on apoptotic cell: a new autoinflammatory trigger in autoimmune vasculitis** *Véronique WITKO-SARSAT, Cochin Institute, Paris, France* **Apoptotic cell-based therapies for transplantation and inflammatory diseases** *Sylvain PERRUCHE, Inserm - University of Besançon, France*
	Meeting Rooms 1-6	Symposia Session – Australia **Novel inflammatory pathways and targets** **New mechanisms of autoinflammation** *Seth MASTERS, WEHI* **Disordered haematopoiesis and cardiovascular disease** *Andrew MURPHY, Baker Institute* **A new GM-CSF-dependent pathway in inflammation** *Adrian ACHUTHAN, University of Melbourne* **Neural regulation of immunity after stroke** *Connie WONG, Monash University*

Sunday, July 9th		
14:30-15:45	Balmoral	**Oral Presentations**
	Sandringham	**Oral Presentations**
	Meeting Rooms 1-6	**Oral Presentations**
	Monarch Suite	**Coffee Break**
16:15-17:45	Balmoral	Symposia Session – BRAZIL **Neuroinflammation** ***Chair:*** *José Carlos ALVES FILHO (School of Medicine Ribeirao Preto, FMRP-University of São Paulo -Ribeirão Preto, SP, Brazil)* **Neuro-immune-glial interactions mediate herpetic neuralgia** *Thiago M. CUNHA (USP-Ribeirão Preto, SP)* **Neuropathic pain: role of spinal cord oligodendrocyte-derived IL-33** *Waldiceu Ap. VERRI JUNIOR (Universidade Estadual de Londrina, Center of Biological Science, Department of Pathological Science, Londrina, PR, Brazil)* **Contributions of peripheral and spinal inflammatory signalling to chronic pain states** *Victoria CHAPMAN (University of Nottingham, Nottingham, UK)*
	Sandringham	Symposia Session – CANADA **The microbiome as a drug target** ***Co-Chairs:*** *Dr. Nathalie VERGNOLLE (France) & Dr. Jason MCDOUGALL (Canada)* **Microbiota proteases and inhibitors** *Dr. Nathalie VERGNOLLE (France)* **The microbiome as a target for anti-inflammatories** *Dr. John L. WALLACE (Canada)* **Microbiome pathobionts: From new disease mechanisms to therapeutics** *Dr. Andre BURET (Canada)*
	Meeting Rooms 1-6	Symposia Session - ISBD & BUS **Rare Inflammatory Diseases: what can we learn?** **Sponsored by International Association for Behcet's Disease and Birdshot Uveitis Society** **Is HLA-B*51 the Causative Gene in Behcet's Disease** *Graham WALLACE (Birmingham University)* **Amplified inflammatory responses and thrombosis Behçet's Syndrome** *Dorian HASKARD (Imperial College London)* **Immune Dysfunction: the role of polymorphic ERAP1 in autoinflammatory disease** *Dr. Edd JAMES (University of Southampton, UK)* **A multiomics approach to rare disease research** *Dr. Jonas KUIPER (Utrecht University, Netherlands)*
17:45-18:45	Monarch Suite	**Posters**

Monday, July 10th, 2017		
09:30-10:30	**Balmoral**	Plenary Lecture **The regulation of inflammation by epithelia and local T cells** *Adrian HAYDAY FRS (Crick Institute)*
	Monarch Suite	**Coffee Break**
11:00-12:00	**Balmoral**	Plenary Lecture **Innate Immune memory** *Mihai NETEA (Radboud University, Holland)*
	Monarch Suite	**Lunch & Posters**
13:00-14:30	**Balmoral**	Symposia Session – BRAZIL **Resolution and Repair** ***Chair:*** *Fernando CUNHA (School of Medicine Ribeirao Preto, FMRP-University of São Paulo -Ribeirão Preto, SP, Brazil)* **Angiotensin1-7 and acetate – novel resolvers of inflammation.** *Mauro TEIXEIRA (UFMG, Belo Horizonte)* **Pro-resolution effect of 15d-PGJ2 on allergen-induced lung inflammation and remodeling** *Marco Aurélio MARTINS (Fiocruz, Rio de Janeiro)* **Immunoresolvents at the intersection between brain and immune system** *Jesmond DALLI (William Harvey Research Institute, London, UK)*
	Sandringham	Symposia Session – JAPAN **Comprehensive perspectives of systemic and organ specific inflammatory responses** **Interaction between the immune system and bone metabolism** *Dr. Hiroshi TAKAYANAGI (Univeristy of Tokyo)* **Regulation of intestinal inflammation** *Dr. Kiyoshi TAKEDA (Osaka University)* **Pathogenesis of inflammatory skin diseases** *Dr. Kenji KABASHIMA (Kyoto University)* **Interplay between chronic inflammation and metabolic syndrome** *Dr. Yoshihiro OGAWA (Kyushu University)*
	Meeting Rooms 1-6	**Oral Presentations**
14:30-15:45	**Balmoral**	**Oral Presentations**
	Sandringham	**Oral Presentations**
	Meeting Rooms 1-6	**Oral Presentations**
	Monarch Suite	**Coffee Break**

Monday, July 10th, 2017		
16:15-17:45	**Balmoral**	Sponsored Symposia - RSPCA **3Rs in inflammation research** **Introduction to the EWG process** *Elliot LILLEY (RSPCA)* **The 3Rs in Sepsis reserach** *Manasi NANDI (KCL)* **The 3Rs in Experimental autoimmune encephalomyelitis research** *Daniel ANTHONY (Oxford)* **The 3Rs in Rheumatoid arthritis research** *Michael SEED (UEL)*
	Sandringham	Symposia Session – Italy **Targets of Inflammation** **Resolution Pharmacology - therapeutic innovation in Inflammation** *Mauro PERRETTI (London, UK)* **Inflammation, a key event in development of neurodegenerative diseases** *Salvatore CUZZOCREA (Messina, Italy)* **Defining the role of glucocorticoids in inflammation with cell specific glucocorticoid receptor knockout mice** *John CIDLOWSKI (NC, USA)* **Glucocorticoid Induced Leucine Zipper (GILZ) in control of inflammation and tumor growth** *Carlo RICCARDI (Perugia, Italy)*
	Meeting Rooms 1-6	Symposia Session *(Young Investigator oral presentations)*
17:45-18:45	**Monarch Suite**	**Posters**

Tuesday, July 11th, 2017		
09:30-10:30	**Balmoral**	Plenary Lecture **Microbiome & Immunity** *Fiona POWRIE FRS (Oxford University)*
	Monarch Suite	**Coffee Break**
11:00-12:00	**Balmoral**	Plenary Lecture **Intestinal fibrosis in Inflammatory Bowel Disease** *Tom HOLVOET (Ghent University)*
		Lunch
13:00-14:30	**Balmoral**	Symposia Session - USA **Autophagy and Immune-regulation** **Autophagy proteins in endo- and exocytosis** *Dr. Christian MÜNZ, Institute of Experimental Immunology, University of Zurich* **Autophagy determines immune metabolism during neutrophil differentiation** *Dr. Katja SIMON, Kennedy Institute of Rheumatology, Oxford* **Impaired autophagy in Crohn's disease** *Dr. Arthur KASER, University of Cambridge*
	Sandringham	Symposia Session - Novel therapies **Redox therapeutics for inflammatory disease** **Novel isozyme-specific NOX inhibitors – pre-clinical & clinical studies in inflammatory diseases** *Dr. Philippe WIESEL , Genkyotex* **Myeloperoxidase, Friend or Foe. Studies in Models of Human Autoimmune Diseases** *Prof. Stephen HOLDSWORTH, Monash University* **Novel Nrf2 Activators for the Treatment of Chronic Inflammatory Diseases** *Dr. Colin MEYER, Reata Pharmaceuticals, Inc.* **Pre-clinical studies of novel anti-inflammatory hydrogen sulfide donors** *Prof. Matt WHITEMAN, University of Exeter, UK*
	Meeting Rooms 1-6	**Oral Presentations**
14:30-15:45	**Balmoral**	**Oral Presentations**
	Sandringham	**Oral Presentations**
	Meeting Rooms 1-6	**Oral Presentations**
	Monarch Suite	**Coffee Break**
16:15-16:30	**Balmoral**	**Young Investigator & Lifetime Achievement Awards**
16:30-17:15	**Balmoral**	**Lifetime Achievement Lecture**
17:15 - 18:00	**Balmoral**	Plenary Lecture **Inflammation & Metabolism** *Luke O'NEIL FRS (Trinity College Dublin)*

Wednesday, July 12th, 2017		
08:30-09:30	**Balmoral**	**Women in Science Award & Lecture**
09:30-10:30	**Balmoral**	Plenary Lecture **Cancer & Inflammation** *Fiona WATT FRS (Kings College London)*
	Monarch Suite	**Coffee Break**
11:00-12:30	**Balmoral**	Sposored Symposia - GSK-EPINOVA **Epigenetics and Inflammation** **Transcriptional mechanisms underlying inflammation** *Rahul ROYCHOUDHURI, Babraham Institute* **Epigenetic control of human inflammatory disease phenotypes** *Rab PRINJHA, Epigenetics DPU, GSK* **The transcription factor T-bet as a master regulator of mucosal homeostasis in health and disease** *Graham LORD, King's College London*
	Sandringham	Symposia Session – **Integrative Inflammatory Mechanisms** **Immunopathophysiology of Proinflammatory Mechanisms** *Valeriy CHERESHNEV* **Inflammation and Shock: Interaction of SIRS and other Complications** *Irina FOMOCHKINA* **Autoantibodies to beta1-adrenoreceptors and cardiac arrhythmias: breakthroughs and pitfalls** *Kirill ZYKOV* **Inflammation and Systemic Typical Pathological Processes: Interaction and Conflict of Regulating Programs** *Leonid CHURILOV*
	Meeting Rooms 1-6	**Oral Presentations**
12:30-13:15	**Balmoral**	Presidents Lecture & Closing Address **Inflammageing & future therapies** *Peter BARNES FRS (Imperial Collge London)*

OP-01	Decreased neutrophil migration and activation in aging: A protective role for the adenosine a2a receptor	*Marc Pouliot*			
OP-02	Inflammageing contributes to age-related impairments in the maintenance of immunological memory in the bone marrow	*Luca Pangrazzi*			
OP-03	Age dependent effect on post-stroke infection	*Shu Wen Wen*			
OP-04	Senescent endothelium maintains the anti-inflammatory vascular phenotype: identification of a vascular protective gene	*Jennifer R Gamble*			
OP-05	Obesity, ageing and sarcopenia: 'Resistin-ance' is futile	*Mary Oleary*			
OP-06	Survival of exhausted and highly differentiated CD8+ T cells in the human bone marrow and the effects of aging and Cytomegalovirus	*Erin Courtney Naismith*			
OP-07	Effector $CD4^+$ T cells promote glomerular inflammation via recognition of intravascular antigen presented by patrolling monocytes	*Michael Hickey*			
OP-08	Eosinophils have an essential role in cardiac repair following myocardial infarction	*Iqbal Toor*			
OP-09	*Chlamydia trachomatis* Associated Immune Responses, Inflammation and Tissue Pathology is Regulated by Effector Cell Specific MicroRNAs	*Bernard Arulanandam*			
OP-10	Investigating the role of chemerin and the non-signalling chemerin receptor, CCRL2, during an acute inflammatory model of peritonitis	*Sophia Valaris*			
OP-11	Suppressive effects of early-phase administration of intravenous immunoglobulin in recurrent pregnancy loss model mice	*Jun Tanaka*			
OP-12	Parenchymal nitric oxide synthase mediated injury and dysfunction in the acutely inflamed exocrine salivary glands. Bystander role of invasively infiltrating leukocytes	*Abeer Shaalan*			
OP-13	*Escherichia coli* type VI secretion system (T6SS) modulates murine inflammation and innate immune response	*Dalila Juliana Silva Ribeiro*			
OP-14	Pre-hospital immune responses and development of multiple organ dysfunction syndrome following traumatic injury	*Jon Hazeldine*			
OP-15	Elevated CD31 levels on 1,25-dihydroxyvitamin D3 treated $CD11c^+$ dendritic cells restrain $CD4^+$ T cell priming ability	*Iris Mair*			
OP-16	NKTR-358: a selective, first-in-class IL-2 pathway agonist which increases the number and suppressive function of regulatory T cells for the treatment of immune inflammatory disorders	*John L Langowski*			
OP-17	PGE_2 protects against intestinal barrier damage and systemic inflammation through promoting ILC3 response	*Chengcan Yao*			
OP-18	Non-classical monocytes orchestrate inflammatory myeloid retention to mediate tissue damage in immune-complex-mediated glomerulonephritis	*Kevin Woollard*			
OP-19	Mutually counteracting effects of CerS2 and CerS6 in G-CSF signaling	*Jennifer Kurz*			
OP-20	A pro-inflammatory environment modulates the human dermal fibroblast phenotype: implications for chronic wounds	*Aaiad Al Rikabi*			
OP-21	WNT signalling in salivary gland injury and repair	*Araz Ahmed*			
OP-23	NFkappaB and NFAT5 transcription factors are involved in muscle inflammation and function in close interrelationship	*Boel De Paepe*			
OP-24	Macrophages require phosphoSTAT3 activation during efferocytosis to support autophagy and phenotypic conversion in sterile injury	*Lara Campana*			
OP-25	SCIMP is a novel transmembrane TLR adaptor protein that imparts cytokine specificity to TLR4 responses in macrophages	*Matthew J Sweet*			
OP-26	Neuronal Cue on Macrophages to Enhance Neovascularization; Roles of CGRP-RAMP1 signaling	*Masataka Majima*			
OP-27	Secreted Ectodomain of Sialic Acid-Binding Ig-Like Lectin-9 and Monocyte Chemoattractant Protein-1 Synergistically Regenerate Transected Rat Peripheral Nerves by Altering Macrophage Polarity	*Fumiya Kano*			
OP-28	Subtype-selective $GABA_A$ receptor agonist reduces inflammation and improves recovery post-stroke	*Silke Neumann*			
OP-29	Narrowband UVB phototherapy for Clinically Isolated Syndrome: Delivering the benefits of all UVB-induced molecules to early MS patients	*Prue H Hart*			

OP-30	Cytokines as peripheral biomarkers in neurodegenerative disorders: plasma and CSF analysis of patients with mild cognitive impairment	*Bianchi Massimiliano*			
OP-31	The role of chemokines in the pathophysiology of major depressive disorder	*Vladimir M Milenkovic*			
OP-32	Redox-dependent B7-H1 downregulation contributes to liver failure during murine polymicrobial sepsis	*Andreas Von Knethen*			
OP-33	The effect of titanium implant derivatives on Neutrophil Extracellular Trap formation	*Helen Roberts*			
OP-34	Hydroquinone exposure aggravates arthritis in rats	*Cintia Scucuglia Heluany*			
OP-35	Immunomodulatory effect of minocycline in intestinal inflammation	*Natividad Garrido Mesa*			
OP-36	T-Cell TNFα synthesis and monocyte differentiation to functional TNF synthesising macrophages is inhibited by sulphated disaccharides. An explanantion for their anti-rheumatic activity?	*Prabhjot Bajwa*			
OP-37	Selective deletion of T-bet in ILCs regulates intestinal inflammatroy responses	*Natividad Garrido Mesa*			
OP-38	Disarming *Pseudomonas aeruginosa* evasion mechanisms in the lung vasculature with a bi-specific antibody	*Ajitha Thanabalasuriar*			
OP-39	Short chain fatty acids modulate the inflammatory response during bacterial infection	*Marco Aurélio Ramirez Vinolo*			
OP-40	An ecoimmunological approach to study an ancient link between complement and humoral immunity	*Praveen Papareddy*			
OP-41	Serum 3-nitrotyrosine levels increase prior to a sepsis diagnosis in individuals with post-surgery sepsis	*Annie Rose Knight*			
OP-42	Measurement of serum nitrate concentration for the diagnosis of infective gastroenteritis	*Miranda Jane Smallwood*			
OP-43	Dual integrin blockade attenuates fibrotic and vascular alterations in a murine model of sistemic sclerosys	*Gabriele Pizzino*			
OP-44	Blocking the TNF Superfamily member LIGHT to control lung and skin fibrosis	*Rana Herro*			
OP-45	Fibroblast innate memory: tropism, disease-association and consequences	*Thomas Crowley*			
OP-46	Elucidating the mechanisms governing neutrophil reverse migration	*Hannah Isles*			
OP-47	TRPA1 elicits inflammatory effects in chondrocytes and its expression is downregulated by anti-inflammatory drugs dexamethasone and aurothiomalate	*Elina Nummenmaa*			
OP-48	A metabolic study of microbiome modulation in mice	*Caitlin SL Parello*			
OP-49	Protective, anti-inflammatory effects of the microbiome-associated metabolite propionate upon the blood–brain barrier	*Simon McArthur*			
OP-50	Plasmin and Plasminogen induce macrophage reprogramming and regulate key steps of inflammation resolution via Annexin A1	*Michelle A Sugimoto*			
OP-51	The cannabinoid receptor CB2 plays a non-redundant role in neutrophil recruitment in acute inflammation	*David Robert Greaves*			
OP-52	Src homology 2 domain-containing protein tyrosine phosphatase (SHP-1): role in polarization of adipose tissue macrophages and development of FRET based activity reporter	*Farah Khan*			
OP-53	Non-invasive luminol-based bioluminescence imaging as a standardized measure of inflammation in animal models for skin diseases	*Natasja De Bruin*			
OP-54	Inhibition of IκB Kinase attenuates cardiac dysfunction caused by sepsis in mice with pre-existing type 2 diabetes mellitus	*Sura Al Zoubi*			
OP-55	Annexin A1 (ANXA-1)-mimetic peptide controls the inflammatory and fibrotic effects induced by house dust mite (HDM) in mice	*Patricia Machado Rodrigues Silva*			
OP-56	Pre-stimulation with IL-6 metabolically prepares T cells for the antigenic response	*Kalvin Sahota*			
OP-57	HGK/MAP4K4 deficiency induces TRAF2 stabilization and Th17 differentiation leading to T-cell-mediated type 2 diabetes	*Tse Hua Tan*			

OP-58	Synovial fibroblasts display metabolic memory and bioenergetic reprogramming during the transition from resolving to persistent inflammatory disease	*Jane Falconer*			
OP-59	A novel IRAK1/4 dual inhibitor prevents inflammation in rodent models of autoimmune disease and reverses lupus-like disease in NZB/W F1 mice	*Chrystelle Lamagna*			
OP-60	Sensory nerves and mediators have protective roles in Aldara-induced skin inflammation and associated nocifensive behaviours	*Xenia Kodji*			
OP-61	Diet promoted intra-hepatic inflammatory foci representing a model of Nonalcoholic Steatohepatitis (NASH)	*Ghazal Alipour Talesh*			
OP-62	Melanocortin 1 receptor modulates skin repair by driving wound angiogenesis and lymphangiogenesis	*Jenna Cash*			
OP-63	Evidence for an in vivo protective effect of endogenous TRPC5 in rheumatoid- and osteo-arthritis	*Susan D Brain*			
OP-64	Amelioration of experimental arthritic pain and disease by G-CSF receptor blockade	*Ming Chin Lee*			
OP-65	Dimethyl fumarate reduces tactile allodynia in a HCAR2-mediated mechanism in two models of peripheral neuropathic pain	*Livio Luongo*			
OP-66	Pyrazine-fused triterpenoids block TRPA1 ion channel *in vitro* and inhibit TRPA1-mediated acute inflammation *in vivo*	*Mari Hämäläinen*			
OP-67	Loin Pain Haematuria Syndrome (LPHS): in vitro modelling for assessing the contribution of inflammtory components from blood	*Elaine How*			
OP-68	Anti-inflammatory and Antinociceptive potential of Opuntia dillenii and its pure compounds: Opuntiol and Opuntioside via Eicosanoids and Cytokines Inhibition Pathways	*Faheema Siddiqui*			
OP-69	Hydroalcoholic crude extract of *Casearia sylvestris* Sw. reduces chronic post-ischemic pain by activation of pro-resolving pathways	*Anna Paula Piovezan*			
OP-70	Inhibition of IL-13Rα2 protects mice from DSS-induced murine IBD	*Kevin Vannella*			
OP-71	cAMP enhancing drugs salbutamol and rolipram augment the alternative activation of murine macrophages through MKP-1	*Tiina Leppänen*			
OP-72	Comparison of two cell-free assays for anti-drug antibody detection in biologic therapy	*Sorwe Mojtahed Poor*			
OP-73	Blocking IL-17C reverses the disease signature in a mouse model of atopic dermatitis	*Stéphanie Lavazais*			
OP-74	CCR1 and CCL3 in mouse model of Behcet's disease	*Seonghyang Sohn*			
OP-75	Activation of adenosine receptor by PDRN improves skin remodelling in an experimental model of psoriasis-like dermatitis	*Natasha Irrera*			
OP-76	Bruton's tyrosine kinase inhibition reduces the development of diabetic nephropathy by reducing NF-κB and NLRP3 inflammasome activation	*Gareth S D Purvis*			
OP-77	The antimicrobial peptide LL37 underpins thrombotic complications during inflammatory diseases	*Sakthivel Vaiyapuri*			
OP-78	Modeling Psoriasis-Like Inflammation in Rats	*Britnie R James*			
OP-79	Homeostatic cytokines interleukin-7 (IL-7) and IL-15: novel targets to control inflammatory CD4+CD28null T cells and reduce cardiovascular risk in coronary atherosclerosis and rheumatoid arthritis	*Ingrid E Dumitriu*			
OP-80	Human bone marrow adipocytes display distinct functional immune regulatory properties	*Carina Michelle Miggitsch*			

PP-001	Age-associated changes in Natural killer cell cytotoxicity and migration	*Mohammad Ahsan Tariq*			
PP-002	Effect of Bax Inhibitor-1 (BI-1) on aging induced ER stress	*Kashi Raj Bhattarai*			
PP-003	Role of contraceptive hormones on combination antiretroviral treatment induced apoptosis in human cervical cancer cells	*Christopher Mafuva*			
PP-004	Macrophage annexin-A1 is critical in the tumor microenvironment by promoting alternative macrophage polarization	*Leonardo A Moraes*			
PP-005	Anti-cachectic effect of Shenchujianpi-tang, a traditional herbal remedy, is associated with suppression of pro-inflammatory cytokine production and inhibition of autophagy	*Jun Yong Choi*			
PP-007	Downregulated STAT6 in glioma increases hypoxic viability via mTOR/HIF-1α, but decreases STAT6-specific gene expression, leading to a facilitation of cancer cell survival	*Soo Jung Park*			
PP-008	Lipoxin A4 selectively programs the profile of M2 tumor-associated macrophages which favour control of tumor progression	*Natália Mesquita De Brito*			
PP-009	Priming endothelial cells with a melanoma-derived extracellular matrix triggers the activation of αvβ3/VEGFR2 axis	*Renata Machado Brandão Costa*			
PP-010	NADPH oxidase 4 contributes to immune tolerance of oral cancer by recruiting regulatory T cells	*Wei Gao*			
PP-011	Regulation of Natural Killer Cells by Long Pentraxin-3 in Oral Cancer	*Thian Sze Wong*			
PP-012	Cardiac melanocytes influence atrial reactive oxygen species involved with electrical and structural remodeling in mice	*Hayoung Hwang*			
PP-013	The role of the G-actin sequestering peptide, thymosin-beta4, in macrophage function	*Elisavet Vasilopoulou*			
PP-016	Influence of VSIG4 Gene Polymorphism on The Clinical Expression of Rheumatoid Arthritis	*Iara José Messias Reason*			
PP-017	MASP2 Intronic Polymorphisms Increase The Susceptibility To Endemic Pemphigus Foliaceous	*Iara José Messias Reason*			
PP-018	Association between Complement Receptor 1 polymorphisms and leprosy in Brazil	*Iara José Messias Reason*			
PP-019	FCN1 Polymorphisms and Rheumatic Fever in Brazilian Patients	*Iara José Messias Reason*			
PP-020	Pemphigus Foliaceus and The Lectin Pathway of Complement: Evidence for An Association With Masp1 Polymorphisms	*Iara José Messias Reason*			
PP-021	Role of Lectin Pathway Components in Suscetibility to HIV, AIDS and Hepatitis Coinfection	*Iara José Messias Reason*			
PP-022	Ficolin-3 Association with Viral Hepatitis in HIV Infected Patients	*Iara José Messias Reason*			
PP-023	Association Between FCN3 intronic Polymorphisms and Pemphigus Foliaceous	*Iara José Messias Reason*			
PP-024	Genetic polymorphisms of the complement system influence susceptibility to pemphigus foliaceous	*Iara José Messias Reason*			
PP-025	TMEM203: a putative co-regulator of STING-mediated innate immunity	*Yang Li*			
PP-026	Effect of decreased BCAA synthesis through disruption of ilvC gene on the virulence of Streptococcus pneumoniae	*Gyu Lee Kim*			
PP-027	Induction of atopic dermatitis (AD)-like symptoms in NC/Nga mutant mice with repeated topical applications of the hapten, oxazolone	*Harunor Rashid*			
PP-028	Activity of inflammation and various parts of immune response depending on the duration of disease and frequency of exacerbations in chronic inflammatory diseases	*Mykola O Klymenko*			
PP-030	Case of Angiolymphoid Hyperplasia with Eosinophilia Associated with Anti-TNF Inhibitor	*Neeraj Singh*			
PP-031	MiRNA regulation of CXCL12β during inflammation	*Raju Ranjha*			
PP-032	Aberrant expression of interleukin-10 and activation-induced cytidine deaminase in B cells from patients with Behçet's disease	*Jeong Yun Yoon*			
PP-033	M1-polarized macrophages control mycobacterial survival by induction of endoplasmic reticulum stress-mediated apoptosis	*Yun Ji Lim*			
PP-034	Downregulation of tumor necrosis factor and interleukin-1 by nanoprotein encapsulated resveratol in murine and human monocyte cell line	*Seonghoon Park*			

PP-035	Involvement of prostaglandin terminal synthases PGIS and mPGES-1 in contact hypersensitivity	*Shuntaro Hara*			
PP-036	Periostin deficiency exacerbates joint inflammation and bone destruction in mouse models of rheumatoid arthritis	*Yun Hong Cheon*			
PP-037	Pneumococcal pep27 mutant immunization confers a wide range of protection with long-term immunity via activation of adaptive immune response	*Gyu Lee Kim*			
PP-038	Enhancing effects of Korean red ginseng on pneumococcal pep27 mutant immunization	*Sion Lee*			
PP-039	Neutrophil Phenotype and Function in Ocular Inflammatory disorders	*Mariam Murad*			
PP-040	Immune response profile in natural dentine repair	*Vitor De Carvalho Moreno Das Neves*			
PP-041	Prophylactic and therapeutic effects of three different pharmacological agents in the key-hole limpet hemocyanin (KLH)-induced delayed-type hypersensitivity (DTH) model in mice	*Harunor Rashid*			
PP-042	Platelet-mediated activation of neutrophils results in coagulopathy and contributes to lung pathogenesis associated with virus infection	*Seok Joo Kim*			
PP-043	A multifunctional approach towards testing immunomodulatory effects of new drugs	*Susanne Schiffmann*			
PP-044	Roles of macrophage migration inhibitory factor in cartilage tissue engineering	*Yuko Fujihara*			
PP-045	Interrelationships between inflammation and humoral immune response to enterobacterial lipopolysaccharides in patients with rheumatoid arthritis	*Anatolii Kubyshkin*			
PP-046	Reducing virulence factors and increasing inflammatory responses by pneumococcal Δpep27	*Sejin Kim*			
PP-047	Leukotriene (LT) B4 metabolites inhibit the LTB4-mediated functions of human neutrophils	*Caroline Turcotte*			
PP-049	Ficolin-1 serum levels in patients with HIV infection from Brazil	*Iara José Messias Reason*			
PP-050	Mannose binding lectin (MBL) and Pentraxin 3 plasma levels as biomarkers of Diabetic Retinopathy	*Iara José Messias Reason*			
PP-051	Ablating Ifitm genes potentiates inflammatory responses in the mouse	*Shau Kwaun Chen*			
PP-052	Synthetic tripeptide WOL074-029 shows potent systemic and local anti-inflammatory capacities	*Ana Kilic*			
PP-053	Impact of Toll-like receptor 4-mediated signalling in the modulation of platelet activation, haemostasis, and thrombosis	*Thomas Vallance*			
PP-054	CD39 contributes sepsis-induced immunosuppression by expanding the regulatory T cell population	*Daniele Bernardo Nascimento*			
PP-055	Immature single and double positive thymocytes contribute to septic thymus involution	*Tilo Knape*			
PP-056	The AIM2 Inflammasome Preferentially Engages Active but Unprocessed Caspase-1 to Induce Noncanonical Activation of the NLRP3 Inflammasome	*Dario S Zamboni*			
PP-057	Are 3σ or more levels of power achievable in integrative models of inflammation?	*Michael Seed*			
PP-058	Clinical features of drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome in Korea	*Jinyong Lee*			
PP-061	Parapancreatic adipose tissue immunometabolism in experimental rats with streptozotocin-induced diabetes and after introduction of metformin	*Denis Putilin*			
PP-062	Molecular characterization of metabolic health in young adults by expression of sirt1, circulating miRNAs, cytokines and adipokines	*Alejandro Méndez Mancilla*			
PP-063	Zinc Supplementation Prevents pneumococcal sepsis, pneumonia with alcoholic lung disorder	*Seungyeop Lee*			
PP-064	Decreased expression of CDKN2A/2B associate with T cell imbalance in human diabetes and coronary artery disease	*Herminia González Navarro*			
PP-065	Genetic inactivation of the cytokine LIGHT(TNFSF14) decreases insulin resistance in mice fed a high-fat high-cholesterol diet	*Herminia González Navarro*			
PP-066	Involvement of glycolysis and pentose phosphate pathway in formation of neutrophil extracellular traps by septic neutrophils	*Elzbieta Kolaczkowska*			

PP-067	Adenylate kinase from Streptococcus pneumoniae is essential for growth through its catalytic activity	*Bo Gyeong Kim*			
PP-068	Anti-inflammatory effects of traditional herbal extracts from Stellaria dichotoma var. lanceolata on Mycobacterium abscessus-infected murine macrophages	*Sujin Bae*			
PP-069	Orphan nuclear receptor Small Heterodimer Partner mediates host resistance to infection with Toxoplasma gondii	*Jina Lee*			
PP-070	Induction of reactive oxygen species is important to suppress the intracellular survival of Mycobacterium smegmatis via ER stress responses	*Seonhwa Kim*			
PP-072	Inhibition of autolysis by lipase LipA in Streptococcus pneumoniae sepsis	*Gyu Lee Kim*			
PP-073	Visceral leishmaniasis patients: the role of different immune cell populations	*Gabriela Pessenda*			
PP-074	Capsular Polysaccharide Synthesis by Glucosyltransferase (GtfA) in Streptococcus pneumoniae	*Sion Lee*			
PP-075	Mannose Binding Lectin (MBL) deficiency and risk to Tuberculosis infection in Ankylosing Spondylitis patients	*Iara José Messias Reason*			
PP-076	Zika Virus Induces Inflammasome Activation in BMDMs of C57BL/6 Mice	*Taline Monteiro Klein*			
PP-077	Translational features of a model of hRSV infection in BALB/c mice: efficacy of Ribavirin, Rupintrivir and Oseltamivir	*Dinko Relkovic*			
PP-078	Clinical utility of IL28B genotypes in chronic hepatitis B with entecavir monotherapy	*Jaekyun Choi*			
PP-080	Melatonin Treatment Modulates Intestinal Microbiota on DSS Colitis	*Young Sook Park*			
PP-081	Women's pregnancy history may influence Alzheimer's risk through alterations in immune function	*Molly Fox*			
PP-082	The synthetic form of malaria pigment hemozoin induces NF-kappa B-mediated neuroinflammation in BV2 microglia: implications for cerebral malaria	*Olumayokun Olajide*			
PP-083	TRPV1 in Allergic Contact Dermatitis	*Francis Fu Yuen Lam*			
PP-084	Anti-inflammatory and neuroprotective effects of co-ultraPEALut in a mouse model of vascular dementia	*Rosalba Siracusa*			
PP-085	Pseudobulbar Affect Associated with Autoimmune Encephalitis	*Neeraj Singh*			
PP-086	Harmane ameliorates depressive-like behaviors in chronic mild stress-treated rats: The possible involvement of the neuroinflammatory pathway	*Feyza Aricioglu*			
PP-087	Annexin A1 controls PPARγ and CD36 expressions by BV2 cells	*Sandra Poliselli Farsky*			
PP-088	EHD1 modulates IL-6/JAK2/STAT3 signaling in a lipid raft dependent manner	*Joo Hong Woo*			
PP-089	Dimethyl fumarate attenuates neuroinflammation and neurobehavioral deficits induced by experimental traumatic brain injury	*Emanuela Esposito*			
PP-090	Inhibition of mammalian target of rapamycin (mTOR) improves neurobehavioral deficit and modulates inflammatory response after traumatic brain injury	*Michela Campolo*			
PP-091	Multiple mechanisms of dimethyl fumarate in amyloid β-induced neurotoxicity in human neuronal cells	*Marika Lanza*			
PP-092	The effect of antidepressant-acting NOS inhibitors on Nod-like receptor protein 1 (NLRP1) inflammasome-mediated sterile neuroinflammation in a rat model of depression	*Ceren Sahin Ozkartal*			
PP-093	α-Chlorofatty acid induces endoplasmic reticulum stress and inflammation in brain microvascular endothelial cells	*Nora Kogelnik*			
PP-094	Multi-tracer brain imaging detects regional microglial over-activation in a mouse model of systemic inflammation	*Dávid Szöllösi*			
PP-095	Activating transcription factor-3 regulates inflammatory cytokines against lung infection by activating the MAPK-jnk pathway	*Seungyeop Lee*			
PP-096	Evidence for gender-dependent in experimental pneumococcal meningitis	*Seungyeop Lee*			
PP-097	Effects of GYY-4137 on K/BxN serum transfer induced arthritic TRPA1 WT and KO mice	*István Zoárd Bátai*			

PP-098	Protective role of p-coumaric acid in diabetes-induced periodontitis and alveolar bone loss in mice	*Govinda Bhattarai*			
PP-099	Bax inhibitor-1 inhibits acetaminophen- induced hepatotoxicity by reducing oxidative ER stress through regulating the RIDD activity of IRE1α	*Raghupatil Junjappa*			
PP-100	Protective effects of Dunaliella against to suppression of co-administration of ethanol and DEHP on glutathione peroxidase activity and total antioxidative capacity in HepG2 Cells	*Jong Yuh Cherng*			
PP-101	Inhibitory effects of Cordyceps extract on UV-induced MMP-1 expression and degradation of collagen	*Mei Fen Shih*			
PP-102	Approaches to the regulation of chronic low-intensity inflammation that follow metabolic disorders	*Anatolii Kubyshkin*			
PP-103	Intestinal anti-inflammatory activity of Musa spp (AAA) involves incresead production of short-chain fatty acids	*Luiz Claudio Di Stasi*			
PP-104	LRRK2 G2019S mutation disrupt ER calcium homeostasis and increase ER stress-induced cell death by inhibiting SERCA activity	*Ji Hye Han*			
PP-105	Optimization of a chronic colitis model in mice induced by dextran sulphate sodium (DSS)	*Harunor Rashid*			
PP-106	Effect of Korean red ginseng extracts on drug-drug interaction	*Sejin Kim*			
PP-107	The role of nonspecific proteinases in the development of inflammation of the paranasal sinuses	*Anatolii Kubyshkin*			
PP-108	Accelerated development of aging-associated and instability-induced osteoarthritis in 12/15-lipoxygenase deficient mice	*Hassan Fahmi*			
PP-109	A new inflammation resource: Introducing the IUPHAR Guide to IMMUNOPHARMACOLOGY (GtoImmuPdb)	*Elena Faccenda*			
PP-110	Immunomodulation of human complement system by supercritical extracts of Musa paradisiaca L. inflorescence	*Iara José Messias Reason*			
PP-111	Regulatory role of peripheral peptidergic sensory nerves in the proteoglycane-induced chronic autoimmune arthritis model of the mouse	*Adam Horvath*			
PP-112	Validation of an electrochemiluminescence-based ELISA for high-sensitivity measurement of the oxidative stress biomarker 3-nitrotyrosine	*Annie Rose Knight*			
PP-113	The development of translational biomarkers as a tool for improving the understanding, diagnosis and treatment of chronic neuropathic pain	*Patrick C Mchugh*			
PP-114	Involvement of Th1cell-dependent responses in the transition of nerve injury-induced acute pain to a chronic pain state	*Xinwen Zhang*			
PP-115	Antinociceptive Effects of Methanol Extract of Callophyllum inophyllum: Possible Roles of the Opioidergic, Adrenergic and Cholinergic Pathways	*Olubori Adebukola Mujeedat*			
PP-116	Gastrointestinal safety and efficacy of long-term GCSB-5 use in patients with osteoarthritis: A 24-week, multicenter study	*Joon Soon Kang*			
PP-118	Dimethyl trisulfide and sodium polysulfide both ameliorate mechanical hyperalgesia induced by carrageenan in a TRPA1 receptor-dependent manner	*Gabor Pozsgai*			
PP-119	Adipokine contribution to enhanced inflammatory pain in rodent models of obesity	*Sharron Dolan*			
PP-120	Pilot epidemiological survey of patients suffering from Loin Pain Haematuria Syndrome (LPHS)	*Michael Seed*			
PP-121	IFNβ is produced by resolution-phase macrophages and promotes the resolution of inflammation	*Amiram Ariel*			
PP-123	Molecular and structural alterations after Bidens pilosa L. supercritical CO2 extract treatment in intestinal inflammation induced by TNBS in rats	*Luiz Claudio Di Stasi*			
PP-124	The effect of the selective MC3 agonist PG990 on high density human chondrocyte micromass cultures activated by TNF-alpha	*Vedia C Can*			
PP-125	The Effect of oxygen and pentoxifylline on macrophage and T cells in hypoxic condition	*Sung Hyuk Choi*			
PP-126	The non-antibiotic macrolide EM703 improves survival in a model of quinolone-treated Pseudomonas aeruginosa airway infection	*Gopinath Kasetty*			
PP-127	The impact of pentoxyfilline in inflammatory cells interaction	*Sung Hyuk Choi*			

PP-128	Anti-inflammatory activity and inhibition of ICAM-1 expression by aqueous extract from Tragia benthamii Bak	*Olubori Adebukola Mujeedat*			
PP-129	Scalp treatment with Low Level Light Therapy/GentleWaves® down-regulates inflammatory biomarkers CD69, AP1 and mir21 in men with androgenetic alopecia	*Yann Franck Mahé*			
PP-130	Protectin D1n-3 DPA and resolvin D5n-3 DPA are effectors of intestinal protection	*Thomas Gobbetti*			
PP-131	Rebamipide regulates goblet cell differentiation and alleviates radiation induced colitis	*Hyosun Jang*			
PP-132	Transgenic mice expressing human proteinase 3 exhibit sustained neutrophil-associated peritonitis	*Katherine R Martin*			
PP-133	Rebamipide inhibits MMP9 and attenuates the barrier disruption in radiation enteritis	*Sehwan Shim*			
PP-134	Inhibition of inflammasome activation improves lung acute injury induced by carrageenan in a mouse model of pleurisy	*Roberta Fusco*			
PP-135	PEA/PLD association, reduce inflammatory process associated to experimental mouse model of atherosclerosis, induced by carotid artery ligation	*Enrico Gugliandolo*			
PP-136	Lipid mediator class-switching downstream of PGE2 determines the outcome of inflammation resolution in vivo	*Catherine Loynes*			
PP-137	Role of S-nitrosoglutathione reductase (GSNOR) on inflammation	*Toshihiro Tanioka*			
PP-138	Enhanced expression of NLRP3 inflammasome, interleukin-1β, and interleukin-18 in Sjögren's syndrome	*Seong Kyu Kim*			
PP-139	The antioxidant activity of pistachios reduces cardiac tissue injury of acute ischemia/reperfusion (I/R) in diabetic streptozotocin (STZ)-induced hyperglycaemic rats	*Rosanna Di Paola*			
PP-140	Oral administration of linoleic acid induces new vessel formation and improves skin wound healing in diabetic rats	*Hosana Gomes Rodrigues*			
PP-141	Regulation of bone metabolism by Resolvin D1	*Mohamed Benderdour*			
PP-142	Effects of Corticosteroid and Anti-TNF in Murine Collagen-Antibody Induced Arthritis	*Samadfam Rana*			
PP-143	Effects of Corticosteroid in the Rat Ovalbumin Pulmonary Inflammation Model	*Rana Samadfam*			
PP-144	Genistein effects on primary rat chondrocytes: possible use in osteoarthritis	*Federica Mannino*			
PP-145	Podoplanin expression by mesenchymal stromal cells promotes migratory capacity	*Lewis S.C. Ward*			
PP-146	Monitoring of Pneumocystis jirovecii acquired pneumonia by specialized pro-resolving mediators	*Marc Dubourdeau*			
PP-147	Role of TSPO ligands on GPCR and TLR-4 pathways on neutrophils	*Sandra Poliselli Farsky*			
PP-148	Ferric carboxymaltose does not induce apoptosis in human endothelial cells: implications in chronic kidney disease	*Anne M Graham*			
PP-149	YghJ (SslE), a secreted lipoprotein of neonatal septicemic E. coli induces TLR2 mediated inflammation in macrophages with the involvement of NFκB and MAP kinase signaling	*Rima Tapader Ghosh*			
PP-150	Boerhavia diffusa attenuates inflammation and bone damage through inhibiting the activation of nuclear factor-κB in Wistar rats with adjuvant-induced arthritis	*Ritu Karwasra*			
PP-151	6-Hydroxy-5,7-dimethoxy-flavone suppresses the neutrophil respiratory burst via selective PDE4 inhibition to ameliorate acute lung injury	*Tsong Long Hwang*			
PP-152	AMPK-related kinase MPK38/MELK stimulates GPR120-mediated anti-inflammatory pathway by phosphorylating GPR120 at Ser226	*Hyunjung Ha*			
PP-153	Terminalia chebula supplementation with tacrolimus attenuates the overexpression of pro-inflammatory cartilage cytokines and modulates antioxidant status in adjuvant arthritic rats	*Prerna Kalra*			
PP-154	Attenuation of tacrolimus induced nephrotoxicity by Pomegranate via down regulating pro-inflammatory cytokine and inhibiting apoptosis in Wistar rats	*Surender Singh*			
PP-155	p300 and C/EBPβ-regulated IKKβ/NF-κB activation are involved in thrombin-induced IL-8/CXCL8 expression in human lung epithelial cells	*Bing Chang Chen*			

PP-156	A structure-based interaction analysis of the cytoplasmic regulator of the chemokine receptor FROUNT and an anti-inflammatory compound	*Soichiro Ezaki*			
PP-157	Deletion of the prostaglandin D2 receptor DP1 exacerbates aging-associated and instability-induced osteoarthritis	*Hassan Fahmi*			
PP-158	Critical Role of N-terminal Domain and Middle-Domain on Chaperone Activity and Stability of ClpL	*Bo Gyeong Kim*			
PP-159	Human neutrophils express 15-lipoxygenase-2 and metabolize fatty acids and the endocannabinoid anandamide through this pathway	*Caroline Turcotte*			
PP-160	Curcumin potentiates the anti-inflammatory activity of flavocoxid at a post-transcriptional level in human chondrocytes with an inflammatory phenotype	*Natasha Irrera*			
PP-161	Lipopolysaccharide potentiates platelet responses via toll-like receptor 4-stimulated Akt-Erk-PLA2 signalling	*Maria Elisa Lopes Pires*			
PP-162	Acute increase in O-GlcNAc improves survival in mice with LPS-induced endotoxemia	*Rita C Tostes*			
PP-163	Pharmacokinetic interaction of water extract of Andrographis paniculata and ibuprofen in rabbit	*Jutti Levita*			
PP-164	Targeting of viral interleukin-10 using an antibody fragment specific to damaged arthritic cartilage improves its therapeutic potency	*Louise Mary Topping*			
PP-165	Antibodies to posttranslationally modified insulin in Type 1 Diabetes	*Chiara Vinci*			
PP-166	Synthesis and in vitro evaluation of anti-inflammatory activity of imidazopyridine derivatives	*José Antonio Assumpção*			
PP-167	The novel aldehyde trap, ADX-102, reduces inflammation-mediated lung infiltrate in a mouse model of LPS-induced acute lung injury	*Susan Macdonald*			
PP-168	Comprehensive screening of compounds that modulate human macrophage polarization	*Michael John Parnham*			
PP-169	Identification of Anti-Angiogenic Potential and Cellular Mechanism of Napyradiomycin A1 Isolated from the Marine-Derived Streptomyces sp. YP127	*Kyung Hee Kim*			
PP-170	Wound healing activity of pure natural compounds from Ayurvedic medicinal plants used since the 3rd and 4th Century	*Divya K Shah*			
PP-171	Anti-inflammatory effects of silver nanoparticles stabilized in solution by sodium alginate	*Anatolii Kubyshkin*			
PP-172	PR013 Reduces Symptoms of Allergic Conjunctivitis in the Murine CAC™ Model and Demonstrates Excellent Safety in Repeat-Dose Ocular Tolerability Studies	*Mark Sampson*			
PP-173	An in-vivo approach to testing anti-inflammatory agents based on live imaging of granulocyte-macrophage dynamics and marker assessments in zebrafish	*Martina Fenske*			
PP-174	Pharmacological modulation of circulating cathelicidin-related antimicrobial peptide (CRAMP) in the mouse model of imiquimod-induced psoratic skin	*Frederic Machet*			
PP-176	Dramatic elevation in urinary amino terminal titin fragment excretion quantified by immunoassay in Duchenne muscular dystrophy patients and in dystrophin deficient rodents	*Stephen A. Stimpson*			
PP-177	Interest of dextran sulfate on anti-redness treatment	*Hélène Hernandez Pigeon*			
PP-178	Interest of TRP-regulin®, pongamia oil and hesperidin methyl chalcone on anti-redness treatment	*Hélène Hernandez Pigeon*			
PP-179	Associations between an anti-angiogenic vascular endothelial growth factor-A isoform and acute myocardial infarction in humans	*Laura Piqueras*			
PP-182	Pro-resolving agonists of Fpr2 can restrain microglial activity, controlling inflammation and oxidative stress	*Edward S Wickstead*			
PP-183	Emerging role of GABA in gut inflammation	*Surbhi Aggarwal*			
PP-184	Antileishmanial activity evaluation of gallic acid	*Rym Salah Tazdaït*			
PP-185	Achillea fragrantissima Extract Induced Differentiation and Death of Chronic Myeloid Leukemia K562 Cells	*Nabila Al Jaber*			

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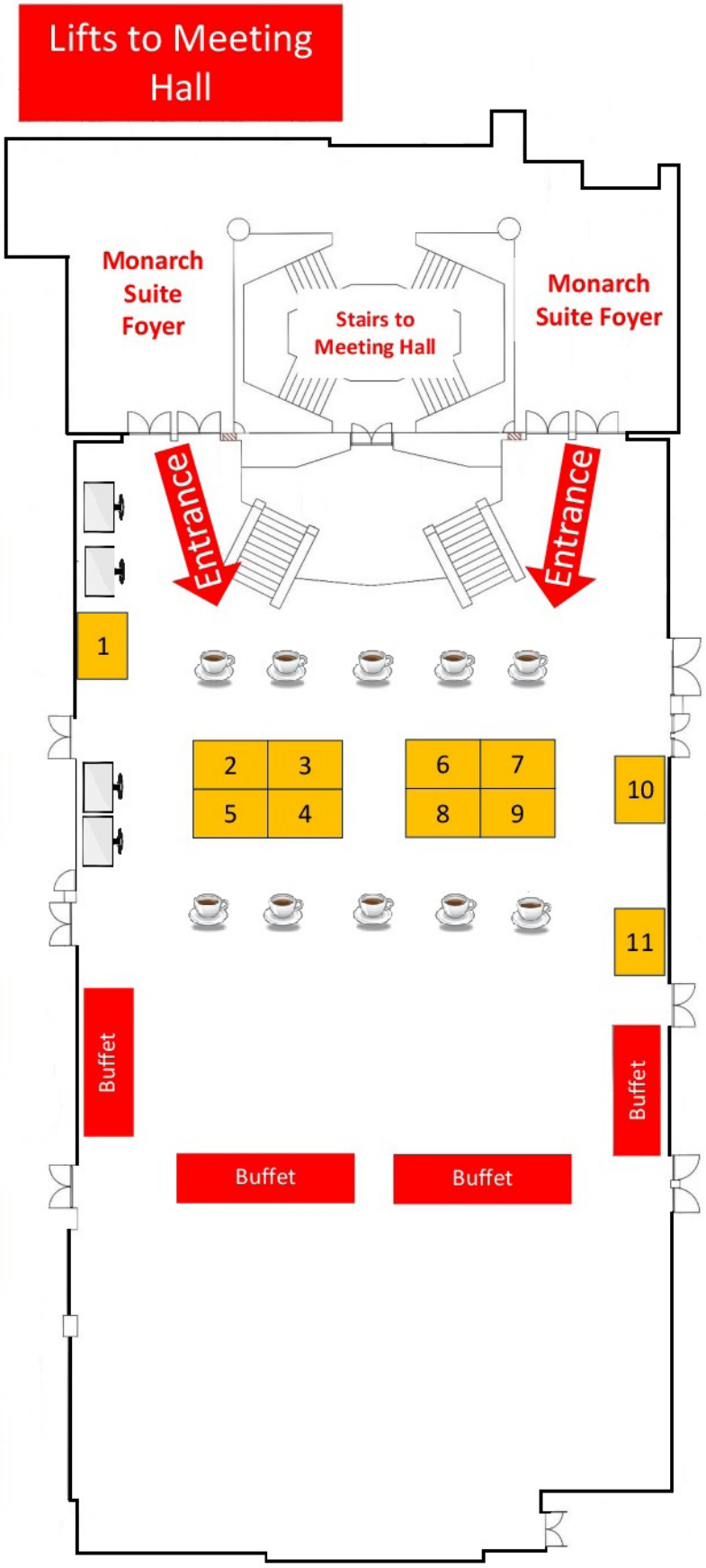


Hilton London Metropole

The Hilton London Metropole is just a two minute walk from both Edgware Road underground stations serving the Bakerloo, Circle, District and Hammersmith & City lines. Discover several attractions only a 10 minute walk away, including Oxford Street shopping, Marble Arch and Hyde Park. Reach both Regent's Park and ZSL London Zoo via a 5-minute underground journey.

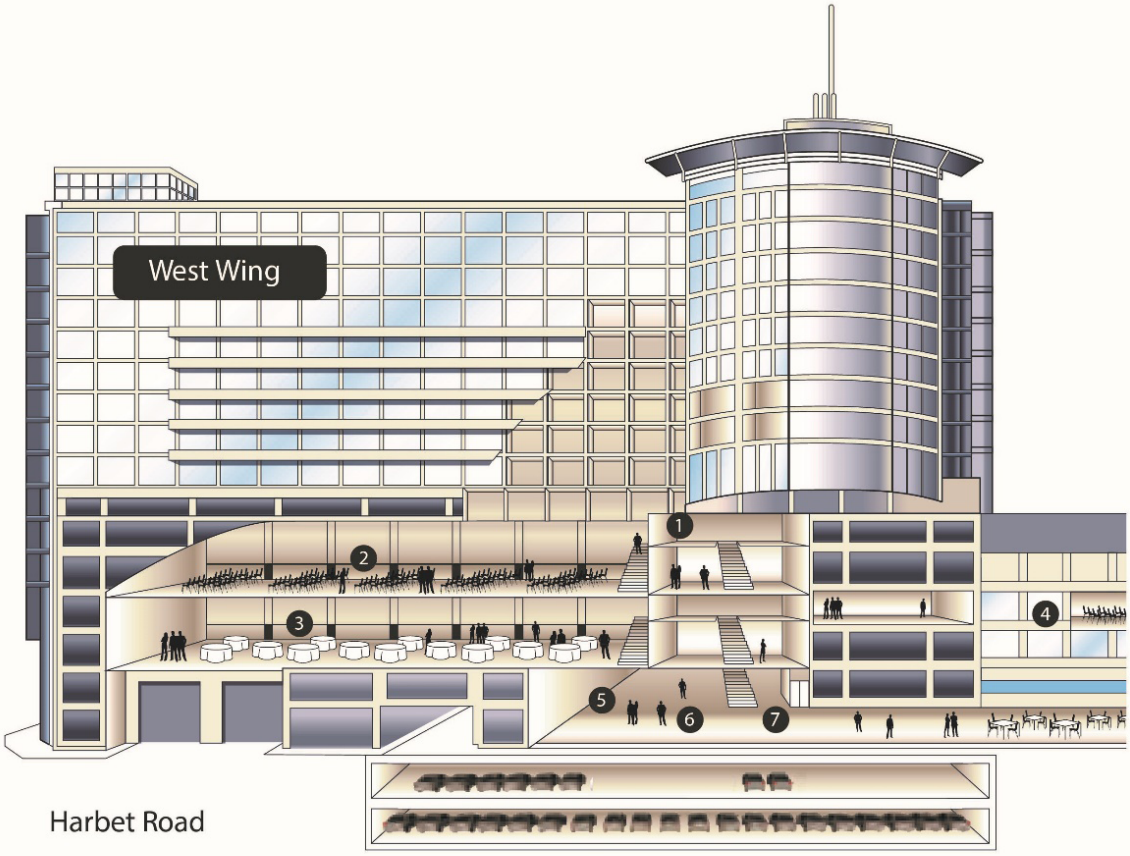
Paddington Station is only a 10 minute walk away and offers easy access to Notting Hill and High Street Kensington.

The Metropole features comfortable, contemporary rooms that will make your stay enjoyable and productive. Guest rooms feature flatscreen TVs with on-demand movies, a comfortable workspace with desk and chair, and mini-bar. The hotel offers numerous options for a bite to eat or a beverage to suit any mood, including the EDG Bar & Lounge, Fiamma Restaurant (with international cuisine), Herb n' Kitchen (for grab and go options), a sports bar, and a whisky lounge in the lobby. Hotel ameneties include a fitness room and pool, laundry/valet service, and concierge and tour desks to make your experience of London as wonderful as possible.

The Hilton London Metropole is easily accessible to Heathrow and Gatwick airports. From Heathrow, the Heathrow Express train line stops at Paddington Station, a 5 minute walk from the hotel. From Gatwick, the Gatwick Express train line stops at Victoria Station, which is 15 minutes from the Metropole by taxi/metro. Delegates arriving by the Eurostar train line will arrive at St. Pancras Station, which is 15 minutes from the hotel by taxi/metro.

West Wing

1. Praed meeting room - Committee Room
2. King's Suite (Sandringham and Balmoral Halls) - Meeting Halls
3. Monarch Suite - Exhibition Area
4. Meeting Rooms 1- 6
5. Congress Registration
6. West Wing Entrance
7. West Wing lifts



Banking
Banking services, ATMs, and currency exchange are widely available around the city and Heathrow Airport, including many international banks such as HSBC and Barclays. Banks are generally open from 09:30 to 14:30 from Monday to Friday and 09:30-12:00 on Saturdays.

Climate & Clothing
The average temperature in London in July is 19 C, with an average high of 23 C and a low of 15 C. There is a 50% chance of rain, with 14 rainy days in the month. Rainfall tends to be light or moderate showers, and cloudy days can also be common.

Currency & Credit Cards
The official currency in England is the Pound Sterling. ATM's are widely available in London, and you should have no problem if you land in the city with nothing but a credit card. VISA and MasterCard credit cards are accepted by almost every merchant, shops, or cafe and restaurant.

Foreign Exchange
ATMs are widely available across the city, making it very straightforward to directly withdraw British Pounds. If you prefer, you may exchange cash at numerous Bureau de Change around the city, including at the airport.

Driving License
You may use your valid driver's license from your country of residence to drive in Great Britain. However, an International Driver's Permit can be useful backup option, and may be required if you are ferrying across the channel or by a rental company if your license is not in English.

Electricity
In the UK, electricity is supplied at 230 Volt / 50Hz AC. Type G outlets are used, with three rectangular prongs.

Emergency Telephone Numbers
Call 112 or 999 for emergency services (police, fire, ambulance).

Insurance
Registration fees do not include insurance of any kind. At the time you register for the Meeting and book your travel, you may wish to take an insurance policy of your choice. This insurance is to be purchased in your country of origin.

Internet Access
There is wireless internet access available at the official hotel.

Smoking
Smoking is not permitted inside of public enclosed or substantially enclosed spaces, including restaurants, public transport vehicles, taxis, and hotels (except within designated smoking roooms.) The fine is £50. You may ask any business or public employee for help locating designated smoking areas.

Tax-free shopping
Under the Retail Export Scheme (also known as the Tax Free Shopping Scheme), you might be eligible for a VAT refund on some goods purchased during your visit to the UK. Ask your retailer about eligibility. You must collect a VAT 407 form from your retailer, and present this form along with your goods at customs for approval when you leave the country. (If these items will be in your checked bag, you must do this before checking the bag.) You may then collect a refund from a refund booth or by mailing the form back to the retailer. For more information, visit: https://www.gov.uk/tax-on-shopping/taxfree-shopping

HALF-DAY LONDON TOUR

£65 pp (10 ppl)

- Panaromic tour of London (Westminster Abbey, Big Ben, Trafalgar Square, Downing Street, London Eye, St.Paul's Cathedral)

LONDON EYE & GREENWICH VILLAGE

£70 (minimum 7 pax)
£90 (minimum 4 pax)
£100 (minimum 1 pax)

- Head off from the port to Greenwich with the boat where the prime meridian is based.
- Lunch break (not included)
- A walk through the world famous Greenwich park and visit the Royal Observatory.

WINDSOR CASTLE&OXFORD&STONEHENGE TOUR

£130.00 pp (minimum 7 pax)
£145.00 pp (minimum 4 pax)
£160.00 pp (minimum 1 pax)

- Starting from hotel, visiting the Windsor Castle, beautiful home of the Queen's favourite weekend residence. Exploring features such as the State Apartments and St George's Chapel
- Lunch break (included)
- After Windsor, visiting the pretty university city of Oxford. Home to England's oldest university and packed full of cloistered buildings and ornate colleges. (the famous Christ Church where the movie Harry Potter has been shot, Bodleian library, Science Museum)

STONEHENGE&BATH TOUR

£125.00 pp (minimum 7 pax)
£140.00 pp (minimum 4 pax)
£160.00 pp (minimum 1 pax)

- Starting from hotel, visiting Stonehenge, where you can visit the awesome 5,000 year old mysterious stone structure that still has the capacity to baffle experts and inspire endless wonder.
- Lunch Break (included)
- After the lunch break, visiting the Georgian city of Bath, also a World Heritage Site. You will have the opportunity the visit the Roman bath.

OXFORD&CAMBRIDGE TOUR

£130.00 pp (minimum 7 pax)
£140.00 pp (minimum 4 pax)
£160.00 pp (minimum 1 pax)

- Starting from the hotel, visiting the pretty university city of Oxford. Home to England's oldest university and packed full of cloistered buildings and ornate colleges. (the famous Christ Church where the movie Harry Potter has been shot, Bodleian library, Science Museum)
- Lunch Break (included)
- After leaving Oxford, head to the equally stunning city of Cambridge. Visiting the location of the University of Cambridge and the famous Senate House once used for gatherings of the Council of the Senate and now a traditional venue for degree ceremonies.

COTSWOLDS-STRATFORD UPON AVON TOUR

£100.00 pp (minimum 7 pax)
£120.00 pp (minimum 4 pax)
£140.00 pp (minimum 1 pax)

- Starting from the hotel, visiting one of the most beautiful places in England-Cotswolds
- Lunch Break (not included)
- After lunch break, visiting the hometown of Britain's most celebrated writer William Shakespeare. There are plenty of houses associated with the writer around the town.

PLENARY & SYMPOSIA ABSTRACTS

Sunday, July 9th

09:30-10:30

AGEING AND INFLAMMATION

Janet M Lord

FMedSci, Institute of Inflammation and Ageing, University of Birmingham, Birmingham B15 2TT, UK.

We are an ageing society, with falling birth rates and increasing life expectancy. However healthy life expectancy is not keeping pace with life expectancy and now on average adults are unwell for the last 2 decades if life. There is thus an urgent need to understand the drivers of ill health in old age. An increase in systemic inflammation, termed Inflammageing, has been shown to be associated with increased risk of a range of age-related diseases including cardiovascular disease, sarcopaenia and dementia. The causes of this inflammation are varied and include increased sedentary behaviour and associated adiposity, a build up of senescent cells (which are pro-inflammatory) as well as age-related changes to the immune system. Our work has shown that adults who maintain a high level of physical activity show no signs of inflammageing and also display few signs of the ageing phenotype (1). We have also reported that age-related changes to the immune system, including reduced neutrophil migrational accuracy (2) and basal activation of monocytes (3) contribute to inflammageing. Importantly we have revealed that the dysregulated migrational behaviour, caused by constitutive activation of PI3kinase delta, can be corrected to improve immunity in older adults (2).

References

1. Pollock RD, Carter S, Velloso CP, Duggal NA, Lord JM, Lazarus NR, Harridge SR (2015) An investigation into the relationship between age and physiological function in highly active older adults. *J Physiol* 593:657-680.
2. Sapey E, Greenwood H, Walton G, Mann E, Love A, Aaronson N, Insall RH, Stockley RA, Lord JM (2014) Phosphoinositide 3 kinase inhibition restores neutrophil accuracy in the elderly: towards targeted treatments for immunesenescence. *Blood* 123:239-248.
3. Arranz L, Lord JM, De la Fuente M (2010). Preserved ex vivo inflammatory status and cytokine responses in naturally long-lived mice. *AGE.* 32:451-466.

11:00-12:00

TARGETING THE INNATE AND ADAPTIVE IMMUNITY IN COMBATING NEURODEGENERATIVE DISEASES

Michal Schwartz

The Weizmann Institute of Science, Rehovot, Israel, 76100; Michal.schwartz@weizmann.ac.il

Immunosureveilence is needed for supporting brain repair and functional plasticity. It involves the microglia, the brain resident immune myeloid cells, circulating and monocytes and CD4+ T cells. The blood-cerebrospinal fluid-barrier (BCSFB), namely the choroid plexus epithelium (CP) was identified as an immune-brain interface, and as a gate that "ticketing" the leukocytes to allow their entry to the CNS. In analyzing how the activity of this interface determines the fate of the brain, we discovered by immunogenomic and by immunohistochemistry that in aging and in Alzheimer's disease (AD) mouse this interface is suppressed with respect to its ability to allow communication between the brain and the circulating leukocytes. We further found that by transiently reducing systemic immune suppression, increased IFN-γ availability at the CP, thereby activated the CP to express trafficking molecules, and in turn led to recruitment of immune regulatory cells to sites of brain pathology. Immune activation could be achieved by blocking inhibitory immune checkpoints, regulatory pathways which maintain systemic immune homeostasis and tolerance. Among such inhibitory checkpoints, PD-1/PD-L1 pathway has been tested. Antibodies directed to this pathway, in several mouse models of AD, was found to be effective in reversing cognitive loss, restoring immunological brain homeostasis as determined by the inflammatory molecular profile, and reduced disease pathology. Our emerging new understanding on the fate of microglia in AD, suggest that microglial are also kept under tight regulation by brain checkpoint mechanisms, manipulation of which could be an additional target in understanding and developing immunotherapy in AD.

13:00-14:30

SYMPOSIA SESSION – BIRAS
INFLAMMAGEING

An increased lifespan brings with it modifications to the immune system that result in a reduced ability to fight and conquer infection. The ensuing chronic inflammatory state has been termed Inflammageing, and represents a significant risk factor for increased mortality/morbidity in the elderly. In this session cellular responses to aging will be disseminated at the level of gene splicing and mitochondrial function as well as the altered functionality of both the adaptive and the innate immune systems during ageing. All of these strategies are uncovering potential new therapeutics aimed at targeting the process of Inflammageing.

Gene regulation is critical for maintaining cellular function as we age. Gene splicing is a key component in this regulation with malfunctions in the proteins that regulate splicing associated with age-related diseases such as Alzheimer's disease. Prof Lorna Harries and her group have shown that the expression of splicing factor transcripts is altered with aging and senescence and may be further modulated by the pro-inflammatory state associated with the ageing phenotype.

Cellular senescence is a key factor in age-related tissue degeneration. Mitochondria play an essential role in energy generation, cell signalling and differentitation in eukaryotic cells. Dr Joao Passos and his team have also uncovered a critical role for mitochondria in cellular senescence with elimination of mitochondria resulting in a reduction in cellular senescence and the associated pro-inflammatory phenotype.

Prof Arne Akbar will focus on the adaptive immune system and how its functions decline as we age, which can result in the reactivation of latent viruses, such as varicella zoster. Using a human model, Prof Akbar has uncovered deficits in T cell migration, dendritic cell maturation as well as alterations in key pro-inflammatory signalling cascades that together result in decreased antigen-specific immunity in the elderly.

Neutrophil function also alters as we age, with impairment of some responses (migration) and enhancement of others (degranulation). This impairs our ability to fight infection as we age and also increases the risk of collateral damage of healthy tissue. Dr

Elizabeth Sapey's team has investigated neutrophil function in young and old adults suffering from both chronic conditions such as COPD as well as infections such as pneumonia. Importantly, they are uncovering therapeutics interventions that act to restore neutrophil function.

SASP, SPLICING REGULATION AND RESCUE OF SENESCENCE IN PRIMARY HUMAN CELLS

Eva Latorre[1], Vishal Birar[2], Richard GA Faragher[2], Elizabeth Ostler[2] and Lorna W. Harries[1]

[1]RNA-mediated disease mechanisms group, University of Exeter Medical School, UK
[2]School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, UK, BN2 4GJ

Human ageing is characterised by chronic inflammation (inflammaging) and also by progressive changes in the expression of proteins regulating alternative splicing. Correct regulation of splicing processes is critical for cellular plasticity and adaptability. Alternative splicing occurs for the vast majority of genes in the human genome and allows the production of alternatively-expressed transcripts in a co-ordinated and regulated fashion. We have recently determined that transcripts involved in these processes are amongst the most deregulated mRNAs during human aging in population studies, and also in cells that have undergone replicative senescence in vitro. These changes are also associated with age- or senescence-related changes to the precise composition of different messenger RNA isoforms produced from our genes. In vitro work indicates that the expression of splicing regulator proteins is responsive to the cytokines IL1β, TNFα and IFNγ, indicating that inflammaging processes may contribute to this phenomenon. Furthermore, treatment of senescent primary human fibroblasts with small molecules that moderate the SASP, or manipulation of cellular signalling pathways that induce the SASP is capable of restoring splicing factor expression and rescuing multiple features of cellular senescence. Our data indicate that inflammaging processes may contribute to cellular senescence and ageing phenotypes in man through moderation of alternative splicing processes.

INHIBITING INFLAMMATION TO ENHANCE HUMAN IMMUNITY IN VIVO

Prof Arne Akbar
University College London

Age related immune decline leads to re-activation of latent varicella zoster virus (VZV) resulting in herpes zoster (HZ) or shingles. We investigated the decreased immunity during ageing using cutaneous VZV antigen challenge in humans as an experimental model. Decreased skin responses during ageing were associated with reduced T cell infiltration and dendritic cell maturation. The transcriptional profiling of skin from the challenge site indicated that while the signalling pathways that were activated were similar, they were attenuated globally during ageing, especially at later time points, suggesting a defect in immune amplification. This decreased gene activation was associated with significantly greater proportions of $FoxP3^+$ regulatory T cells and higher PD-1 inhibitory receptor expression on T cells in the skin. Paradoxically the injection of saline into the skin of old subjects induced elevated p38 MAP kinase related pro-inflammatory cytokine gene expression including *type 1 IFN, TNF-α, IL6 and IL12* by monocyte/macrophages. Furthermore, the reduced response to VZV challenge was highly correlated with individual inflammatory mediators or a combined index of inflammatory gene expression. To test the hypothesis that increased propensity to inflammation attenuates antigen-specific cutaneous immunity we treated old subjects orally with a small molecule p38 MAPkinase inhibitor (Losmapimod), that inhibits pro-inflammatory cytokine secretion *in vivo*, before cutaneous VZV antigen challenge. The short-term inhibition of p38 MAPkinase significantly enhanced the response to VZV antigen challenge in older subjects. Therefore anti-inflammatory intervention may counteract decreased antigen-specific immunity that may be utilized to promote the ability to combat cutaneous malignancy and infections during ageing.

NEUTROPHIL DYSFUNCTION WITH AGEING, INFLAMMATION AND INFECTION

Dr Elizabeth Sapey
Reader in Respiratory Medicine and Consultant in Respiratory Research
University of Birmingham

Our population is ageing and chronic non-communicable diseases associated with ageing, such as chronic obstructive pulmonary disease (COPD), are the leading cause of mortality worldwide and are associated with huge economic costs. Pneumonia is the leading infectious cause of death in developed countries and deaths are highest in the elderly with mortality rates not improved over the last decade.

Neutrophils are key effector cells during bacterial infections, with evidence of sub-optimal immune responses in the elderly. *In vitro,* age is associated with reduced neutrophil migratory accuracy, phagocytosis and NETosis, although degranulation appears increased. Poor accuracy, reduced bactericidal function and enhanced degranulation may delay pathogen clearance but increase by-stander tissue damage, and may contribute to the pathogenesis of chronic inflammatory diseases.

With the continued emergence of antibiotic resistance, there is great interest in harnessing immune responses to improve outcomes during severe infections but prevent immune cell associated tissue damage.

We studied neutrophil functions in health, in chronic lung diseases including COPD, during simple lower respiratory tract infections, pneumonia and pneumonia associated sepsis in young and old adults and describe deteriorating neutrophil targeting in the elderly, resulting in prolonged immunoparesis. Neutrophil functions can be restored using therapeutic interventions during health, chronic disease and mild to moderate infection, but not during more severe sepsis episodes. Targeted mechanisms are cell based, including surface adhesion molecule expression, PI3K signalling and small GTPase signalling, suggesting immunomodulation may be an effective option in infection and inflammation.

TARGETING INFLAMMATION TO COMBAT CELLULAR AGEING: MITOCHONDRIA AS POSSIBLE INTRACELLULAR ALLIES?

João F. Passos
Newcastle University
Newcastle University Institute for Ageing and Institute for Cell and Molecular Biosciences
Newcastle upon Tyne

Cellular senescence, the irreversible loss of proliferating capacity of somatic cells, is an important tumour suppressor mechanism but also a driver of age-related diseases. These somehow contradictory functions are dependent on the development of the so-called senescent phenotype, which involves over-production of pro-inflammatory and pro-oxidant signals.

The exact mechanisms underlying the induction of the senescent phenotype remains incompletely understood. Our lab has shown a critical role for mitochondria in cell senescence. In multiple models of cellular senescence, we found that elimination of mitochondria reduced a spectrum of senescence effectors and phenotypes, including secretion of pro-inflammatory and pro-oxidant signals. Furthermore, absence of mitochondria also reduced senescence-associated beta-galactosidase (SA β-Gal) activity, expression of the cyclin-dependent kinase inhibitors p21 and p16, while preserving growth arrest. Mitochondria-depleted senescent cells exhibited enhanced glycolytic activity and increased ATP production. Mechanistically, we show that mTORC1 integrates signals from the DNA damage response towards PGC-1β-dependent mitochondrial biogenesis, playing a causal role in the development of senescence. Finally, we demonstrate that inhibition of mitochondrial content in vivo, by either rapamycin treatment or PGC-1β deletion, prevents the age-dependent increase in senescence in mouse tissues and reduces its pro-inflammatory phenotype. Our results suggest mitochondria as a candidate target for interventions to reduce the deleterious impact of senescent cells in ageing tissues.

SYMPOSIA SESSION – GREMI
CELL DEATH & INFLAMMATION

This session is devoted to exploring "**Cell death and inflammation**" and aims to spotlight a few selected issues that might provide future opportunities to modulate inflammation.

It is now well established that there are different forms of cell death that can affect the outcome of the inflammatory and the immune process. Apoptosis, the best-described form of programmed cell death mediated by the caspases is characterized by typical biocemical and morphological features. Neutrophils are key anti-infectious actors in host defense but are also inflammatory cells able to mediate tissue damage. Their apoptosis and elimination must be tightly regulated to avoid uncontrolled inflammation. The recent advances in our understanding of the mechanisms governing neutrophil apoptotic pathways will be reviewed and its therapeutic manipulation by roscovitine to resolve inflammation will be presented. For instance, the generation of "eat-me signals" that stimulate apoptotic cell phagocytosis by macrophages or surrounding cells is a key mechanism to avoid autoimmunity. Because the cytoplasmic contents of apoptotic cells are not spilled into the extracellular medium, apoptosis does not trigger an inflammatory response and, hence, is considered immunologically silent. Two example showing that subversion of these mechanisms could lead to inflammation and autoimmunity will be given. The first example will emphasized the importance of the C1q complement component in lupus. The second exemple will show that proteinase 3, the autoantigen in vasculitis associated with ANCA (anti-neutrophil cytoplasmic antibody) expressed at the membrane of apoptotic cells could act trigger macrophage activation and promote autoimmunity. Finally, the therapeutic potential of the microenvironnement of apoptotic cells to dampen inflammation will be illustrated in different pathophysiological situations.

This restricted selection of topics dealing with cell apoptosis will highlights the tight connections between immune cell activation and cell death. This overview also exposes the wide ranges of ways in which inflammation could be controlled as a innovative therapeutic approach.

ROSCOVITINE AS A NEW ANTI-INFLAMMATORY THERAPY PROMOTING NEUTROPHIL APOPTOSIS

Adriano Rossi
MRC Centre for Inflammation Research, Queen's Medical Research Institute, The University of Edinburgh, Scotland, UK.

(R)-Roscovitine (Seliciclib, CYC202, 2-(R)-(1-Ethyl-2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine) is one of the best characterized pharmacological inhibitors of cyclin-dependent kinases (CDKs). It is undergoing phase II clinical trials as a drug candidate for several diseases where the immune response plays a key role (e.g., cancer, Cushing's disease and rheumatoid arthritis) and has the potential to be tested in other diseases including cystic fibrosis, idiopathic pulmonary fibrosis and acute lung injury (Meijer et al., J Innate Immunity, 2016;8(4):330-49). Our laboratory first reported that roscovitine, has powerful pro-apoptotic effects on human neutrophils (Rossi et al., Nat Med, 2006;12(9):1056-64); a finding that has subsequently been confirmed and investigated by several other groups. Apoptosis, or programmed cell death, is a physiological form of 'silent' cell death that is important for normal homeostasis that 'marks' the cell for rapid clearance by phagocytic cells such as macrophages; a process often termed efferocytosis. We hypothesised that pharmacological induction of neutrophil apoptosis during established neutrophilic inflammation would enhance inflammation resolution. We tested this hypothesis in several mouse models and showed that roscovitine markedly enhances resolution of established neutrophil-dependent inflammation in carrageenan-elicited acute pleurisy, bleomycin-induced lung injury, and passively-induced arthritis. We subsequently demonstrated *in vivo* that caspase inhibitor drugs prevent R-roscovitine-enhanced resolution of inflammation. Together, these findings indicate that this CDK inhibitor augments caspase-dependent neutrophil apoptosis to enhance the resolution of established neutrophilic inflammation. Subsequent experiments using roscovitine and other CDK inhibitor drugs have shown that CDK9 is the likely important target responsible for the observed effects. Thus, the phosphorylation of RNA polymerase II by CDK9 is inhibited by R-roscovitine and that specific effects on neutrophil transcriptional capacity are responsible for the induced neutrophil apoptosis. Evidence suggest that inhibition of CDK9 prevents the synthesis of the anti-apoptotic protein Mcl-1 (critical for neutrophil survival) and potentially other key proteins (Leitch et al., Cell Death Differ. 2012;19(12):1950-61). It has been demonstrated that

roscovitine has powerful pro-resolution and anti-inflammatory effects in other lung models of inflammation and in experimental pneumococcal meningitis (see Leitch et al., Br J Pharmacol, 2009;158(4):1004-16). Roscovitine and similar compounds have, perhaps rather surprising, several anti-inflammatory effects that make them interesting candidate drugs for the treatment of inflammatory disease.

C1Q, CELL DEATH AND INFLAMMATION

Marina Botto
Imperial College London, UK

In humans, homozygous deficiency of the first component of the complement system, C1q, is the most powerful susceptibility genetic factor for the development of systemic lupus erythematosus (SLE). The vast majority of patients with C1q deficiency develop a syndrome closely related to SLE. The disease is typically of early onset and is often very severe. Although the phenotype of disease varies between patients the fact that C1q deficiency is sufficient to cause SLE in almost all humans identifies a pivotal role for this molecule. The challenge is to identify the relevant physiological activity that can explain this strong association.

One of the hypotheses to explain the heightened susceptibility to the development of SLE in the absence of C1q invokes an important role for complement in the physiological waste-disposal mechanisms of dying cells and immune complexes. In the lecture we will review the *in vitro* and *in vivo* evidence for a role of C1q in the clearance of dying cells. However, impaired clearance of such cells is, on its own, insufficient to produce autoimmunity. The data available from knockout mice emphasize that susceptibility to an autoimmune disease might depend on many factors in addition to the defective removal of dying cells.

In summary, it is clear that the traditional view of the role of complement in SLE needs revision. Complement activation in SLE has been viewed as a major cause of tissue injury. Instead, evidence is emerging that complement may play a protective role rather than an exclusively pro-inflammatory role in tissue injury. There is much work still to be done in order to fully understand the immunobiology of complement in relation to SLE.

PROTEINASE 3 ON APOPTOTIC CELLS: A NEW AUTOINFLAMMATORY TRIGGER IN AUTOIMMUNE VASCULITIS

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Neutrophils have a major role in the pathophysiology of vasculitis associated with autoantibodies against neutrophils called ANCA (antineutrophil cytoplasmic antibodies). Indeed, they are involved both in the mechanisms of endothelial injury and in the immune deregulation associated with these diseases. The neutrophil granule proteins proteinase 3 (PR3) and myeloperoxidase (MPO) are the two main target antigens for ANCA. This selectivity is surprising regarding the diversity of the proteins stored within cytosolic granules. In the group of autoimmune vasculitides associated with ANCA, granulomatosis with polyangiitis (GPA) illustrates the concept that autoimmunity can develop against one specific neutrophil protein, namely proteinase 3, one of the four serine protease homologs contained in azurophilic granules, which can by itself, instigates an immune dysregulation.

Inflammation in GPA is sustained and fails to resolve, leading to vessel necrosis, granuloma formation and ultimately promotion of auto-immunity. Indeed, a delay in the phagocytosis of apoptotic cells may favour auto-immunity. Interestingly, PR3 expressed on the membrane of apoptotic cells triggers a pro-inflammatory response in macrophages via their secretion of inflammatory cytokines, (including interleukin-1β), chemokines and the expression of nitric oxide synthase 2. This PR3 effect is dependent on its membrane anchorage and its enzymatic activity, and strongly suggests another novel "auto-inflammatory" component in this disease. Notably, the microenvironment produced by the macrophages after the phagocytosis of apoptotic cells expressing PR3 could regulate pDCs to polarize T Helper lymphocytes toward the Th9/Th2 phenotype. This function can completely abrogate the generation of regulatory T cells, thus favouring auto-immunity. Most importantly, a similar T cell polarization was found in patients with GPA. Finally, macrophages, pDCs and T cells are all found in close proximity in the granulomatous lesions in lungs from these patients. In GPA, the auto-antigen therefore appears to play a double role, acting as an auto-antigen and a danger signal disturbing the resolution of inflammation and promoting auto-immunity.

APOPTOTIC CELL-BASED THERAPIES FOR TRANSPLANTATION AND INFLAMMATORY DISEASES

Sylvain Perruche
Inserm – University of Besançon, France

Immune-mediated inflammatory diseases have in common a dysregulation of the immune response. Resolution of inflammation is a powerful process involving a numerous of factors including specialized pro-resolutive lipid mediators and proteins such as TGF-β which act all together on the inflammatory pathways, resulting in inflammation termination and the initiation of tissue healing. A critical step of inflammation resolution is efferocytosis of apoptotic effector cells by phagocytes which produce factors allowing the termination of inflammation and initiating tissue healing. TGF-β has been shown has mandatory in such process since it is produced by apoptotic cell and phagocytes clearing them. We hypothesize whether the reintroduction of pro-resolutive factors issued from apoptotic cell efferocytosis would allow a termination of ongoing inflammation. Injection of such resolutome in ongoing collagen-induced experimental arthritis allowed the long term reduction of clinical symptoms and was accompanied by the emergence of highly collagen-specific Tregs and antigen presenting cells (APC) reprograming through a tolerogenic state. When injected in ongoing T cell-transfer-induced Inflammatory Bowel Disease experimental model, again, clinical symptoms and colon mucosa lesions were severely reduced and this was accompanied by a higher proliferation, migration and wound healing capacities of both epithelial cells and fibroblasts. TGF-β has been evaluated in such settings and demonstrated a prominent role in the resolution of the adaptive immune response, notably in the reprogramming of APC and regulatory T cell emergence. So far the pro-resolutive factors issued from apoptotic cell efferocytosis demonstrated the potential to terminate ongoing inflammation. This opens clinical drug development opportunities in the treatment of refractory chronic inflammatory diseases.

SYMPOSIA SESSION – AUSTRALIA
NOVEL INFLAMMATORY PATHWAYS AND TARGETS

NEW MECHANISMS OF INFLAMMASOME ACTIVATION AND AUTOINFLAMMATION.

Seth L. Masters
Inflammation Division, The Walter and Eliza Hall Institute, Australia

The innate immune system is the first line of defence against infection. Our genome encodes cell surface innate immune sensors that directly detect pathogens, but also a series of cytoplasmic sensors that can form inflammasome complexes. As we recently discussed in *Nature Reviews Immunology*, most inflammasome sensors do not directly detect pathogens, but instead monitor homeostasis-associated molecular patterns in the cell, and alert the host to danger indirectly (1). This enables a single sensor to detect a wide variety of invading pathogens, however leaves the host open to inadvertent activation of the pathway causing sterile autoinflammatory disease.

We have now identified actin polymerisation as a key homeostatic process monitored by the inflammasome sensor Pyrin (2). Specifically, mutations in the actin-depolymerizing cofactor Wdr1, lead to increased actin polymerisation, and trigger Pyrin in mice (2) and humans (3). We have also identified mutations in Pyrin itself which determine how this inflammasome is activated mechanistically, and results in a novel autoinflammatory skin disease (4). We show that these patients can be treated by targeting the downstream cytokine produced by the inflammasome, IL-1b.

References

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DISORDERED HAEMATOPOIESIS AND CARDIOVASCULAR DISEASE

Andrew Murphy
Baker Institute

Atherosclerosis, the major underlying cause of CVD, is characterized by a lipid-driven infiltration of inflammatory cells in large and medium arteries. Many cardiovascular risk-factors are linked with increased levels of circulating monocytes and neutrophils, which impact atherosclerotic lesion severity. In particular, changes in plasma lipids and metabolic abnormalities associated with diabetes, alter haematopoiesis and promote atherogenesis. Dietary factors are also closely linked with the incidence of CVD, particularly diets rich in salt. A high-salt diet (HSD) has recently been shown to alter the immune response in both mice and humans by promoting inflammatory and suppressing anti-inflammatory immune cells. We hypothesized that a HSD would drive Th17 cells to promote haematopoietic stem progenitor cells (HSPCs) mobilisation, monocytosis and increased atherosclerosis. A HSD significantly promoted rupture-prone atherosclerotic lesion formation with increased macrophage and lipid content in the sinus. These changes were associated with monocytosis and neutrophilia, but not plasma cholesterol or hypertension. We found HSPC mobilization from the bone marrow (BM) to the spleen where more monocytes were produced. HSPC mobilisation appeared to be caused by signals emanating from the splenic macrophages and dendritic cells, which promote a breakdown of the BM osteoblast HSPC niche. This haematopoietic phenotype and accelerated atherogenesis could be inhibited by administration of IL-17 neutralizing antibodies. To date our findings reveal a novel pathway initiated by a high salt diet that act via the haematopoietic system to induce atherosclerosis, largely independent of hypertension.

A NEW GM-CSF-DEPENDENT PATHWAY IN INFLAMMATION

Adrian Achuthan, Ming-Chin Lee, Reem Saleh, Andrew Fleetwood, Andrew Cook and John Hamilton
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GM-CSF plays a key role in rheumatoid arthritis, as evidenced by promising recent clinical trial data targetting GM-CSF or its receptor. However, blockade of its function could lead to undesirable side-effects creating a need to delineate downstream pathways and mediators. We report here that GM-CSF drives CCL17 production via a new interferon regulatory factor 4 (IRF4)-dependent pathway in human monocytes and murine macrophages, as well as in vivo. Moreover, we provide evidence for the first time that GM-CSF controls IRF4 expression via regulating the expression and activity of JMJD3, which demethylases trimethylated-H3K27. Importantly, in arthritis and pain models IRF4-regulated CCL17 formation can mediate the proinflammatory and algesic actions of GM-CSF. Evidence will also presented for new CCL17 functions in inflammatory arthritis and pain. The delineated pathway potentially provides new therapeutic options for the treatment of inflammatory diseases and their associated pain.

NEURAL REGULATION OF IMMUNITY AFTER STROKE

Connie H. Y. Wong
Centre for Inflammatory Diseases, Department of Medicine, School of Clinical Sciences, Monash University, Clayton, Victoria 3168, Australia.

Stroke is highly prevalent and is one of the leading contributors to morbidity and mortality worldwide. Despite the debilitating neurological deficits, the major cause of death after stroke is bacterial infection. Post-stroke infection has traditionally been associated with precipitants such as aspiration, indwelling catheters, immobility related to severe strokes or other health co-morbidities. Although such factors may play a role, we have demonstrated that stroke impairs immune cell function critical in the regulation of antibacterial defence in a sympathetic nervous

system (SNS)-dependent manner. In addition, our latest findings revealed that neural control of the intestinal tissue contribute significantly to bacterial translocation and dissemination from the gut to peripheral organs to result in fatal infections. Furthermore, the microbial communities within the mucosa of gastrointestinal tract were significantly different between sham-operated and post-stroke mice at 24 h following surgery. The differences in microbiota composition were substantial in all sections of the gastrointestinal tract and were significant even at the phylum level. Analysis of the predicted functional potential of the altered mucosal microbiota after stroke revealed significant increases in pathways associated with infectious diseases, membrane transport, xenobiotic degradation, lipid metabolism and signalling related KEGG pathways. Taken together, our studies uncovered stroke induces far-reaching and robust changes to the peripheral immunity and intestinal mucosal microbiota, and a better understanding of the precise molecular events leading up to stroke-induced immune and microbiota changes may represent novel therapy targets to improve patient outcomes.

16:00-17:30

SYMPOSIA SESSION – BRAZIL
NEUROINFLAMMATION

NEURO-IMMUNE-GLIAL INTERACTIONS MEDIATE HERPETIC NEURALGIA

Thiago Cunha
Ribeirao Preto Medical School, University of Sao Paulo Brazil

Herpetic neuralgia is the most important symptom of herpes zoster disease, which is caused by Varicella zoster. Nevertheless, the pathophysiological mechanisms involved in herpetic neuralgia are not totally elucidated. Here, we examined the neuro-immune interactions at the sensory ganglia that account for the genesis of herpetic neuralgia by using a murine model of Herpes simplex virus type-1 (HSV-1) infection. The cutaneous HSV-1 infection of mice results in the development of a zosteriform-like skin lesion followed by a time-dependent increase in pain-like responses (mechanical allodynia). Leukocytes, composed mainly of macrophages and neutrophils, infiltrate infected DRGs and account for the development of herpetic neuralgia. Infiltrating leukocytes are responsible for driving the production of TNF, which in turn mediates development of herpetic neuralgia through down-regulation of the inwardly rectifying K+ channel, Kir4.1, in satellite glial cells. These results revealed that neuro-immune interactions at the sensory ganglia play a critical role in the genesis of herpetic neuralgia. In conclusion, the present study elucidates novel mechanisms involved in the genesis of herpetic pain and open new avenues in its control.

NEUROPATHIC PAIN: ROLE OF SPINAL CORD OLIGODENDROCYTE-DERIVED IL-33

Waldiceu A. Verri Jr, PhD
Associate Professor at the State University of Londrina (UEL), Brazil

Interleukin-33 (IL-33) and its receptor ST2 belong to the IL-1 family of cytokines. Initial studies found the prominent role of IL-33 in Th2 immune responses. However, over the years, it has become clear that it is rather a pleiotropic cytokine regulating the function of varied cell types in most immune responses. Herein, we will focus in the role of IL-33/ST2 in the regulation of pain. IL-33/ST2 signalling mediates pain in inflammation, cancer and neuropathy. In special, we will discuss the identification of a new function of spinal cord oligodendrocytes. In the chronic constriction injury-induced neuropathic pain model, spinal cord oligodendrocytes release IL-33. In turn, IL-33 orchestrates spinal cord neuroinflammatory response to induce hyperalgesia. Therefore, IL-33/ST2 signalling is a conceivable target to reduce pain as well as revealed a previous unrecognized role of spinal cord oligodendrocytes in neuropathic pain.

Funding: MCTI/ SETI/ Fundação Araucaria/ Parana State Government, FAPESP, CNPq, CAPES, FINEP (Brazil), 2010 International Association for the Study of Pain (IASP) Early Career Grant funded by the Scan Design Foundation by INGER & JENS BRUUN, European Union Seventh Framework Programme (TIMER), and The Centre National de la Recherche Scientifique International.

CONTRIBUTIONS OF PERIPHERAL AND SPINAL INFLAMMATORY SIGNALLING TO CHRONIC PAIN STATES

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Chronic pain impacts upon the lives of around 20% of the adult population in developed countries. Lack of progress in the development of new effective analgesics which reverse established chronic pain means that chronic pain is a major burden on the individual, families and society. Unlike acute pain, which is protective and essential for survival, chronic pain arises due to pathophysiological changes in the central nervous system and the resultant pain is not beneficial or protective. Decades of research have shown that the mechanisms of chronic pain often transcend specific disease conditions, and involve plasticity in the peripheral and central signaling of nociceptive messages and a complex interplay between neurons and immune cells. A common feature of many chronic pain states is sustained localised inflammatory signalling, which influences the properties of ion channels and receptors expressed by sensory nerves and neurones. This presentation will discuss the potential roles of the specialised pro-resolvin molecules, which as to curtail inflammation, and consider whether deficits in this endogenous response contribute to chronic pain responses. Our recent findings that exogenous systemic administration of a precursor for the D-series resolvin 17-hydroxy docosahexaenoic acid (17-HDHA) has robust inhibitory effects on established pain responses in two clinically relevant models of OA pain, with no effect on the extent of joint pathology will be discussed. The clinical relevance of this work will be highlighted by our recent finding that higher blood levels of a resolvin precursor were significantly associated with the intensity of chronic pain in knee OA patients.

SYMPOSIA SESSION – CANADA
THE MICROBIOME AS A DRUG TARGET

Increasingly the microbiome, and particularly that resident within the gastrointestinal tract, has a significant impact on many physiological and pathophysiological processes. Drugs that can perturbate the intestinal microbiome, such as antibiotics and probiotics, have been shown to be capable of producing long-term changes that effect the health of an organism. This symposium will focus on the effects of certain drugs to significantly impact the microbiome so as to alter intestinal and extra-intestinal function. It will also focus on the ability of some endogenous transmitters, including hydrogen sulfide, to significantly modify the intestinal microbiome.

MICROBIOTA PROTEASES AND INHIBITORS

Nathalie Vergnolle
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Imbalance between proteases and their inhibitors play a crucial in the development of mucosal inflammation. This has been particularly studied in the context of Inflammatory Bowel Diseases (IBD) and Irritable Bowel Syndrome (IBS), which are considered as chronic inflammatory and chronic pain disorders respectively. An increased proteolytic activity has been detected at the mucosal surface in colonic tissues of IBD and IBS patients. The source of proteases and inhibitors that regulate proteolytic balance at the intestinal mucosa surface has been recently investigated, pointing to both a microbial and host origin. Knowledge on the type of proteases and inhibitors, their source and their potential role in mucosal inflammation will be presented and discussed. Further, microbiota manipulation for the expression of proteases or inhibitors will be evoked as potential new therapeutic approach to treat inflammation and pain associated with IBD and IBS.

THE MICROBIOME AS A TARGET FOR ANTI-INFLAMMATORY AND GASTRO-PROTECTIVE THERAPIES

John L. Wallace
Department of Physiology & Pharmacology, University of Calgary, Canada

The gastrointestinal (GI) microbiome is increasingly recognized as an important, dynamic 'organ' that contributes significantly to health and disease. For example, enteric bacteria contribute significantly to the ulcers and bleeding induced in the GI tract by non-steroidal anti-inflammatory drugs (NSAIDs), in part through the impact of intestinal bacteria on metabolism of bile and of enterohepatic circulation of drugs. Drugs designed to protect the GI tract from damage induced by various aggressive factors, including NSAIDs, can cause significant dysbiosis, which in turn can contribute to an increase in susceptibility to intestinal damage. For example, proton pump inhibitors, which were designed to protect the upper GI tract from acid-related damage, can markedly increase the severity of hemorrhagic injury in the small intestine, and cause microscopic colitis. Hydrogen sulfide (H2S) is a major product of the microbiome, and can exert significant effects on a wide range of physiological process. H2S has been shown to exert numerous beneficial effects in the GI tract, such as reducing inflammation and accelerating repair of damaged tissue. The GI epithelium is very efficient in oxidizing H2S, which results in significant generation of ATP. H2S donors can also significantly modify the nature of bacterial colonization of the GI tract. Novel H2S-releasing anti-inflammatory drugs have been shown to exert significant protective effects in the GI tract, some of which may be attributable to effects on the intestinal microbiome.

Stats on Microscopic colitis from AJP paper under review.

MICROBIOME PATHOBIONTS: FROM NEW DISEASE MECHANISMS TO THERAPEUTICS

Andre G. Buret, Jean-Paul Motta, John L. Wallace
University of Calgary, Inflammation Research Network, Calgary (AB), Canada

Microbiota dysbiosis has been implicated in a broad range of disorders, in the gut as well as at extra-intestinal sites. A large body of scientific evidence describes alterations in relative abundance of microbiota representatives in these conditions. Little is known of the role played by disruptions of the microbiota phenotype or function. H_2S is an important regulator of mucosal homeostasis. We hypothesized that H_2S promotes resolution of colonic inflammation through actions on microbiota biofilm, pathobiont release, and the mucus barrier. The aim of these experiments was to characterize disruptions in gut microbiota biofilms and mucus, to assess production of invasive pathobionts during colitis, and to determine potential therapeutic benefits of hydrogen sulfide (H_2S). Because microbiota dysbiosis is a prominent feature of Inflammatory Bowel Disease (IBD), the present studies investigated various models of experimental colitis. In rats and mice, experimental DNBS-colitis fragmented the microbiota biofilm lining the colonic mucosa and induced the release of invasive pathobionts, while disrupting mucus barrier function and causing mucosal inflammation (revealed in histopathological scores and high myeloperoxidase concentrations). Inflammatory effects were worse in mice genetically deficient for cystathionine gamma-lyase, a key enzyme for H_2S production. Ex vivo, colonic microbiota biofilms from human tissue biopsies obtained from patients with ulcerative colitis released microbiota pathobionts that translocated through human intestinal epithelial monolayers, para-cellularly and trans-cellularly, and increased the release of pro-inflammatory CXCL-8.

Effects of H_2S donors (DADS, diallyl disulfide) were tested. DADS (once daily by oral gavage, 7 days) inhibited the inflammation, as well as the fragmentation of colonic microbiota biofilms and pathobiont release, and restored the mucus barrier in rats with DNBS colitis. H_2S donors also promoted the growth of human microbiota biofilms in vitro, and had anti-bacterial activity against planktonic *E. coli* and *Salmonella typhimurium*.

In conclusion, colonic microbiota form a continuous microbiota biofilm coating the underlining mucus and tissue in vivo. During colitis, the mucus barrier is broken, and these biofilms are fragmented and release planktonic pathobionts that invade the tissues. Endogenous H_2S controls DNBS-induced colonic inflammation. Administrations of exogenous H_2S donors offers therapeutic benefits in colitis at least in part by correcting microbiota biofilm disruptions and protecting against the release of invasive pathobionts.

SYMPOSIA SESSION – ISBD & BUS

RARE INFLAMMATORY DISEASES: WHAT CAN WE LEARN?

SPONSORED BY INTERNATIONAL ASSOCIATION FOR BEHCET'S DISEASE AND BIRDSHOT UVEITIS SOCIETY

IS HLA-B*51 THE CAUSATIVE GENE IN BEHCET'S DISEASE

Graham Wallace
Institute of Inflammation and Ageing, University of Birmingham

Behcet's Disease (BD) is a multisystemic autoinflammatory disease characterised by mucosal ulceration and vasculitis. The cause of BD is unknown but a genetic basis for the disease has been reported. The strongest association in all studies is the MHC class I molecule HLA-B*51. MHC class I molecules present endogenous antigens to cytotoxic T cells and inhabit NK cells killing via interaction with killing inhibitory receptors (KIR). In this presentation the potential role of HLA-B*51 in BD will be discussed including interaction with KIR3DL1 alleles and other MHC class I alleles that influence the risk of BD. Finally, BD has particular geographical spread leading to it being named the Silk Road Disease. Such a distribution is matched by the prevalence of HLA-B*51 and the possible reason for this association will be discussed.

AMPLIFIED INFLAMMATORY RESPONSES AND THROMBOSIS BEHÇET'S SYNDROME

Dorian O. Haskard F MedSci
National Heart and Lung Institute, Imperial College London

Behçet's syndrome (BS) is a multisystem inflammatory disease affecting mucocutaneous tissues, the eyes and various internal organs. The talk will highlight two recent research projects addressing central hallmarks of the condition, namely exaggerated inflammatory responses and venous thrombosis. In the first project, we have dissected cytokine release from human monocyte-derived macrophages (mph) and found that mph derived from BS patients release significantly more CXCL10 chemokine compared to cells from healthy controls in response to interferon gamma stimulation and that this can be attributed to an abnormality in the post-transcriptional regulation of CXCL10 mRNA translation. In the second study, we have addressed the role of plasma microparticles (MP) expressing tissue factor (TF, coagulation factor III) and tissue factor pathway inhibitor (TFPI). We found that BS patients with a history of thrombosis not only have increased circulating numbers of MP expressing TF but have a relative defect in MP expressing TFPI. An imbalance between pro- and anti-inflammatory mediators may therefore underpin the thrombotic tendency. Further research into these abnormalities may lead to more effective rational treatment of BS. Furthermore, an analysis of this rare condition may help our understanding of the links between inflammation and thrombosis in general.

IMMUNE DYSFUNCTION: THE ROLE OF POLYMORPHIC ERAP1 IN AUTOINFLAMMATORY DISEASE.

Edd James
Department of Medicine, University of Southampton, UK

The MHC class I antigen processing pathway generates antigenic peptide epitopes to present at the cell surface to cytotoxic T cells (CD8+). A key final step in the pathway is the trimming of N-terminal residues to generate peptides that are an optimal length for loading onto MHC class I, performed by the endoplasmic reticulum resident aminopeptidase, ERAP1. This trimming is critical for the generation of the antigenic peptide repertoire. However, ERAP1 can also destroy antigenic epitopes by trimming them to lengths too small for MHC class I binding leading to its characterisation as an antigenic peptide editor. We have identified that ERAP1 is highly polymorphic forming distinct allotypes which display three generic trimming activities (efficient, hypo- and hyperfunctional) based on the precise substrate specificity of each allotype. We have shown that individuals with chronic autoinflammatory disease that have a strong genetic linkage with particular MHC class I molecules (e.g. ankylosing spondylitis) can be identified by the identity and trimming function of the ERAP1 allotype combinations they express. We are currently investigating how these ERAP1 molecules impact on disease to identify ways to modulate ERAP1 activity as a therapeutic strategy.

Monday, July 10th

SYMPOSIA SESSION - ISBD & BUS
RARE INFLAMMATORY DISEASES: WHAT CAN WE LEARN?

09:30-10:30

THE REGULATION OF INFLAMMATION BY EPITHELIA AND LOCAL T CELLS

Adrian Hayday, FRS, F.Med.Sci

The thesis of conventional immunology is centralised control whereby responses to infection within tissues are decided within lymph nodes, from which effector T lymphocytes are despatched to quell regional disturbances. But this cannot explain the observation that many tissues at steady state are T cell-rich, a fact that raises many questions. For example, do such cells simply provide responses to infection or do they provide more generalised means to sustain tissue integrity and organ function? Likewise, how are such cells able to respond to acute stress but not drive constitutive tissue inflammation? And, how do immune cell–tissue interactions relate to organ physiology? To approach these questions, we have employed molecular genetics to identify key receptor-ligand axes that tissues use to communicate with local T cell compartments at rest, upon infection and upon non-infectious dysregulation. These axes show how epithelial cells at body surfaces selectively regulate specific T cell subtypes, thereby establishing the appropriate organ-specific composition and activities of local T cells. The molecules mediating epithelial control over tissue T cell immunity offer hope for highly localised clinical regulation of inflammation and other diseases.

diMarco Barros, Roberts et al.,Cell 167, 203-218

www.immunophenotype.org

11:00-12:00

TRAINED IMMUNITY: A MEMORY FOR INNATE HOST DEFENSE

Mihai G. Netea

Department of Medicine, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands

The inability of innate immunity to build an immunological memory, considered one of the main characteristics differentiating it from adaptive immunity, has been recently challenged by studies in plants, invertebrates, and mammals. Long-term reprogramming of innate immunity, that induces adaptive traits and has been termed *trained immunity* characterizes prototypical innate immune cells such as natural killer cells and monocytes, and provides protection against reinfection in a T/B-cell-independent manner. In contrast, *trained immunity* has been shown to be able to induce protection against reinfection in a monocyte-independent manner. Non-specific protective effects dependent on *trained immunity* have also been shown to be induced after BCG vaccination in humans. Specific signaling mechanisms including the dectin-1/Raf1 and NOD2-mediated pathways induce trained immunity, through induction of histone modifications (methylation, acetylation) and epigenetic reprogramming of monocyte function. Complex immunological and metabolic circuits link cell stimulation to a long-term epigenetic reprogramming of its function. The concept of *trained immunity* represents a paradigm change in immunity and its putative role in infection and inflammation may represent the next step in the design of future vaccines and immunotherapeutic approaches.

13:00-14:30

SYMPOSIA SESSION – JAPAN
COMPREHENSIVE PERSPECTIVES OF SYSTEMIC AND ORGAN SPECIFIC INFLAMMATORY RESPONSES

In the recent several years, there has been great progress in understanding pathogenesis of, and drug targets for, inflammatory diseases. The studies have focused not only on a specific inflammatory organ by itself but also on interplays between organs, which involve multiple factors like genetic predisposition, infection, dietary habit, and environmental insults. For instance, to reveal the pathogenesis of intestinal inflammatory disorders, alteration of several factors such as host genetics, commensal bacteria, and diet-derived metabolites have been taken into consideration. Another example is the research on atopic dermatitis, in which a primary pathogenic role of skin barrier deficiency has been extensively studied. In addition, recent studies draw increasing attention to a close positive association between inflammation and fibrosis that leads to the destruction of organ architecture and impairment of organ function. This line of research gives us a hint to understand organ damage regulated by various inflammatory mediators. The last but not least example is the tight link between immune/ inflammation and bone disease progression. An interdisciplinary research, osteoimmunology, has been conducted for the molecular understanding of the interplay between the immune and skeletal systems. This symposium is aimed to share recent advancements in those fields including newly identified molecules and novel approaches, and bring together basic scientists, clinical researchers, and industry investigators for discussing the inflammatory responses from organ-specific and systemic points of view.

INTERACTION BETWEEN THE IMMUNE SYSTEM AND BONE

Hiroshi Takayanagi, M. D., Ph. D. Professor

Department of Immunology, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo, Japan

Bone cells and immune cells share the same microenvironments in the bone marrow, communicating through various factors. Osteoblasts, osteoclasts and osteocytes are not only degrading or forming bone but have distinct roles in the immune regulation. The interdisciplinary filed, osteoimmunology, studying the interaction and shared molecules between bone and immune systems is important for many fields including medical researches such as rheumatology and dental researches in addition to basic researches on bone and immune systems (*Nat Rev Rheumatol 5, 667-76, 2009*). Here I summarize the recent advance in the field of osteoimmunology and its relevance in the studies on autoimmune diseases such as rheumatoid arthritis.

Self-tolerance to immune reactions is established by negative selection of self-reactive T cells in the thymus. This process is dependent on promiscuous expression of tissue-restricted antigens (TRAs) in medullary thymic epithelial cells (mTECs), leading to the elimination of T cells that respond to self-antigens. The transcriptional regulator Aire has been thought to be sufficient for the induction of TRAs, but we found that the transcription factor Fezf2 directly regulates various TRA genes in mTECs independently of Aire and suppresses autoimmunity (*Cell* 163, 975-87, 2015). I will discuss the mechanism of central tolerance in the context of transcriptional control of TRA expression.

REGULATION OF INTESTINAL INFLAMMATION

Kiyoshi Takeda, M. D., Ph. D. Professor
Department of Microbiology and Immunology, Immunology Frontier Research Center, Graduate School of Medicine, Osaka University, Japan

Intestine is a unique tissue, where many commensal bacteria, called microbiota, inhabit. Therefore, intestinal mucosa is protected from microbiota as well as pathogenic bacteria by several types of barriers. One of these barriers is constructed by mucus layers, composed of inner and outer mucus layers in the colon. Microbiota is present in the outer mucus layer, whereas there is no microbiota in the inner mucus layer. Separation of microbiota from the intestinal epithelial cells contributes to prevention of intestinal inflammation. Indeed, invasion of bacteria into the colonic epithelial surface was shown in several mouse models of intestinal inflammation. However, the precise mechanisms by which the inner mucus layer is free of microbiota in the colon remain unknown.

Ly6/PLAUR domain-containing protein 8 (Lypd8), which was selectively expressed on the uppermost layer of colonic glands, was a highly glycosylated GPI-anchored protein and secreted into the colonic lumen, particularly the inner mucus layer. In mice lacking Lypd8, bacterial free space in the inner mucus layer disappeared and they were highly susceptible to intestinal inflammation. On the intestinal epithelial cell layer of the colon of the mutant mice, flagellated bacteria such as *Escherichia*, *Helicobacter* and *Proteus* were present. Depletion of these bacteria by antibiotics restored the bacterial free space in the inner mucus layer and ameliorated the intestinal inflammation of the mutant mice. Lypd8 bound to bacterial flagella and suppressed motile activity of flagellated bacteria. Thus, Lypd8 mediates segregation of microbiota from the intestinal epithelial layer in the colon, and thereby contributes to the prevention of intestinal inflammation.

LIVE IMAGING OF SKIN IMMUNE RESPONSES

Kenji Kabashima, M. D., Ph. D. Professor
Department of Dermatology Graduate School of Medicine and Faculty of Medicine, Kyoto University, Japan

Various immune cells orchestrate cutaneous immune responses to external stimuli. To capture such dynamic phenomena, intravital imaging is an important technique and it may provide substantial information that is not available using conventional histological analysis. Multiphoton microscopy enables the direct, three-dimensional, and minimally invasive imaging of biological samples with high spatio-temporal resolution, and it has now become the leading method for *in-vivo* imaging studies. Using fluorescent dyes and transgenic reporter animals, both skin structures and cell- and humor-mediated cutaneous immune responses have been visualized.

Through the detailed examination of the elicitation phase of contact hypersensitivity as a murine model of contact dermatitis, we demonstrated the formation of sequential leukocyte clusters at the postcapillary venules. The structure does not exist in the steady state, but is 'induced' in response to local inflammatory conditions. Herein, we propose that this structure to be termed as 'inducible SALT (iSALT)'. In this symposium, I will introduce cutaneous immune responses to external stimuli (such as haptens) in the perspective of iSALT.

ROLE OF LOCAL CELL-CELL INTERACTION AND SYSTEMIC ORGAN NETWORK IN METABOLIC DISEASES

Yoshihiro Ogawa, M. D., Ph. D. Professor
Department of Medical and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University;
Department of Molecular and Cellular Metabolism, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University;
AMED-CREST, Japan

Energy homeostasis is maintained locally through parenchymal-stromal cell interaction and systemically through organ network. In obese adipose tissue, saturated fatty acids, which are released as a danger signal from hypertrophied adipocytes, stimulates a pathogen sensor TLR4 in the infiltrating macrophages, thus establishing a vicious cycle between adipocytes and macrophages to stimulate adipose tissue inflammation. Histologically, macrophages aggregate to constitute crown-like structures (CLS), where they are thought to scavenge the residual lipid droplets of dead adipocytes.

Free fatty acids, when released from obese visceral fat depots, are transported in large quantities to the liver via the portal vein, where they are accumulated as ectopic fat, thus developing non-alcoholic fatty liver disease (NAFLD). There is a unique histological feature termed "hepatic CLS (hCLS)" in non-alcoholic steatohepatitis (NASH) liver, where macrophages aggregate to surround dead hepatocytes with large lipid droplets. Notably, the number of hCLS is positively correlated with the extent of liver fibrosis. Our data suggest that hCLS serves as an origin of hepatic inflammation and fibrosis during the progression from simple steatosis to NASH.

Sodium glucose cotransporter 2 (SGLT2) inhibitors, an oral antidiabetic drug, promotes the urinary excretion of glucose by blocking its reabsorption in renal proximal tubules. Inhibition of SGLT2 is expected to lower body weight because of urinary calorie loss. Interestingly, SGLT2 inhibition improves hepatic steatosis in obese mice in parallel with increased adiposity, suggesting that SGLT2 inhibition induces the "healthy" adipose tissue expansion and prevents ectopic fat accumulation in the liver.

Here I will discuss how NASH develops through the dysregulation of local parenchymal-stromal cell interaction and systemic organ network.

SYMPOSIA SESSION – BRAZIL
RESOLUTION AND REPAIR

ANGIOTENSIN1-7 AND ACETATE – NOVEL RESOLVERS OF INFLAMMATION

Mauro M Teixeira
Departamento de Bioquimica e Imunologia, ICB, Universidade Federal de Minas Gerais, Brazil

The resolution of inflammation is an active process that relies on the release of molecules that actively decrease production of pro-inflammatory molecules, decrease leukocyte influx, induce leukocyte apoptosis and efferocytosis, and polarize macrophages to pro-resolving phenotypes. Molecules known to resolve inflammation include annexin-A1 and the pro-resolving lipid mediators. Here, we will providence evidence that short chain fatty acids (SCFA) decrease inflammatory responses in the tissue because they actively induce the resolution of inflammation. In

addition, evidence will be provided that angiotensin 1-7 activates the Mas receptor to induce resolution of neutrophlic and eosinophilic inflammation. Together, these experiments add to the body of existing pro-resolving mediators and suggest that metabolic products (such as acetate and angiotensin 1-7) may have profound effects on the inflammatory response by the return of tissue to homestasis.

PRO-RESOLUTION EFFECT OF 15D-PGJ2 ON ALLERGEN-INDUCED LUNG INFLAMMATION AND REMODELING

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15-deoxy-Δ-^{12,14}-prostaglandin J_2 (15d-PGJ_2) has been described as an anti-inflammatory lipid mediator in several *in vitro* and *in vivo* studies, but its effect on ongoing allergic pulmonary inflammation and remodeling remains elusive. We investigated here the therapeutic potential of 15d-PGJ_2 based on murine models of allergic asthma triggered by either ovalbumin (OVA) or house dust mite extract (HDM). 15d-PGJ_2 treatments were given via subcutaneous injection or intranasal instillation, respectively. Analyzes were carried out 24 h after the last allergen provocation. Irrespective of the route of administration, 15d-PGJ_2 significantly inhibited the peribronchial accumulation of eosinophils and neutrophils, subepithelial fibrosis and also mucus exacerbation caused by either OVA or HDM challenge. The protective effect of 15d-PGJ_2 occurred in parallel with inhibition of allergen-induced AHR and lung tissue production of pro-inflammatory cytokines, such as IL-5, IL-13, IL-17 and TNF-α. Finally,15d-PGJ_2 was found effective in inhibiting NF-kB phosphorylation upon HDM challenge as measured by Western blotting. In conclusion, our findings suggest that 15d-PGJ_2 can reduce crucial features of asthma, including AHR, lung inflammation and remodeling in distinct murine models of the disease. These effects are associated with a decrease in lung tissue generation of pro-inflammatory cytokines by a mechanism related to down-regulation of NF-κB phosphorylation.

IMMUNORESOLVENTS AT THE INTERSECTION BETWEEN BRAIN AND IMMUNE SYSTEM

Jesmond Dalli

William Harvey Research Institute, London, UK

Resolving inflammatory exudates produce chemical mediators that regulate inflammation, stimulate resolution and tissue regeneration. These mediators include the lipoxin, resolvin, protectin and maresin families and are collectively called specialized pro-resolving mediators (SPM). These mediators actively regulate leukocyte responses counter-regulating the production pro-inflammatory signals, promoting their differentiation to a protective phenotype and orchestrating tissue cellular trafficking. We recently found that disruption of the vagal system reduced peritoneal RORγt$^+$CD335$^+$ Group 3 innate lymphoid cells (ILC-3) and altered peritoneal macrophage responses. It also led to an inflammatory peritoneal lipid mediator profile with reduced levels of the novel immunoresolvent protectin conjugate in tissue regeneration (PCTR)1, elevated inflammation-initiating eicosanoids and delayed the resolution of *Escherichia coli* infections. Administration of PCTR1 or ILC-3 to vagotomised mice restored tissue resolution tone and host responses to *E. coli* infections. Together these findings elucidate a novel host protective circuit mediated by ILC-3-derived pro-resolving mediators including PCTR1 that is under local neuronal control and regulates tissue resolution tone and myeloid cell response.

16:00-17:30

SYMPOSIA SESSION – ITALIAN SOCIETY OF PHARMACOLOGY
TARGETS OF INFLAMMATION

RESOLUTION PHARMACOLOGY - THERAPEUTIC INNOVATION IN INFLAMMATION

Mauro Perretti

William Harvey Research Institute, Queen Mary University of London

Inflammation is a defensive response of the body against pathogens and injury where immune cells and soluble factors take part to neutralize the injurious agent and initiate tissue repair. However, a loss of regulation of these mechanisms can prevent the final ending of inflammation leading to fibrosis and chronic conditions. As such de-regulated inflammatory processes contribute to a variety of chronic diseases including those classically assigned as 'inflammatory' and those which have long considered to be of different etiology, e.g. cardiovascular diseases like atherosclerosis or CNS pathologies like Alzheimer's disease.

Immune cells like the macrophage and the neutrophil are pivotal players in organizing the onset of inflammatory reaction and, at the same time, they are also central in promoting its resolution, thus ensuring tight spatial and temporal control within the host. Neutrophils may enable this biological action through the release of microvesicles [1] or *via* apoptosis [2], thus impacting on the microenvironment. An example of the latter is the switch of macrophage phenotype upon phagocytosis of apoptotic neutrophils, a change in phenotype that is required to enable correct engagement of resolution mechanisms. Aside cellular processes, soluble pro-resolving mediators act on specific receptors (e.g. FPRs or MCRs) to signal on macrophages and neutrophils to evoke pro-resolving and tissue-protective effects.

Work over the last decade has led to the definition of the biology of resolution, with the detailed investigation of the bioactions of pro-resolving mediators and receptors that are engaged to activate tissue-specific molecular and cellular mechanisms. We propose time is now ready to harness this biology for therapeutic innovation, that is the device of strategies to develop novel medicines for the treatment of chronic inflammatory diseases enabling the establishment of a new branch of pharmacology we term 'Resolution Pharmacology' [3,4].

Funds supporting this research have been received from the Medical Research Council UK (project MR/K013068/1), Wellcome Trust (programme 086867) and BBSRC (studentship BB/K011782/1).

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INFLAMMATION, A KEY EVENT IN DEVELOPMENT OF NEURODEGENERATIVE DISEASES

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The degeneration of the central nervous system (CNS) is characterized by chronic progressive loss of the structure and functions of neuronal materials, resulting in functional and mental impairments. Immune activation in the CNS, always present in immune-mediated disorders, and neurodegenerative diseases, involves microglia and astrocytes which constitute the resident immune cells of the CNS and play an important role in the regulation of homeostasis of the brain during development, adulthood and aging. In the CNS, microglia constantly survey the microenvironment by producing factors that influence surrounding astrocytes and neurons, particularly in response to pathogen invasion or tissue damage thereby promoting an inflammatory response that further engages a self-limiting response through the immune system and initiates tissue repair. However, inflammation that may result in the production of neurotoxic factors amplifying the disease states, indicates the persistence of inflammatory stimuli or failure in normal resolution mechanisms. Accordingly, specific inducers of inflammation associated with neurodegenerative diseases converge in mechanisms responsible in the sensing, transduction and amplification of the inflammatory processes that result in the production of cytokines and interleukins. These neurotoxic mediators are, in general, associated with several neurodegenerative diseases including Alzheimer's disease (AD), multiple sclerosis (MS), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), which are commonly linked to intracellular mechanisms such as the degradation of protein, the dysfunction of mitochondria, the defects of axonal transport and apoptosis. The emerging evidence on the sustained inflammatory response associated with the contribution of microglia and astrocytes in disease progression, suggest contributory important roles of effectors derived from innate and acquired immune systems in neuronal dysfunction and death.

The available evidences convincingly demonstrate that neuroinflammation is a crucial factor in the onset and progression of neurodegeneration and neuronal loss in neurodegenerative diseases. Suppression of neuroinflammation can ameliorate neurodegenerative disease symptoms and reduce the extent of neurodegeneration. However, it is necessary to find newer therapeutic agents to repair the damaged neurons, and to regenerate new neurons at the site of neuronal damage in the CNS.

MOLECULAR BATTLES BETWEEN GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS IN THE HEART REGULATE CARDIOVASCULAR HEALTH AND DISEASE

Robert H. Oakley, Diana Cruz-Topete, Bo He, and John A. Cidlowski

The Signal Transduction Laboratory, NIH/NIEHS

Heart failure is one of the leading causes of death in the Western world, and stress is increasingly associated with adverse cardiac outcomes. Glucocorticoids are primary stress hormones that regulate inflammation and homeostasis through two closely related nuclear receptors, the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). Cardiomyocytes express both receptors but little is known concerning their coordinated actions in heart physiology and pathology. To examine the in vivo function of glucocorticoid signaling in the heart, we generated mice with cardiomyocyte-specific deletion of GR (cardioGRKO), MR (cardioMRKO), or both GR and MR (cardioGRMRdKO). The cardioMRKO mice exhibited normal heart function whereas the cardioGRKO mice spontaneously developed cardiac hypertrophy and left ventricular systolic dysfunction. Surprisingly, the cardioGRMRdKO mice were protected from cardiac disease, despite having pathological gene changes that were present in the GR deficient hearts. Genome-wide microarray analysis identified a set of cardioprotective gene changes that occurred uniquely in the double knockout hearts. Re-installation of MR into the cardioGRMRdKO hearts by adeno-associated virus gene transfer completely reversed the cardioprotective gene changes and resulted in cardiac dysfunction. These findings reveal not only a deleterious role for cardiac MR signaling when unopposed by GR but also the molecular targets of MR that contribute to cardiac pathology. Therapies that shift the balance of cardiomyocyte glucocorticoid signaling to favor more GR and less MR activity may provide an improved approach for treating heart disease.

GLUCOCORTICOID INDUCED LEUCINE ZIPPER (GILZ) IN CONTROL OF INFLAMMATION AND TUMOR GROWTH

Carlo Riccardi

Department of Medicine, Section of Pharmacology, University of Perugia, Italy

Glucocorticoids (GCs) exert important therapeutic effects in many inflammatory/autoimmune and degenerative diseases. Most of GC effects are receptor (GR) mediated and relate to regulation of gene transcription, consistent with the capability of endogenous GCs to mediate environment-induced epigenetic effects.

GCs rapidly increase the transcription of GC-induced leucine zipper (GILZ) and its transcriptional variant long (L)-GILZ. GILZ and L-GILZ mediates several anti-inflammatory and immunomodulatory GCs functions. In particular, GILZ attenuates inflammation mainly by inhibition of nuclear factor κB (NF-κB), MAP kinase pathway, induction of Anxa1 and increase of GC-induced Treg development (1-2). GILZ and L-GILZ contribute to the control of T and B lymphocyte activation, proliferation and survival. Consistent with these inhibitory effects on T and B lymphocytes, there is an increased severity of inflammation and colitis in GILZ-KO while a reduction in GILZ-TG. Notably, in vivo administration of GILZ fusion protein inhibits the disease

development in various colitis models (3). Results indicate GILZ as a mediator of GC activity, provide new means to predict sensitivity to treatment with GC and outline new anti-inflammatory therapeutic approaches.

The GILZ/L-GILZ system is also important in control of tumor cell growth. In fact, several functions of GILZ and L-GILZ are mediated by their interaction with crucial transcription factors, including p53, Ras/Raf/MEK/ERK pathway and CEBPA. As an example, L-GILZ inhibits MAPK pathway, hence triggering anti-proliferative and apoptotic pathways, with consequent inhibition of human thyroid tumor growth. In addition, deletion of GILZ and L-GILZ increases B-cell survival, NF-kB transcriptional activity and Bcl-2 expression, resulting in B-cell lymphocytosis, thus indicating GILZ can also be important in leukemogenesis. Consistent, GILZ deficiency while delays tumor development in a mouse model of acute myeloid leukemia, induced by leukemogenic CEBPA mutation, decreases the number of pre-leukemic hematopoietic stem cells (HSC).

In conclusion our results indicate the GILZ/L-GILZ system as a mediator of anti-inflammatory effects of GCs. Moreover, the capability of the GILZ/L-GILZ system to modulate cell proliferation and survival, suggests its role in control of tumor growth.

References

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Tuesday, July 11th

11:00-12:00

INTESTINAL FIBROSIS IN INFLAMMATORY BOWEL DISEASE

Tom Holvoet
Ghent University

Inflammatory Bowel Diseases (IBDs) are a group of relapsing-remitting inflammatory disorders mainly affecting the small and large intestine, and include Crohn's disease and ulcerative colitis. Crohn's disease is characterized by transmural bowel inflammation as opposed to ulcerative colitis where inflammatory lesions are limited to the superficial mucosa. In both IBDs, recurrent episodes of inflammation, followed by mucosal healing cause (sub)mucosal deposition of extracellular matrix components, which progressively leads to structural fibrosis. Ultimately, up to one third of CD patients develop an end-stage fibrotic disease characterized by intestinal strictures, luminal stenosis and organ failure. This comorbidity is currently untreatable, and cannot be predicted, posing a major clinical problem in IBD management. In this talk we will provide an overview of the pathophysiology and molecular mechanisms contributing to intestinal fibrosis in IBD. Targets for potential therapies will be highlighted, such as the rho kinases, mediating cytoskeleton organization and pro-fibrotic signaling pathways in effector cells involved in fibrosis. Compounds inhibiting these kinases are emerging as a promising anti-fibrotic treatment option.

13:00-14:30

SYMPOSIA SESSION – USA
AUTOPHAGY AND IMMUNE-REGULATION

Autophagy, a constitutive intracellular degradation pathway, is involved in modulation of cell metabolism, cell survival, and host defense. Moreover, autophagy has a fundamental role in innate and adaptive immune cells activation and homeostasis. Lack of autophagy has been shown to be related to several inflammatory syndromes and autoimmune diseases. Therefore, strategies to induce autophagy can be an innovative therapeutic approach to re-establish immune-regulation and tolerance.

AUTOPHAGY PROTEINS IN ENDOCYTOSIS AND EXOCYTOSIS

Monica Loi, Laure-Anne Ligeon, Maria Pena, Heike Nowag and Christian Münz
Viral Immunobiology, Institute of Experimental Immunology, University of Zürich, Switzerland

Macroautophagy describes the degradation of cytoplasmic contents in lysosomes. In recent years it has become apparent that modules of the molecular machinery of macroautophagy can serve other purposes in endo- and exocytosis. Along these lines viruses have been found to acquire autophagic membranes during their exocytosis. We could demonstrate that such membranes are stabilized by lytic EBV replication in infected epithelial and B cells. Inhibition of autophagic membrane formation compromises infectious particle production and leads to the accumulation of viral DNA in the cytosol. Atg8/LC3, an essential macroautophagy protein and substrate anchor on autophagic membranes, was

found in virus preparations, suggesting that EBV recruits Atg8/LC3 coupled membranes to its envelope in the cytosol. Our data indicate that EBV subverts macroautophagy and uses autophagic membranes for efficient envelope acquisition during exocytosis.

In addition, the macroautophagy machinery has been implicated in regulation of phagocytosis. We recently reported that this machinery assists in the internalization of MHC class I molecules. In the absence of the autophagy factors Atg5 and Atg7, MHC class I surface levels are elevated due to decreased endocytosis and degradation. In the absence of Atg-dependent MHC class I internalization, dendritic cells stimulate $CD8^+$ T cell responses more efficiently *in vitro* and *in vivo*. During viral infections, lack of Atg5 results in enhanced influenza and LCMV specific $CD8^+$ T cell responses *in vivo*. Elevated influenza-specific $CD8^+$ T cell responses were associated with better immune control of this infection. Thus, the macroautophagy machinery compromises MHC class I restricted antigen presentation.

IMPAIRED AUTOPHAGY IN CROHN'S DISEASE

Arthur Kaser

The ATG16L1-T300A polymorphism has identified impaired autophagy as a major pathomechanism in Crohn's disease. However, it has remained unclear how this would predispose to intestinal inflammation. We discovered that with increasing age, mice with intestinal epithelial cell-specific *Atg16l1* deletion develop transmural, fissuring, discontinuous ileitis phenocopying Crohn's disease. This ileitis was driven by hyperactivation of the unfolded protein response (UPR) sensor IRE1alpha. IRE1alpha accumulated at the intestinal crypt base, both in old *Atg16l1* mutant mice, and in Crohn's disease patients homozygous for *ATG16L1*-T300A; this is consistent with Paneth cells experiencing endoplasmic reticulum (ER) stress under such conditions. Indeed, IRE1alpha also did drive the Crohn's disease-like ileitis we had previously reported to develop in young *Atg16l1;Xbp1* mice, in which the UPR transcription factor X box binding protein 1 is additionally deleted in the intestinal epithelium. Mechanistically, Optineurin - a cargo receptor for selective autophagy - was involved in controlling IRE1alpha degradation under conditions of ER stress. This suggests that defective autophagy in intestinal epithelial cells may predispose to Crohn's disease ileitis via impaired clearance of large IRE1alpha clusters forming in Paneth cells experiencing high levels of ER stress.

AUTOPHAGY DETERMINES IMMUNE METABOLISM DURING NEUTROPHIL DIFFERENTIATION

Katharina Simon
The Kennedy Institute of Rheumatology
University of Oxford, UK

Recent studies have revealed a fundamental role for cellular metabolism in the differentiation of immune/ hematopoietic cells. For example hematopoietic stem cells that are unable to undergo mitochondrial respiration fail to differentiate. Moreover cellular function of immune cells is critically dependent on specific metabolic pathways. When remodeling of the mitochondrial tubular network is enforced in T cells, allowing for mitochondrial respiration, effector T cells are turned into memory T cells.

Our own research has revealed a key role for autophagy in making the decision between two main energy-generating pathways, ie mitochondrial respiration and glycolysis. Autophagy is the main cellular recycling pathway providing essential metabolites and maintaining mitochondrial quality. On the whole, deficient autophagy shifts the cellular metabolism towards glycolysis, away from mitochondrial respiration, impacting on differentiation and function. Here I will present data on the effect of autophagy on the different metabolic pathways that are required for neutrophil differentiation.

Funded by Wellcome Trust Investigator Award.

16:45 - 17:15

A PLAYER AND WITNESS OF PART OF THE HISTORY OF INFLAMMATION

Boris Bernardo Vargaftig
Department of Pharmacology, Institut of Biomedical Sciences, University of S. Paulo

In this presentation, I shall summarize 40 years of research on inflammation in 4 institutions: in the Universities of São Paulo and Campinas, Brazil, in laboratories from Organon and Merrell International and at the Institut Pasteur, in France. I will show the reasons of my early interest in venoms as tools for investigation, their role for the discovery of important peptide and lipid mediators and in inducing tissue lesions. Their use in the early 70-ies and the study of the mediators they release led to the discovery of the mode of action of aspirin-like drugs, which I claim. I will also recall the potential role of Platelet-activating Factor in inflammation, even though its antagonists have not show clinical efficacy when tested. These investigations led to my involvement with the discovery of a BCG derivative effective against experimental airways allergy and, in collaboration with the laboratory of airways inflammation in S. Paulo (Prof. W. Tavares de Lima), to the study of the mechanisms of lung inflammation after gut isquemia and reperfusion, and recently to the discovery of its inhibition by estrogens both in male and female mice. This suggests the possible use of female hormones in acute human pathologies.

Keywords: inflammation by venoms, SRS-C, prostaglandins, aspirin-like drugs, PAF, BCG

17:15-18:00

FRONTIERS IN INFLAMMATION RESEARCH: FROM NLRP3 TO METABOLIC REPROGRAMMING

Luke A.J. O'Neill
School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland.

The discovery of the NLRP3 inflammasome provided an important component in the inflammatory process, revealing a key mechanisms underlying the induction of the central pro-inflammatory cytokine IL-1beta as well as IL-18 and a type of inflammatory cell death called pyroptosis. NLRP3 therefore emerged as a compelling therapeutic target for several inflammatory diseases, raning from gout to osteoarthritis and even neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease. NLRP3 was also strongly implicated in metabolic diseases such as atherosclerosis and NASH. Work on NLRP3, IL-1beta and metabolism formed part of the renaissance of interest in immunometabolism,

which in this context means intracellular metabolic changes occurring in immunity that are governing for immune and inflammatory effector mechanisms. Metabolic changes triggered by innate immune receptors have become a particular focus for researchers interested in immunity and inflammation. This area has direct relevance to inflammatory diseases such as rheumatoid arthritis since the rheumatoid joint is known to undergo a range of metabolic alterations and enzymes in glycolysis are well known autoantigens. Furthermore metabolites from the Krebs Cycle such as succinate have been found to be elevated in rheumatoid synovial fluid and act via the receptor SUCRN1 on macrophages to boost IL-1beta production. LPS-activated macrophages undergo metabolic reprogramming with a major increase in glycolysis, which is required for ATP production and also the generation of biosynthetic intermediates. Changes in the Krebs cycle also occur such that intermediates such as citrate are withdrawn for lipid biosynthesis. We have found a key role for citrate in the induction of a range of inflammatory genes and a complex mechanism involving malonylation of GAPDH leading to enhanced translation of a range of mRNA including those encoding TNF and COX2 has been revealed. We have also found a role for the Krebs cycle intermediate succinate in activated macrophages. Succinate induces HIF-1alpha and it's target genes, which include that encoding IL-1beta, can act on the aforementioned succinate receptor SUCRN1 on cells (which can synergise with TLRs) also can be oxidised by Succinate Dehydrogenase which because of the high mitochondrial membrane potential leads to reverse electron transport (RET) via Complex I in the mitochondria. This drives ROS production with inflammatory consequences. Succinate might therefore act as important signal for inflammation. We have also found that inhibition of SDH leads to IL-10 production, indicating that this enzyme is a key arbiter of cytokine production. These insights are providing a new view of metabolism in immunity and inflammation and might indicate new therapeutic approaches.

Wednesday, July 12th

09:30-10:30

CONTRIBUTION OF DIFFERENT EPIDERMAL CELL POPULATIONS TO INFLAMMATION-ASSOCIATED CANCERS

Fiona M. Watt

Centre for Stem Cells and Regenerative Medicine, King's College London, Floor 28, Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT, UK

Although epidermal maintenance depends on stem cell self-renewal, there is good evidence that the post-mitotic, differentiated cell layers also contribute to homeostasis and tumour formation. I will describe recent work from our laboratory in which we have examined the interactions between epidermal stem cells, differentiated cells and immune cells during tumour formation. Our results highlight the importance of macrophages and regulatory T cells in the model systems.

11:00-12:30

SYMPOSIA SESSION – RUSSIA
INTEGRATIVE INFLAMMATORY MECHANISMS

Symposium organized by the Russian Inflammation Society in the framework of the XIII World Congress on Inflammation (London, 8-12 July 2017) is devoted to the general mechanisms of inflammation development and integrative mechanisms that underlie the inflammation. In particular, the presented reports will include data on the interaction of immunological mechanisms in the development of inflammation, the role of inflammation in the development of noninflammatory pathology, the role of regulatory inflammatory mechanisms in the development of systemic typical pathological processes, the phenomenon of the systemic inflammatory response syndrome and its role in shock progression.

IMMUNOPATHOPHYSIOLOGY OF SYSTEMIC INFLAMMATION

Valery Chereshnev[1,2], Eugeny Gusev[1,2], Natalia Zotova[1,2], Margarita Chereshneva[1]

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[2] Federal State Autonomous Educational Institution of Higher Professional Education "Ural Federal University named after the first President of Russia B. N. Yeltsin", Yekaterinburg, Russian Federation

Pathogenesis of many critical septic as well as noninfectious states currently associates with development of systemic inflammation (SI). Systemic inflammation as a pathonomy term has not been formalized yet and corresponds to synonym of such clinical notions as SIRS or systemic inflammatory response. Systemic inflammatory response is multifaceted though and protective, it is generally not a mechanism of shock and multiple organ dysfunctions. Therefore there is a necessity to consider systemic inflammation as a separate form of common pathological process. And this form differs from "classical" inflammation (in traditional meaning) by a number of attributes. Fundamental signs of SI, from our point of view, are the following: 1) Systemic

alteration – an entry of microbial antigens and tissue destruction products into the bloodstream as well as other systemic changes of homeostasis; these lead to development of systemic proinflammatory cell stress. 2) Endothelial cells of microvessels and paravascular connective tissue, intravascular leukocytes, and plasma factors of all vitals are the basic cell elements of SI development. 3) Secondary systemic alteration phenomenon is the driving force of systemic inflammation evolution; the phenomenon is associated with oxygen transport disturbances and other manifestation of microcirculation disorders. 4) Systemic inflammation clinically appears as an unstable complex of resuscitation syndromes. 5) To detect SI a nature of systemic inflammatory response to be specified, notably the origin of cytokines and other cell stress molecules, if they are local (from inflammation focus) or systemic origin. The specification is accessible under differentiation of levels of these factors in the blood as well as under application of integral SI criteria. Integral criteria should reflect the intensity of some SI phenomena. Subject to above we defined Systemic inflammation as a "typical, multi-syndrome, phase-specific pathological process, developing from systemic damage and characterized by the total inflammatory reactivity of endotheliocytes, plasma and blood cell factors, connective tissue and, at the final stage, by microcirculatory disorders in vital organs and tissues."

Key words: systemic inflammation, SIRS, pathophysiology.

INFLAMMATION AND SHOCK: INTERACTION OF SIRS AND OTHER COMPLICATIONS

Iryna Fomochkina, Vladimir Kharchenko, Mikhail Fedosov, Anatolii Kubyshkin, Anatolii Pisarev, Alexey Beketov
Medical Academy of V.I. Vernadsky Crimea Federal University

The most common causes of complications in patients with critical states of different etiology are acute respiratory distress syndrome (ARDS), DIC, and multisystem organ dysfunction syndrome (MODS). On the other hand, there is a well-known a close connection between the above-mentioned syndromes and SIRS in sepsis due to the common pathogenetic mechanisms. However, the role of SIRS, as well as link between plasma cascade inflammatory systems, in the pathogenesis of complications of critical illness of different etiology has yet to be established.

Clinical and experimental studies have been performed using experimental model (170 Wistar rats) of ischemia-reperfusion injury (IR) and 58 patients with critical states of different etiology (subsequently divided according to the outcome into 2 groups: recovery and death) to study the activity of nonspecific proteases: trypsin- (TLA) and elastase-like activity (ELA), antitrypsin (ATA) and acidstable inhibitors (ASI); concentration of the main proinflammatory cytokines (IL-1ß, IL-6, TNF-α); gene expression of caspase-3 in cells of vessels, lungs and kidney of IR rats; parameters of hemostasis, and clinical signs of SIRS.

We defined that serum levels of IL-6 and TNF-α were elevated in IR rats. Also, the local (the supernatant fraction of rat hind-limb muscles homogenate and bronchoalveolar lavage at ARDS, confirmed morphologically) increase in the proteolytic activity, and decrease in inhibitory activity. At the blood serum level, IR was manifested by an increase in TLA 3 times in 12 hours, a decrease in ELA (with subsequent growth in 48 hours following revascularization), a diminish in ATA and ASI, indicating their increased consumption. Evidence for pathogenetically similar changes has been obtained by results of studying of the blood coagulation system: there was coagulopathy of consumption when deploying a full-fledged IR. Increase in the expression of caspase-3 in the cells of the hind limbs and lung in 6 hours following revascularization was also established, that indicate the initiation of the proapoptotic signalling pathway that results in ARDS (it is confirmed by significant expression of caspase-3 in comparison with the kidney) and may be one of a key factors for the development of MODS in IR. Critical states in clinic were accompanied by a significant increase in a levels of proinflammatory cytokines and the activity of proteases. Moreover, in cases of the favourable outcome the level of cytokines and proteases' activity wasn't significant with the tendency to reduce gradually within 5 days; while in cases with fatal outcome, considerable initial activation of cytokines and proteases was identified with the tendency towards their further progressive growth.

Thus, our results to provide a sounder evidence about the key role of SIRS in the subsequent development of complications (DIC, ARDS and MODS) of various critical illness. "Cytokine" and "protease storm" may be a key element of distant effects in organs' damage, which triggers may be implemented through the initiation of inflammatory reactions, and activation of apoptosis. Moreover, depending on the etiopathogenesis of the critical state, it is possible to distinguish a primary or secondary option of development of SIRS, which implies the possibility of different treatment strategy for the prevention of complications.

Key words: SIRS, proteases, cytokines, caspase, MODS, ARDS

AUTOANTIBODIES TO BETA1-ADRENORECEPTORS AND CARDIAC ARRHYTHMIAS: BREAKTHROUGHS AND PITFALLS

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Growing evidence indicates an important role of autoantibodies against β1-adrenergic receptors (β1AR) in pathogenesis of cardiovascular diseases. This report will review the current evidence for the significance of autoantibodies in the development of cardiac arrhythmias. In particular the role of autoantibodies to β1AR in patients with «idiopathic» ventricular arrhythmias with no signs of organic heart disease will be thoroughly discussed. One of the main problems is the absence of the reliable method of detection of the autoantibodies to β1AR as classical enzyme-linked immunosorbent assay (ELISA) with synthetic peptide, mimicking the region of second extracellular loop of β1AR, and developed recently competitive cell-based ELISA have significant limitations. Currently some treatment strategies are proposed, such as immunoadsorption and application of synthetic mimics of the epitope, but the results of these approaches are inconsistent. Future directions in laboratory diagnostics of this condition and possible role of autoantibodies to β1AR in pathogenesis of cardiac arrhythmias will be discussed in the lecture.

Key words: cardiac arrhythmias, adrenergic receptors

INFLAMMATION AND SYSTEMIC TYPICAL PATHOLOGICAL PROCESSES: INTERACTION AND CONFLICT OF REGULATING PROGRAMS

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Inflammation - is a typical pathological process developing in a vascularized organs and tissues in response to a local injury. Being relatively independent of central systemic regulation, during a disease of the whole organism, inflammation never works alone, but always coincides and interacts it time with other programmed systemic defensive responses, which predominantly obey to centralized programme management (stress, fever, acute phase response etc.). The matter of interest is pathologic informatics and logistics of resources' distribution during healthy balance and unhealthy conflicts of these local and systemic defensive mechanisms. According the rule of systemic-local defensive balance, neither in health, nor in standard course of typical pathological processes systemic neuroendocrine regulation and local autacoid-mediated mechanisms confront: Autacoids are effective within closest distance only; Systemic signals do not unify optimal and topically versatile work of local defensive mechanisms, because their access to inflammation foci is limited with functional barriers. Structural and functional integrity of connective tissue is essential for correct combination of local peripheral and systemic central signals and separation of their domains. Both excessive central interference in local autonomy, and disproportionate systemic effects of inflammatory autacoids could be highly pathogenic and lead to shock – in acute – and to accelerated ageing – in chronic situations. The presentation discusses these "conflict of Kremlin and Kolkhoz" in pathophysiology of acute severe combined trauma and early-complicated metabolic syndrome. Interaction of orthophlogosis and stress, as 2 main partially contradictory anti-shock mechanisms is discussed in acute and chronic disorders. The energetic and plastic resources in disease are limited. Various programme managers in a disease tend to direct them preferencially to different addressees Stress and acute phase response are compared in a presentation as different managers for in vivo logistics of fuel and bricks. Shock and metabolic syndrome are interpreted as examples of typical conflicts between local and systemic regulation mechanisms in organism. The concept is illustrated by authors' original data on Clinical Pathophysiology of shock, early forms of metabolic syndrome, marfanoid dysplasiae of connective tissue and chronic autoimmune thyroiditis. Some evidences are obtained, that witness for marfanoid phenotype and chronic disequilibrium between local, autacoid-mediated and systemic, hormone-mediated regulation, typical for inherited connective tissue disorders, may promote the early transition of juvenile Simpson-Page' syndrome into early-complicated metabolic syndrome

Key words: inflammation, stress, shock, acute phase response

ORAL PRESENTATIONS

Ageing

OP-01

DECREASED NEUTROPHIL MIGRATION AND ACTIVATION IN AGING: A PROTECTIVE ROLE FOR THE ADENOSINE A2A RECEPTOR

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Background: Aging can be accompanied by an increase in markers of innate immunity and by a decline in adaptive immunity. Polymorphonuclear leukocytes (neutrophils) accumulate at sites of injury during the acute phase of inflammation, and the adenosine A2A receptor (A2AR) signaling pathway is a pivotal modulator of neutrophil functions. However, how aging affects neutrophil functions remains unclear.

Methods: In this study, we monitored through aging an acute inflammatory response and measured the infiltration and viability of neutrophils into dorsal air pouches, their activation, and the accumulation of cytokines in the microenvironment, in wild-type (WT)- and A2AR-knock out (KO) mice of three different age groups.

Results: Mice steadily gained weight throughout the study, but A2AR-KO mice became up to 12% lighter than WT. Numbers of migrated neutrophils decreased by at least 50% with aging while their mortality increased, more severely so in A2AR-KO mice. Levels of elastase and of total proteins in the air pouch exudates also diminished in aged mice, and were even lower in A2AR-KO mice. Dorsal cavity levels of TNF, IL-6, IL-10, CXCL1 and CCL2-4 and G-CSF decayed with aging, often by more than 75%, and the decay occurred more rapidly in A2AR-KO mice, while CXCL1-3 levels were largely spared. A similar pattern of cytokines was obtained in the dorsal pouch from young mice when neutrophils were prevented from migrating to the pouch. Local effects of aging were not observed systemically.

Conclusion: Aging negatively impacts acute inflammatory reponses and neutrophil-related activities; a functional A2AR signaling pathway protects against some of these age-associated symptoms.

Keywords: Neutrophils, aging, cytokines, gene expression, adenosine, in vivo

Ageing

OP-02

INFLAMMAGEING CONTRIBUTES TO AGE-RELATED IMPAIRMENTS IN THE MAINTENANCE OF IMMUNOLOGICAL MEMORY IN THE BONE MARROW

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Aging induces a basal level of inflammation throughout the body, a condition known as inflammageing, which contributes to immunosenescence. New strategies to counteract immunosenescence in the elderly are needed, in particular by improving the maintenance of immunological memory. It has been demonstrated that memory T cells and long-lived plasma cells home to bone marrow niches, well organized structures which promote the long-term survival of these cells through homeostatic proliferation. CD4+ and CD8+ effector memory T cell survival is promoted by IL-7 and IL-15. IL-7 is mostly important for long-lived memory T cells while IL-15 is required also for the maintenance of highly differentiated and senescent T cells, accumulation of which is associated with mortality in old age. The expression of effector memory T cell and proinflammatory factors were investigated in bone marrow mononuclear cells (BMMC) finding that, with age, IL-7 decreases while IL-15 and proinflammatory cytokines IL-6, TNFα, IFNγ and IL1β increase. Stimulation of peripheral blood mononuclear cells (PBMC) with IFNγ and TNF leads to increased IL-15 expression in myeloid cell types. A correlation was found between ROS levels and expression of IL-15 in myeloid cells. Incubation of stimulated PBMCs with ROS scavengers N-acetylcysteine and vitamin C completely neutralized the effects of proinflammatory molecules. Similar results could be obtained in Superoxide dismutase 1 (SOD 1) knockout mice, which show increased ROS levels in the bone marrow compared to WT controls. This results indicate that inflammageing and oxidative stress contribute to age-related impairments in the production of survival molecules important for the maintenance of immunological memory in the bone marrow. Antioxidant treatment may be a valid strategy to counteract immunosenescence by reducing the level of proinflammatory cytokines in old age.

Keywords: bone marrow, immunological memory, inflammageing, oxidative stress

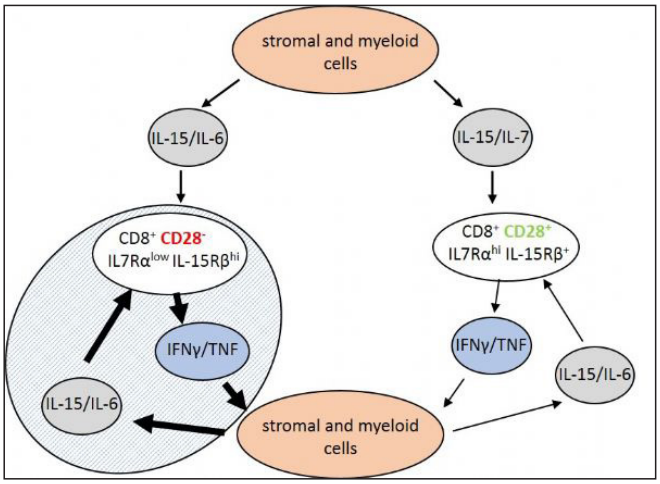

Figure 1. Pangrazzi
Working hypothesis of our study: il-15 and IL-6 produced by stromal and myeloid cells promote the maintenance of CD28- T cells which contribute to inflammageing in the bone marrow.

Ageing

OP-03

AGE DEPENDENT EFFECT ON POST-STROKE INFECTION

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Bacterial infection (e.g., pneumonia) is a leading cause of death among stroke patients, with elderly patients often presenting greater neurological impairment and more debilitating outcomes after stroke. While age is a known risk factor for post-stroke infection, the underlying mechanism for exacerbated infection in the elderly remains unclear. Therefore, we evaluated the effect of age on post-stroke infection rates, with an overall focus on immunological changes at the site of bacterial infection in young (8-10 weeks) and aged (12 months) C57Bl/6 male mice following ischemic stroke or sham-surgery. Despite similar brain infarct sizes after stroke, aged mice showed enhanced neurological impairment compared to young cohorts. Subsequent analysis of lung homogenates in aged mice demonstrated a 100-fold increase of culturable bacteria, suggesting a stroke-induced impairment of antimicrobial defence. Further characterisation of immunological changes at the site of infection showed increased neutrophilic infiltration into lungs in aged mice compared to young cohorts post-stroke, as measured by myeloperoxidase (MPO) levels and confirmed by flow cytometry. This effect correlated with increased expression of CXCL1 and CXCL2, chemokines known for its potent neutrophil recruitment activity. Additionally, lungs of aged mice displayed higher production of pro-inflammatory cytokines IL-1β and TGF-β than young mice after ischemic stroke, whereby these cytokines have the potential to induce tissue damage and fibrosis. Our preliminary results demonstrate the inability of aged mice to resolve post-stroke bacterial infection despite excessive activation of innate immunity. We provide evidence that the risk of bacterial infection after stroke is age dependent, and that onset is contributed by impaired innate immune response at the site of infection.

Keywords: stroke, age, infection

Ageing

OP-04

SENESCENT ENDOTHELIUM MAINTAINS THE ANTI-INFLAMMATORY VASCULAR PHENOTYPE: IDENTIFICATION OF A VASCULAR PROTECTIVE GENE

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One of the major roles of endothelial cells is to maintain the anti-inflammatory, impermeable barrier of blood vessels. The ageing process is epidemiologically and causally linked to ongoing inflammation suggesting a dysfunction of aged endothelium.

We have identified a novel senescent phenotype in endothelial cells, an anti-inflammatory senescent cell as defined by their resistance to inflammatory stimuli. These cells do not express adhesion molecules, nor secrete inflammatory cytokines, they fail to support leucocyte adhesion and they form an impermeable barrier. The mechanism of induction of the anti-inflammatory senescent endothelium is mediated through up-regulation of caveolae and suppression of NFkB. The anti-inflammatory senescent phenotype is induced by 3 of the major stresses of age: hypoxia, disturbed flow and oxidative stress as well as by over-expression of the gene, ARHGAP18 (SENEX). ARHGAP18 is a flow responsive gene, and its depletion results in a failure of the endothelium to respond to the protective mechano-transduction mediated through laminar flow and ARHGAP18 knockout mice are rendered susceptible to atherosclerosis and show enhanced tumour growth with increased angiogenesis. Thus, ARHGAP18 is a vascular protective gene acting to maintain barrier integrity and limit inflammation.

Keywords: senescence, endothelium, inflammation, ARHGAP18

Ageing

OP-05

OBESITY, AGEING AND SARCOPENIA: 'RESISTIN-ANCE' IS FUTILE

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Sarcopenia – the age-related loss of muscle mass and quality – is associated with an increased risk of cardiovascular, metabolic and musculoskeletal diseases and thus has profound personal and societal costs for our ageing population. Ageing is also associated with an accumulation of adipose tissue, which is a potent source of pro-inflammatory cytokines (adipokines). Such inflammation is now considered to be one cause of sarcopenia. However, the principal adipokines contributing to sarcopenia and their myogenic and metabolic actions in skeletal muscle are poorly understood.

To address this, we generated conditioned medium (ACM) from the subcutaneous adipose tissue of normal weight (NW, n = 13), overweight (OW, n = 17) and obese (OB, n = 15) older (> 60 yr) human subjects and examined the effect of these ACM on the myogenesis of both young (18-30 yr, n = 3) and old (>60 yr, n = 3) primary human myoblasts. Confluent myoblasts were stimulated with ACM for 8d. OB ACM significantly reduced the thickness of the resulting myotubes (MTT) compared to NW ACM (young 18 ± 4%, $p = 0.02$; old 28 ± 4% $p < 0.0001$). We then quantified 20 known adipokines in the ACM by Luminex and ELISA. The concentrations of resistin and visfatin were significantly greater in OW and OB ACM (resistin 1.8 ± 0.2 ng/mL, $p = 0.04$; visfatin 1.3 ± 0.2 ng/mL, $p = 0.007$), compared to NW ACM (resistin 1.2 ± 0.1 ng/mL visfatin 2.1 ± 0.2 ng/mL).

Confluent myoblasts were differentiated in the presence of 5 ng/mL recombinant resistin or visfatin. Visfatin did not alter MTT or nuclear fusion index (NFI). However, resistin reduced MTT in young and old myotubes (19 ± 5 %, n = 3 in both groups; $p = 0.02$ and 0.004 respectively). NFI was reduced in old myotubes only (31 ± 7%, $p = 0.0003$).

Having established the importance of resistin in primary human myogenesis we characterised its effect on myotube oxidative metabolism. We observed a ~2-fold increase in lipid accumulation in resistin-stimulated young and old myotubes

by Oil Red O staining. Mitochondrial membrane potential (MitoTracker® Orange CM-H2TMRos staining intensity) was decreased in 8 d resistin (5 ng/mL) stimulated myotubes compared to unstimulated controls (10 ± 3%, p=0.055). A mitochondrial stress test (Seahorse XFe96 Analyser) confirmed a 2-fold increase in proton leak (p = 0.055) with such resistin stimulation of myotubes, as well as increases in basal respiration (1.5-fold, p < 0.01) and ATP production (2-fold, p < 0.001).

We have identified resistin as an important adipokine, that is differentially secreted by lean and obese human adipose tissue and which alters myogenesis and myofibre metabolism.

Keywords: Ageing, sarcopenia, adipokine, resistin, myotube, skeletal muscle

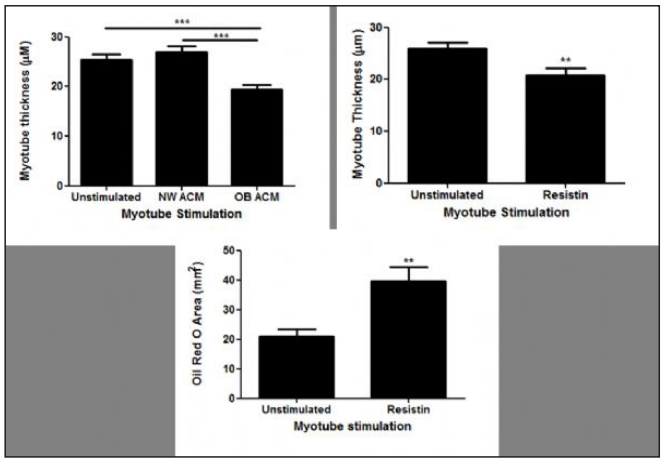

Figure 1. Obese adipose conditioned medium and resistin inhibit in vitro human myogenesis

Subconfluent myoblasts from elderly subjects were switched to differentiation medium containing adipose conditioned medium (ACM) or recombinant resistin (5 ng/mL) for 8 d. The resulting myotubes were fixed, then immunofluorescence stained for desmin and DAPI or stained with Oil Red O and imaged on an epifluorescence microscope. Myotube thickness data represents the mean ± SEM of 450 total measurements taken at 63x magnification from 90 myotubes per treatment condition (comprising 3 biological replicates). Oil Red O Area data represents the mean ± SEM of 45 fields of view per treatment condition (comprising 3 biological replicates). **A)** ACM from obese subjects (OB ACM) reduces myotube thickness compared to ACM from normal weight subjects (NW ACM). **B)** Resistin reduces myotube thickness. **C)** Resistin promotes myotube lipid accumulation. ** = p < 0.001 vs. unstimulated condition *** = p < 0.0001

Ageing

OP-06

SURVIVAL OF EXHAUSTED AND HIGHLY DIFFERENTIATED CD8+ T CELLS IN THE HUMAN BONE MARROW AND THE EFFECTS OF AGING AND CYTOMEGALOVIRUS

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Many antigen experienced immune cells migrate back to the bone marrow, where they can survive in bone marrow niches for extended periods of time. Understanding more about the bone marrow environment, and what allows and supports this long term survival is important for the development of sucessful long lasting vaccinations. IL-7 and IL-15 are survival factors produced by BM stromal cells and myeloid cell types, which are important for the maintenance of memory T cells. Bone Marrow Mononuclear cells (BMMC's) were isolated from human bone marrow samples using collagenase digestion and density gradient centrifugation. Surface and senescence phenotypes were characterised using flow cytometry. Cells were stimulated for 4 hours with PMA Ionomycin for the Intracellular staining of pro-inflammatory and anti-inflammatory cytokine production. Classical markers for exhausted cells such as PD-1, CTLA-4 and CD244 (2B4), and markers for highly differentiated cells such as CD28-, CD57 and KLRG-1 were considered. Exhausted and highly differentiated CD8+ T cells were seen to accumulate in the BM, particularly in old age, and their expression is also influenced by CMV.

Increased IL-15 expression promotes the survival of highly differentiated CD8+CD28- T cells, the presence of which has been associated with reduced efficacy of vaccinations and increased mortality in old age. These highly differentiated cells secrete pro-inflammatory cytokines, and therefore the increased levels of IL-15 which are found in the BM, may indirectly contribute to the inflammatory environment observed in the BM of elderly persons. Cytomegalovirus (CMV) has been considered one of the most important propagators of immunosenescence, with 60 to 100% of adults infected by the virus, depending on geographical location. CMV infection leads to a very prominent T cell response, which occupies >20% of the total CD8+ T cell pool. It has been suggested that CMV driven memory T cell expansions significantly accelerate the age associated loss of naïve T cells and the accumulation of senescent T cells, decreasing de novo immune responses. In this study populations of exhausted and highly differentiated/senescent CD8+ T cells in the BM and the peripheral blood were compared, taking into consideration the impact of ageing, senescence, and Cytomegalovirus (CMV).

The BM environment may promote the long-term survival of exhausted and highly differentiated CD8+ T cell subsets, while simultaneously having a negative impact on "healthy" memory T cell maintenance in old age.

Keywords: Aging, Ageing, Cytomegalovirus, Bone marrow, immunosenescence, senescence

Immune response

OP-07

EFFECTOR CD4⁺ T CELLS PROMOTE GLOMERULAR INFLAMMATION VIA RECOGNITION OF INTRAVASCULAR ANTIGEN PRESENTED BY PATROLLING MONOCYTES

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Effector CD4⁺ T cells are well-recognised for their capacity to respond to antigen and induce antigen-specific inflammation

after they exit the vasculature. However in some diseases, such as T cell-mediated glomerulonephritis, the disease-initiating antigen is often located within the vasculature. The mechanisms of $CD4^+$ T cell antigen recognition under these circumstances are not well understood. Here we examined this issue using intravital multiphoton microscopy of mouse kidneys. Direct visualisation of glomeruli revealed that effector $CD4^+$ T cells undergo spontaneous intravascular retention and migration in uninflamed glomeruli. While, under resting conditions, MHCII was not expressed by intrinsic glomerular cells, intravascular MHCII-expressing immune cells extensively patrolled glomerular capillaries, regularly interacting with intraglomerular $CD4^+$ T cells. Following targeted deposition of cognate antigen in glomeruli, $CD4^+$ T cells trafficking through glomeruli displayed responses consistent with antigen recognition, including increased recruitment, decreased migration and elevated expression of CD69 and IFNγ. Of the $MHCII^+$ leukocytes present in the circulation, both B cells and monocytes underwent retention in glomerular capillaries, although $MHCII^+$ monocytes were typically retained in the glomerulus for a much longer duration, providing them greater opportunity to encounter intraglomerular $CD4^+$ T cells. Furthermore, while the absence of B cells did not affect $CD4^+$ T cell-dependent glomerular inflammation, monocyte depletion attenuated this response. These studies provide evidence that antigen within the glomerular microvasculature is presented to intravascular effector $CD4^+$ T cells by MHCII-expressing monocytes patrolling glomerular capillaries, and that this can lead to initiation of antigen-dependent glomerular inflammation.

Keywords: leukocyte trafficking, in vivo imaging, T cell, dendritic cell, glomerulonephritis

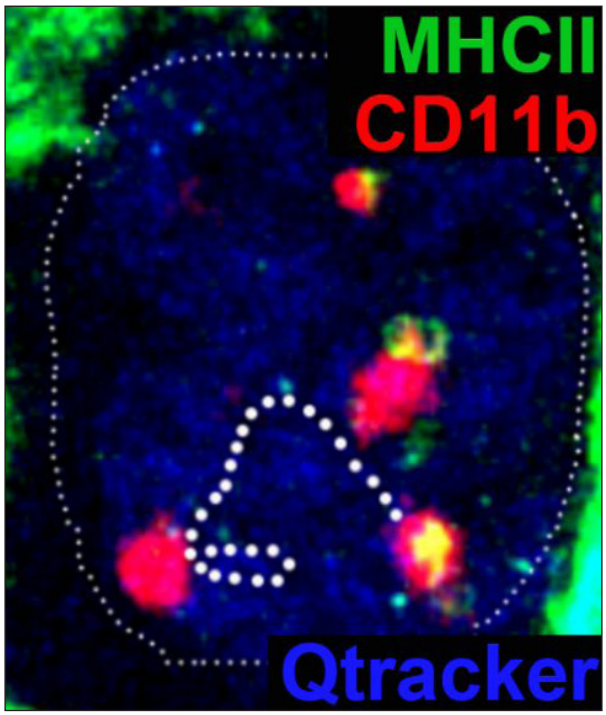

Figure 1. MHCII-expressing monocytes undergo prolonged retention in glomerular capillaries

Multiphoton microscopy image showing the migration path of an MHCII+ (eGFP/green) CD11b+ (red) leukocyte (yellow cell) in the glomerulus. Dotted line shows the cell migration path during a 30 minute observation period.

Immune response

OP-08

EOSINOPHILS HAVE AN ESSENTIAL ROLE IN CARDIAC REPAIR FOLLOWING MYOCARDIAL INFARCTION

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Background: Low peripheral blood eosinophil count is associated with increased risk of mortality in ischaemic heart disease patients. Eosinophils contain preformed IL-4 within their cytoplasmic granules, which promotes an anti-inflammatory response and is associated with tissue repair. Whether eosinophils are recruited to the infarct zone and have a role in regulating infarct repair is currently unknown.

Purpose: This study sought to investigate the role of eosinophils in infarct healing.

Methods: MI was induced by permanent coronary artery ligation in 12-15 week-old male wild-type (WT) BALB/c and eosinophil deficient ΔdblGATA mice. Cardiac function was assessed 7 days later by high-resolution ultrasound. A cohort of 732 patients undergoing emergency percutaneous coronary intervention for ST-elevation myocardial infarction (MI) were followed up for 6 month all-cause mortality.

Results: Histochemical staining ($Siglec\ F^+$) and single cell digestion of infarcted WT BALB/c hearts revealed significant recruitment of ($CD11b^+SiglecF^+Ly6G^{int}$) eosinophils to the infarcted heart. Expression of CD206, a marker for alternatively activated macrophages, was reduced on infarct zone macrophages from eosinophil-deficient ΔdblGATA mice, but was restored by intra-peritoneal eosinophil replenishment. Furthermore, eosinophil deficiency in ΔdblGATA mice led to adverse post-MI cardiac remodelling with greater left ventricular dilatation relative to WT BALB/c mice and worse cardiac function at Day 7 post-MI. Patients with a low eosinophil count at Day 1 following ST-elevation MI had an increased risk of 6 month all-cause mortality (Figure 1). On multivariate analysis the hazard ratio of all-cause mortality in the first tertile of peripheral blood eosinophil count at Day 1 post-MI was 6.97 [2.18 – 22.32] compared to the highest tertile ($p=0.001$). Treatment with IL-4 complexes was able to rescue the adverse cardiac remodelling of eosinophil-deficient ΔdblGATA mice. Expression of plod2, lox and Fmod genes, which are involved in post-translational collagen modification, were increased in hearts from ΔdblGATA mice. This was accompanied with longer infarct length in ΔdblGATA mice compared to WT BALB/c mice ($p=0.01$).

Conclusions: This study provides the first evidence for recruitment of eosinophils to the heart following MI and shows that they are required for alternative activation of infarct zone macrophages and prevention of adverse cardiac remodelling. Eosinophils have an essential role in the tissue repair response following MI, through regulating genes associated with connective tissue biogenesis. Patients with a low eosinophil post-MI may benefit from IL-4 therapy to improve outcome.

Keywords: Eosinophils, myocardial infarction, cardiac remodelling

Low peripheral blood eosinophil count is associated with adverse outcome following ST-elevation MI

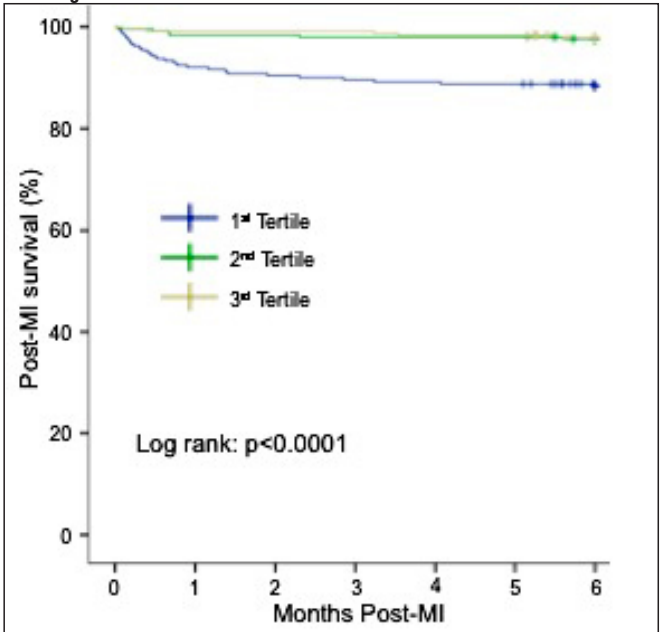

Figure 1: Kaplan-Meier curves for the cumulative probability of all-cause mortality between tertiles of Day 1 post-MI eosinophil count. Eosinophil count: 1st Tertile [≤0.03 x10^9 cells/L]; 2nd Tertile [0.04-0.11 x10^9 cells/L]; 3rd Tertile [≥0.12 x10^9 cells/L].

Immune response

OP-09

CHLAMYDIA TRACHOMATIS ASSOCIATED IMMUNE RESPONSES, INFLAMMATION AND TISSUE PATHOLOGY IS REGULATED BY EFFECTOR CELL SPECIFIC MICRORNAS

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In humans, *Chlamydia trachomatis* (Ct) infection may lead to inflammation associated sequelae including fibrosis and tissue scarring of infected mucosal surfaces. While the role of host molecular regulators namely microRNAs (miRs) is not well examined, the contribution of downstream immune pathways/genes leading to tissue exacerbation and tissue remodeling is known. With growing consensus on the causal link between miRs and immunity, in this study, we determined signatures of host miRs and the mechanistic contribution of selected miRs in immune responses and subsequent development of pathology in *Chlamydia muridarum* (Cm) infection. C57BL/6 wild type (WT) were infected with Cm and cellular infiltrates, miRs and putative targets and tissue pathology was analyzed. *In vivo* and *ex vivo* experiments using miR agonists and antagonists for gain and loss of function respectively were performed. Down-selected miRs were validated in cohorts of Ct infected women with reproductive sequelae including infertility. Our results indicate that Cm infection in WT mice significantly regulated selected miRs. We observed significant upregulation of miR-155 in WT bone marrow derived dendritic cells (DC), and miR-182 in splenic Ag-specific CD4$^+$ T-cells. Using mimics and inhibitors, we determined that miR-155 contributed to DC activation. Co-cultures of miR-155 over-expressed in DC and miR-182 over-expressed in Ag-specific CD4$^+$ T-cells, or miR-155$^{-/-}$ DC with miR-182 inhibitor treated Ag-specific CD4$^+$ T-cells, resulted in IFN-γ production comparable to that of Ag-specific CD4$^+$ T-cells isolated from Cm infected mice. MiR-182 was significantly up-regulated in intranasally vaccinated mice protected against Cm infection. *In vivo* depletion of miR-182 resulted in reduction in Ag-specific IFN-γ and associated tissue damage in Cm infected mice. Importantly, significant regulation of miRs in Ct D infected women was indicative of the translational relevance. To the best of our knowledge, this is the first study to report an interaction of miR-155 and -182 resulting in Ag specific immune responses against an intracellular pathogen.

Keywords: Immune Regulation, MicroRNAs, Chlamydia trachomatis, Protective Immunity, Pathogenesis

Immune response

OP-10

INVESTIGATING THE ROLE OF CHEMERIN AND THE NON-SIGNALLING CHEMERIN RECEPTOR, CCRL2, DURING AN ACUTE INFLAMMATORY MODEL OF PERITONITIS

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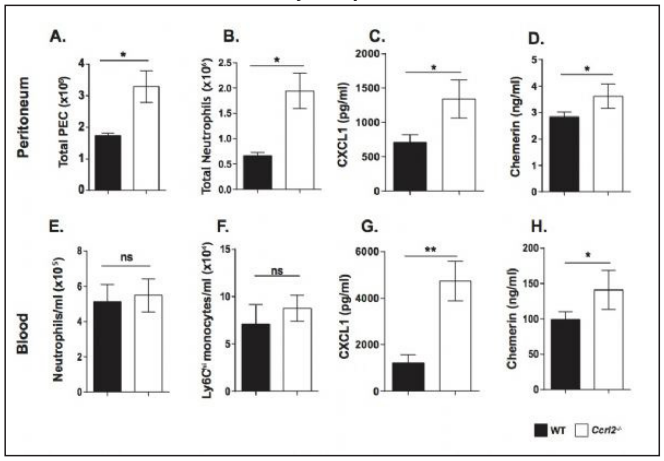
Chemerin is a chemotactic protein that can induce migration of a number of leukocytes including macrophages, immature dendritic cells and NK cells. Chemerin binds to three G protein-coupled receptors (GPCRs), these being: CMKLR1, GPR1 and CCRL2. The exact function of CCRL2 remains unclear, it's rapidly upregulated during inflammation, but it lacks the intracellular DRYLAIV motif required for GPCR downstream signalling. The aim of this study was to investigate what role if any CCRL2 plays during acute inflammation. Using murine models of acute inflammation, we report that mice lacking the *Ccrl2* gene resulted in exaggerated local and systemic inflammatory responses, characterised by increased myeloid cell recruitment. This exaggerated myeloid cell recruitment was associated with increased chemerin and CXCL1 levels. Furthermore, we see that the inflammatory phenotype observed in these mice is dependent on the elevated levels of endogenous chemerin. Antibody neutralisation of chemerin activity in *Ccrl2*-/- mice abrogated the amplified inflammatory responses and administration of recombinant chemerin to WT mice recapitulated the increased myeloid cell recruitment and inflammatory mediator production observed in *Ccrl2*-/- mice. However, chemerin did not directly recruit neutrophils and monocytes but instead we hypothesise that it increases the production of other chemotactic proteins such as CXCL1. Our data is consistent with a model in which CCRL2 maintains free chemerin levels below a pathological threshold

during acute inflammation and further highlights chemerin as a therapeutic target in inflammatory diseases.

Keywords: CCRL2, chemerin, CXCL1

Increased neutrophil recruitment in Ccrl2-/- mice is associated with increased CXCL1 and chemerin levels at early time points.



8-10 week old male Ccrl2-/- or littermate control mice were injected with zymosan i.p. (100 μg/animal) and 2 hours later, animals were sacrificed. Peritoneal cavities were lavaged with ice cold PBS supplemented with 2 mM EDTA. Cells were quantified using counting beads and cell populations were analysed using flow cytometry. (A-B) Total peritoneal cell counts following 2 hour zymosan challenge. (C) CXCL1 and (D) chemerin levels in the peritoneum of Ccrl2-/- and WT mice quantified by ELISA. (E-F) Blood neutrophils and monocytes in WT and Ccrl2-/- mice. (G-H) Plasma levels of CXCL1 and chemerin quantified by ELISA. Data presented as mean ± S.E.M. n=8 animals/group. Statistical significance was assessed using a students' unpaired t-test. * P <= 0.05, **P<=0.01.

Methods: All animal procedures were approved by the Animal Care and Use Committee of our organization. The CBA/J × DBA/2N mating mouse model was employed to analyze RPL. The abortion rate was increased by activating maternal immunity via an intraperitoneal LPS injection, and the preventive effect of IVIg administration on immune reproductive failure was examined. Uterine macrophages and uNK cells were identified as CD45+F40/80+ (macrophages) and CD45+CD14–CD3e–CD49b+ (uNK cells) by flow cytometry or histopathological analysis. Macrophage depletion in the RPL mouse was induced by intravenous injection of dichloromethylene bisphosphonate (clodronate).

Results: The number of CD44^{bright} uNK cells increased from 3 h to 12 h after LPS injection. Morphological change in the fetus was noticed after 18 h. Abortion occurred after 24 h. IVIg was detected in the uterus within an hour after administration. However, IVIg did not affect the number of CD44^{bright} uNK cells until 6 h. IVIg co-localized with macrophages, but not CD44^{bright} uNK cells, in the decidua. The effects of IVIg on both abortion and the CD44^{bright} uNK cell number were annihilated by macrophage depletion in the RPL mouse. Interestingly, macrophage depletion did not affect LPS-induced abortion.

Conclusion: In this study, we observed a time lag in the suppressive effect of IVIg, which did not exist until 6 h after injection. Additionally, IVIg had no effect in macrophage-depleted RPL mice. We suggest that the time lag is due to the fact that IVIg has an indirect effect, via macrophages. On the other hand, macrophages did not affect the abortion in RPL mice. These results revealed that IVIg suppresses abortion via suppressing the increase in CD44^{bright} uNK cells using a pathway unrelated to abortion.

Keywords: Abrtion, IVIg, NK cells, Recurrent Pergnancy Loss, Macrophage

Immune response

OP-11

SUPPRESSIVE EFFECTS OF EARLY-PHASE ADMINISTRATION OF INTRAVENOUS IMMUNOGLOBULIN IN RECURRENT PREGNANCY LOSS MODEL MICE

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Introduction: Women undergoing three or more consecutive spontaneous abortions are believed to suffer from recurrent pregnancy loss (RPL). The triggering mechanism that induces abortion remains unexplained; however, most RPL cases have been explained by autoimmune abnormalities. Intravenous immunoglobulin (IVIg) has been utilized in the treatment of several inflammatory and autoimmune disorders, including RPL. In a previous study, we discovered the presence of two distinct uterine NK (uNK) subsets in the RPL mouse: CD44^{bright} and CD44^{mid}. We observed an increase in the CD44^{bright} uNK subset at abortion in RPL model mice, while the CD44^{mid} uNK subset remained unchanged. Furthermore, the number of CD44^{bright} uNK cells remained unchanged when the abortion rate was reduced by IVIg administration. Unfortunately, the precise mechanism by which IVIg suppresses the increase in CD44^{bright} uNK cells remained unknown. In this study, we evaluated the time course of the IVIg effect on the CD44^{bright} uNK subset in RPL mice.

Immune response

OP-12

PARENCHYMAL NITRIC OXIDE SYNTHASE MEDIATED INJURY AND DYSFUNCTION IN THE ACUTELY INFLAMED EXOCRINE SALIVARY GLANDS. BYSTANDER ROLE OF INVASIVELY INFILTRATING LEUKOCYTES

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Mechanisms of exocrine injury in response to acute inflammation remains unclear. The main aim of this study was to investigate the early, functional and innate immune-mediated responses of salivary glands to a potent inflammagen; polyinosinic:polycytidylic acid (poly(I:C)). Retrograde intraductal cannulation of the non-genetically modified C57/B6 mice enabled studying of exocrine-specific inflammatory responses and ruled out possible extraneous impacts that can arise either from systemic delivery of the inflammagen or autoimmune murine susceptibility.

Functional studies revealed rapid poly (I:C)-induced compromise of the submandibular gland secretory machinery. TLR3 in vivo inhibition is demonstrated for the first time in the salivary glands, and showed the exclusive contribution of this pattern recognition receptor in the perceived loss of function. The first part of the study demonstrated that poly (I:C) induced

a chemokine-mediated, intra-ductal and intra-acinar invasion of acute inflammatory cells, exhibiting myeloperoxidase immunopositivity. Unexpectedly, depletion of this aggressive inflammatory signal using the RB6-8C5 monoclonal antibody did not reverse the submandibular gland secretory dysfunction and implicated an alternative parenchymal signal in deregulating the exocrine secretory machinery.

To verify this assumption, the exclusively glandular, TLR3-induced cytokines were profiled, which revealed the early upregulated expression of inducible nitric oxide synthase (iNOS), side by side the generation of the more potent cytotoxic oxidant peroxynitrite. The following experiments using the selective NOS inhibitor, aminoguanidine were directed toward comprehensively characterizing the sequela of nitrosative injury in the murine salivary glands. Prominently, iNOS dysregulated Ca2+ homeostasis in the parenchymal cells, whereby baseline calcium was increased and carbachol-stimulated calcium release from intracellular stores was markedly diminished. Moreover, the unfolded protein response was rapidly initiated alongside downregulated and aberrant expression of key membranous molecules that are crucial for driving saliva secretion.

Collectively, the novel acute salivary gland injury model based on local infection with poly (I:C), unravels novel dysfunction mechanisms downstream challenging the exocrine innate immunity. In vivo inhibition of TLR3 has never been studied previously in exocrine organs and undoubtedly the functional rescue demonstrated in the salivary glands can be reproduced in similar acutely inflamed secretory epithelia. Furthermore, results of the present study dogmatically disclose the bystander role played by the invasive innate immune cells in acute inflammation of secretory tissues. More importantly, the inducible nitric oxide synthase was demonstrated as one of the earliest parenchymal signals that endogenously interrupt the acini's ability to secrete normally during periods of exocrine acute inflammation.

Keywords: Exocrine, Inflammation, Depletion, Inducible nitric oxide synthase, calcium, unfolded protein response

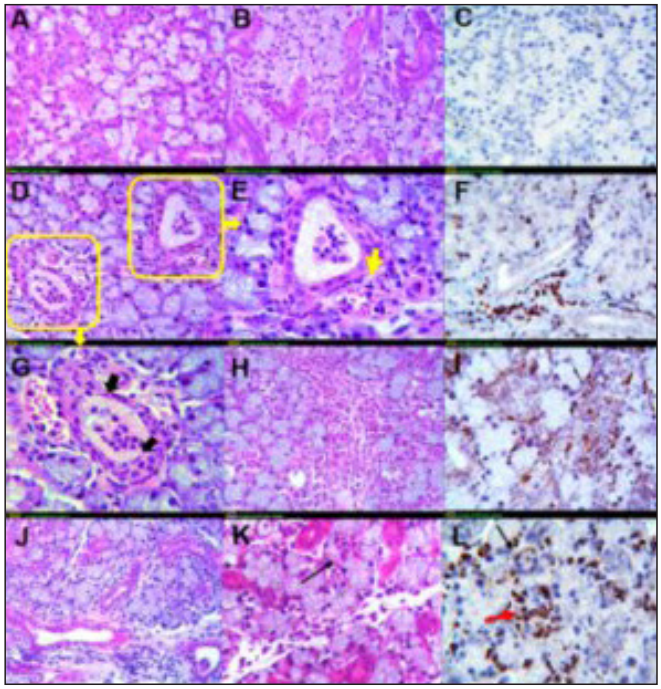

Figure 1. Invasive infiltration of immune cells in the acutely inflamed salivary glands

Immune response

OP-13

ESCHERICHIA COLI TYPE VI SECRETION SYSTEM (T6SS) MODULATES MURINE INFLAMMATION AND INNATE IMMUNE RESPONSE

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Escherichia coli infections remains a public health problem since its higher zoonotic power and the fact that there are still cases with mortality risk in several countries. Secretion systems are described as important cellular apparatus to make bacteria capable of translocate effectors proteins to the host cell. Among all proteins constituent of type six secretion system (T6SS) effectors proteins, ClpV and IcmF are highlighted due to their importance to maintain T6SS functional. However, the role of T6SS in inflammation and innate immune response is still unknown. Therefore, the aim of the present work was to analyze the role of T6SS through ClpV and IcmF in inflammasome activation during innate immune response. Taken together, our results demonstrated that T6SS proteins were crucial to intracellular bacterial replication in macrophages and also played a significant role during modulation of pro inflammatory cytokines release such as IL-12 and IL-6. Presence of ClpV and IcmF interfered in cell redox metabolism and inflammasome activation through caspase-1 cleavage and IL-1 β release. The NLRP3 receptor, as well as caspases 1 and 11, were necessary to increase IL-1β release induced by *E. coli* with a fully functional T6SS. We showed that the presence of caspases 1 and 11 and NLRP3 influenced in lipid antigen presentation, oxygen reactive species generation, nitric oxide and other pro-inflammatory cytokines production in macrophages. The absence of inflammasome components improved bacterial intracellular replication and the colonization of the liver and spleen in mice. We also investigated the role of lipid metabolism during infection through analyses of lipid droplet biogenesis and verified that intracellular bacterial replication can be associated with fatty acid synthase. Therefore, this work characterized the different mechanisms involved in T6SS-mediated innate immune response against a pathogenic strain of *E. coli* and the relevance of inflammasome components in this process.

Keywords: Inflammasome, T6SS, caspases 1 and 11, NLRP3

Immune response

OP-14

PRE-HOSPITAL IMMUNE RESPONSES AND DEVELOPMENT OF MULTIPLE ORGAN DYSFUNCTION SYNDROME FOLLOWING TRAUMATIC INJURY

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Background: Almost all studies that have investigated the immune response to traumatic injury have analysed blood samples acquired from patients post-hospital admission, with first blood draw taking place in the hours or days following injury. Thus, we know little of the immune status of patients prior to hospital admission, and how this might influence patient outcomes. Thus, the objective of this study was to comprehensively assess the immediate, within 1-hour, immune response to trauma and perform exploratory analysis of its relationship with the development of multiple organ dysfunction syndrome (MODS).

Methods and Findings: In collaboration with the West Midlands Air Ambulance Service, the immune and inflammatory response to trauma was analysed in 89 adult trauma patients (mean age 41 years, range 18-90, 75 males) with a mean injury severity score of 24 (range 9-66), from whom blood samples were acquired in the pre-hospital setting within 1-hour of injury (mean time to blood sample 42 minutes, range 17-60 minutes). Within minutes of trauma, a comprehensive leukocytosis, driven by elevated absolute numbers of T cells, B cells, natural killer (NK) cells, NKT cells, monocytes, neutrophils and immature granulocytes was observed. Accompanying this immediate leukocytosis were raised serum pro (e.g. interleukin (IL)-6, IL-8, tumour necrosis factor-alpha) and anti (e.g. IL-10)-inflammatory cytokines and evidence of innate cell activation, which included neutrophil extracellular trap generation and elevated surface expression of toll-like receptor 2 on monocytes and CD11b on neutrophils. Alongside these aspects of immune activation, features consistent with immune compromise were also detected within 1-hour of injury. These included elevated numbers of immune suppressive $CD16^{BRIGHT}$ $CD62L^{DIM}$ neutrophils (82.07 $x10^6$/l ±18.94 healthy controls vs 1092 x 10^6/l ±165 trauma patients, $p<0.0005$) and $CD14^+HLA\text{-}DR^{low/-}$ monocytes (34.96 $x10^6$/l ± 4.48 healthy controls vs 95.72 x 10^6/l ± 8.0 trauma patients, $p<0.05$) and reduced leukocyte pro-inflammatory cytokine secretion in response to lipopolysaccharide stimulation. Exploratory analysis via binary logistic regression found an association between absolute NKT cell numbers within 1-hour of injury and the subsequent development of MODS.

Conclusions: This study has highlighted the dynamic and complex nature of the immune response to trauma, with immune alterations consistent with both activation and suppression evident within 1-hour of injury. The relationship between the ultra-early immune response to trauma, especially NKT cell numbers, and patient outcomes such as MODS warrants further investigation.

Keywords: Innate immunity, Immune activation, Immune suppression, Trauma, Pre-hospital research

Immune response

OP-15

ELEVATED CD31 LEVELS ON 1,25-DIHYDROXYVITAMIN D3 TREATED $CD11C^+$ DENDRITIC CELLS RESTRAIN $CD4^+$ T CELL PRIMING ABILITY

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Dendritic cells are professional antigen presenting cells that initiate and refine adaptive immune responses, and are promising therapeutic targets for autoimmune and chronic inflammatory diseases. Vitamin D, a known epidemiological risk factor for the development of several immune-mediated diseases such as multiple sclerosis and Crohn's disease, influences the development of dendritic cells. However, the mechanism by which vitamin D modulates dendritic cell function is not fully understood.

Here, we demonstrate that generation of CD11c+ bone marrow derived dendritic cells (BMDCs) in the presence of the active vitamin D metabolite, 1,25-dihydroxyvitamin D3 (VitD), drives CD31 expression which acts as a co-inhibitory molecule and dampens the CD4+ T cell priming potential of BMDCs. Analysis of global gene expression by microarray revealed that the expression of six genes was increased in VitD CD11c+BMDCs both prior to and following stimulation with lipopolysaccharides (LPS) when compared to their vehicle-treated counterparts (Veh CD11c+BMDCs). Among these was the platelet endothelial cell adhesion molecule 1 (PECAM-1) also known as CD31, a member of the immunoglobulin superfamily emerging as an immunomodulatory molecule on several immune cell subsets. The generation of CD11c+BMDCs in the presence of 20nM VitD resulted in increased CD31 mRNA as well as protein levels relative to Veh CD11c+BMDCs. Importantly, this effect required VitD treatment at the early stages of BMDC generation; treatment of BMDC with VitD during stimulation with LPS following generation did not affect CD31 expression.

VitD CD11c+BMDCs displayed a less mature phenotype post-stimulation with LPS than their vehicle treated counterparts, and did not prime responder CD4+ T cells as effectively as vehicle treated BMDCs. Notably, lentiviral siRNA knockdown of CD31 in VitD CD11c+BMDCs partially restored their ability to prime CD4+ T cells *in vitro*, resulting in enhanced proliferation (indirectly measured via IL-2 in the cell culture supernatant) and increased pro-inflammatory cytokine production (GM-CSF, IFN-γ, TNF-α) by CD4+ T cells. Likewise, lentivirus-driven overexpression of CD31 in Veh CD11c+BMDCs led to a significant inhibition of

CD4+ T cell priming. This was associated with a lower proportion of responder CD4+ T cells expressing the activation marker CD44 and the proliferation marker Ki-67, as well as reduced production of pro-inflammatory cytokines (GM-CSF, IFN-γ, TNF-α). Lastly, flow cytometric analysis of human mobilised blood-derived CD11c+ dendritic cells generated in the presence or absence of VitD revealed increased CD31 expression levels in VitD treated cells compared to Veh controls. This indicates that VitD-induced upregulation of CD31 is preserved between mice and humans.

In summary, our study demonstrates that VitD induces CD31 expression in CD11c+BMDCs which restrains their ability to prime CD4+ T cells.

Keywords: Dendritic cells, Vitamin D, CD31, T cell priming

Immune response

OP-16

NKTR-358: A SELECTIVE, FIRST-IN-CLASS IL-2 PATHWAY AGONIST WHICH INCREASES THE NUMBER AND SUPPRESSIVE FUNCTION OF REGULATORY T CELLS FOR THE TREATMENT OF IMMUNE INFLAMMATORY DISORDERS

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Impaired IL-2 production and regulatory T cell (Treg) dysfunctions have been identified as key immunological defects leading to the breakdown of self-tolerance, a causative mechanism implicated in autoimmune diseases. Due to IL-2 receptor biology differences, Treg demonstrate greater sensitivity to IL-2 relative to conventional T cells (Tcon), providing a therapeutic window in which low-dose IL-2 can be used to stimulate Tregs for clinical benefit. However, poor pharmacokinetics of IL-2 necessitates daily delivery, adverse events are dose-limiting, and Treg increases are modest and short-lived. Nektar Therapeutics is developing NKTR-358, a novel product which utilizes the aldesleukin (Proleukin®) amino acid sequence chemically conjugated with stable polyethylene glycol (PEG) moieties, intended for low dose subcutaneous administration to selectively restore Treg homeostasis with no impact on Tcon function. NKTR-358 has greatly attenuated affinity for human IL-2Rβ relative to IL-2Rα and IL-2Rαβ complexes, suggesting biological engagement favors activation of Treg which express the high affinity IL-2Rαβγ over Tcon, which express the low-affinity IL-2Rβγ. In vitro and in vivo studies support this finding. NKTR-358 treatment of cynomolgus and human peripheral blood mononuclear cells or human whole blood demonstrated that Treg were far more sensitive to NKTR-358 relative to all other lymphocyte subsets. This preferential activity combined with prolonged exposure in vivo led to significant Treg mobilization in blood and spleen following a single subcutaneous administration in mice. Increases in Treg were sustained for 7 to 10 days and were concomitant with increases in cytometric markers of activation and increased suppressive capacity in an ex vivo functional assay. In cynomolgous monkey, a single administration led to increased Treg mobilization and activity which were sustained for over 14 days, a response superior in magnitude, duration, and specificity compared to an equivalent total dose of rhIL-2 administered daily for five days. In a mouse model of cutaneous hypersensitivity, NKTR-358 administration suppressed the inflammatory response to antigenic rechallenge of keyhole limpet hemocyanin, an effect which was antigen-specific and associated with establishment of Treg memory. Similar results were achieved in cynomolgus monkey using tetanus toxoid. Finally, NKTR-358 was efficacious in the mouse MRL/MpJ-Faslpr model of systemic lupus erythematosus (SLE), as repeat administration over 12 weeks sustained Treg elevation, significantly reduced blood urea nitrogen, and returned urine protein levels and kidney histopathology to normal. Currently, NKTR-358 is being studied in a Phase 1 study in healthy subjects to measure Treg mobilization, functional activity, pharmacokinetics and safety, with the goal of establishing a range of dose levels to be advanced into a multiple-ascending dose trial in patients with SLE planned for the second half of 2017.

Keywords: Treg, lupus, IL-2, SLE, PEG, autoimmune

Immune response

OP-17

PGE_2 PROTECTS AGAINST INTESTINAL BARRIER DAMAGE AND SYSTEMIC INFLAMMATION THROUGH PROMOTING ILC3 RESPONSE

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Systemic inflammation, resulted from the massive release of pro-inflammatory molecules into the circulatory system, is a major risk factor for severe illness, but the precise mechanisms underlying its control are not fully understood. Prostaglandins are bioactive lipid mediators that are generated from arachidonic acid by cyclooxygenases in response to various noxious stimuli such as infection and play critical pathological roles in development of inflammatory diseases. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat acute inflammation (e.g., pain/fever) by inhibiting synthesis of prostaglandins, but NSAIDs have also severe adverse effects (e.g., the best-known gastrointestinal bleeding). Recent clinical observations have also suggested that use of NSAID during evolving bacterial infection is associated with more critical illness. We have observed that the prostaglandin E_2 (PGE_2) signalling pathway, through its receptor EP4, is down-regulated in human systemic inflammatory disease. Mice with reduced PGE_2 synthesis develop augmented systemic inflammatory responses. This was associated with disruption of gut barrier gene expression and translocation of gut bacteria, which can be prevented by treatment with EP4 agonists or antibiotic therapies. Mechanistically, we have demonstrated that PGE_2-EP4 signaling acts directly on type 3 innate lymphoid cells (ILC3s), promoting their maintenance and driving them to produce IL-22 in intestines under homeostatic conditions. While exogenous IL-22 protected against intestinal barrier damage and inflammation, disruption of the ILC–IL-22 axis impairs PGE_2-mediated inhibition of systemic inflammation. Hence, the ILC–IL-22 axis is essential in protecting against gut barrier dysfunction, enabling PGE_2-EP4 signaling to impede systemic inflammation. (Ref: Duffin, et al (2016) Science 351,1333-1338)

Keywords: Systemic inflammation, Group 3 Innate lymphoid cells (ILC3s), IL-22, gut barrier damage, Prostaglandin E2

Immune response

OP-18

NON-CLASSICAL MONOCYTES ORCHESTRATE INFLAMMATORY MYELOID RETENTION TO MEDIATE TISSUE DAMAGE IN IMMUNE-COMPLEX-MEDIATED GLOMERULONEPHRITIS

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Objectives: Recent elegant work using a mouse model of glomerulonephritis (GN) suggests non-classical monocytes patrol the capillaries of glomeruli and mediate an inflammatory response through prolonged interactions with neutrophils during GN. However, this model does not produce histopathological features of glomerular inflammation that resemble human disease. Therefore, we used intravital confocal microscopy in a rat model of severe crescentic GN (nephrotoxic nephritis, NTN) to directly visualise the dynamic changes that occur in monocyte subset behaviour and leukocyte-vascular interactions during glomerular inflammation in vivo. NTN in rats is more reproducible and histopathologically translatable to human GN than the mouse model.

Methods: Intravital imaging was performed using confocal microscopy in a new transgenic rat, hCD68-GFP WKY reporter rats to enable tracking of GFPpos monocytes within glomerular capillaries. Myeloid subpopulations (classical monocytes, non-classical monocytes and neutrophils) were identified in rats using FACS and molecular phenotyping. Non-classical (LinnegCD68posCD43highCX3CR1highCCR2low) and classical monocytes (LinnegCD68posCD43lowCX3CR1lowCCR2high) were labelled in-vivo and neutrophils identified as LinnegCD68negCD43highCXCR2high cells. These myeloid subpopulations were tracked in real-time and their behaviour and function analysed in situ during disease.

Results: Rat myeloid subpopulations are homologous to human cells, which display both a murine and human phenotype. Their blood frequency and distribution change during NTN. Within the glomeruli, during NTN, there was increased recruitment of non-classical monocytes but their dwell time did not change significantly. Conversely, neutrophils and classical monocytes underwent increased retention, with marked increases in their dwell times, but without increased recruitment. No transendothelial migration of any myeloid population was noticeable during disease. Non-classical monocytes displayed a distinct migratory behaviour, scanning glomerular capillaries for prolonged periods of time, even in the absence of inflammation. Neutrophils underwent stationary retention at the endothelial interface during NTN, illustrated by a significant fall in their confinement ratio. Further work has identified the subset specific cytokine response to immobilised immune complexes in-vitro and human immunohistochemistry has confirmed the spatial distribution of myeloid subsets during human crescentic glomerulonephritis.

Conclusion: Using this innovative in vivo imaging technique in a clinically relevant model of GN, our data suggest that non-classical monocytes play a role in orchestrating the inflammatory response to immune complex deposition within glomerular capillaries and may drive changes in inflammatory myeloid behavior that mediates glomerular damage. We define the spatial and temporal pattern of myeloid recruitment and retention during early and late crescentic GN.

Keywords: Monocytes, Neutrophils, Immune Complexes, Vascular Inflammation, Glomerulonephritis

Signalling molecules and pathways

OP-19

MUTUALLY COUNTERACTING EFFECTS OF CERS2 AND CERS6 IN G-CSF SIGNALING

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Multiple sclerosis (MS) is a neurodegenerative autoimmune disease characterized by infiltration of immune cells into the central nervous system. Infiltrating immune cells produce chemokines to recruit further immune cells and release inflammatory cytokines and cytotoxic substances, resulting in the death of oligodendrocytes and subsequently leading to demyelination. In experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, we observed that the genetic deletion of ceramide synthase 2 (CerS2) and ceramide synthase 6 (CerS6), respectively, suppresses and promotes EAE pathology. Ceramide synthases synthesize ceramides of defined acyl chain lengths, which act as second messengers in cell signaling. Whereas CerS2 produces primarily very long chain C24-ceramides, CerS6 forms long chain C16-ceramides. We observed that the effect on EAE pathology was due to a ceramide-dependent regulation of neutrophil migration. This migration was controlled by granulocyte colony stimulating factor (G-CSF) induced expression of C-X-C motif chemokine receptor 2 (CXCR2).

In general, binding of G-CSF to its receptor G-CSF-R leads to phosphorylation and thereby activation of Lyn kinase. This results in activation of multiple intracellular signaling proteins, including the signal transducers and activators of transcription 3 (STAT3), which results in the expression of genes such as CXCR2.

We found that the lack of very long chain ceramides prevents the phosphorylation of Lyn kinase and the CXCR2 expression, but not as expected the phosphorylation of STAT3. In contrast, the lack of long chain ceramides reduces the phosphorylation of STAT3, but unexpectedly increases the CXCR2 expression. To understand these conflicting data, we investigated the translocation behavior of CXCR2 and G-CSF-R into detergent-resistant membranes (DRMs) in wild type, CerS2 and CerS6 null bone marrow cells (BMCs). We observed that both receptors translocated into the DRMs independent of the ceramide status. Since the translocation of the receptors is ceramide-independent, we suppose that altered membrane properties due to the lack of ceramides of specific chain length in CerS2 and Cer6 deficient mice are responsible for the observed effects on G-CSF signaling. Modified G-CSF signaling possibly influences neutrophil migration by altering CXCR2 expression and thereby, the clinical outcome of EAE pathology.

Keywords: ceramides, experimental autoimmune encephalomyelitis, G-CSF signaling, detergent-resistant membranes

Signalling molecules and pathways

OP-20

A PRO-INFLAMMATORY ENVIRONMENT MODULATES THE HUMAN DERMAL FIBROBLAST PHENOTYPE: IMPLICATIONS FOR CHRONIC WOUNDS

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During wound healing the dermal fibroblast plays a pivotal role by providing a scaffold for tissue regeneration and re-epithelialization. In chronically inflamed non-healing wounds, an excessive pro-inflammatory microenvironment resulting in a lengthened inflammatory phase can lead to an imbalance between the production and degradation of the extracellular matrix (ECM). Over expression of pro-inflammatory cytokines may significantly alter the dermal fibroblast phenotype and impair activation, migration, proliferation and function, leading to delayed healing.

Hypothesis: The dermal fibroblast acts as a gatekeeper of the inflammatory response in human skin during wound healing - loss of function can lead to chronic non-healing wounds. Therefore, the aim of this study was to identify any detrimental changes in the functional properties and the secretory phenotype of primary cultures of dermal fibroblasts derived from human skin in response to different concentrations, and length of exposure to inflammatory cytokines such as TNF-α.

Primary cultures of papillary dermal fibroblasts were established from female facial skin (donors aged 52-64yrs). Cells were incubated +/-TNF-α (2.5, 25 or 250ng/ml) to determine its effects on viability, proliferation, migration (in a scratch wound assay), the secretion of active MMP-2 and MMP-9 by zymography and the induction of β-galactosidase as a marker of senescence.

Pre-incubation with all 3 concentrations of TNF-α for 3 days caused a significant reduction in proliferation by day 14 despite the presence of 10% FBS. TNF-α inhibited dermal fibroblast migration in a scratch wound assay and increased the secretion of active MMP-2 and MMP-9 in a dose-dependent manner. In serum-free conditions, all concentrations of TNF-α reduced cell number, with no effect on viability. Dermal fibroblasts exposed to 250ng/ml TNF-α led to an increase in the expression of β-galactosidase.

Exposure to TNF-α impaired dermal fibroblast proliferation and migration, without reducing cell viability; this is likely due to an increase in the number of senescent cells, which is supported by an increase in the secretion of active MMP-2 and MMP-9. The senescence associated secretory phenotype (SASP) is known to propagate inflammation and may contribute to excessive inflammation in chronic wounds. Deciphering and identifying differences in the molecular and functional properties of dermal fibroblasts in response to inflammatory cytokines will further our understanding of the fundamental differences between normal physiological wound healing, chronic non-healing wounds and degenerative fibrotic diseases.

Keywords: dermal fibroblasts, MMPs, SASP, TNF-alpha, wound healing

Signalling molecules and pathways

OP-21

WNT SIGNALLING IN SALIVARY GLAND INJURY AND REPAIR

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WNT signalling is activated by injury and plays a crucial role in injury repair in a number of adult organs. We have investigated the role of Wnt signalling in salivary gland injury and injury repair, using reversible ligation of the main excretory duct of the submandibular gland (SMG) as an injury model.

Using the Axin2CreERT2; R26 mTmG reporter mouse line for canonical WNT/β-catenin signalling, we found a significant increase in WNT/β-catenin signalling within the stroma of the submandibular salivary gland (SMG) after injury. Axin2+ stromal cells were elongated cells located within the gland capsule, septa, and around the excretory ducts and blood vessels. Further investigation revealed the majority of stromal cells with active WNT/β-catenin signalling after SMG injury were CD45+ inflammatory cells mostly represented by F4/80+ macrophages. We identified two peaks of Axin2 expression on day 3 and day 6 after ligation, which correlate with peaks of F4/80 expression. We surveyed expression of the 19 Wnt ligands after injury and discovered WNT1, 2, 2b, 5b, 7b, 9a, and 11 are upregulated on day 3 and 6 after SMG injury. WNT1, 7b and 9a have been reported to be secreted by macrophages, which suggests that after SMG injury, infiltrating macrophages may secrete WNT ligands and respond to them through autocrine signalling. We are currently investigating whether this process is crucial for the repair of the injured gland.

Keywords: WNT Signalling, Salivary glands, Macrophages, Inflammatory cells, Stroma, Sub mandibular gland

Signalling molecules and pathways

OP-23

NFKAPPAB AND NFAT5 TRANSCRIPTION FACTORS ARE INVOLVED IN MUSCLE INFLAMMATION AND FUNCTION IN CLOSE INTERRELATIONSHIP

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The nuclear factor kappaB (NFkappaB)-and nuclear factor of activated T-cells (NFAT)-families of transcription factors share many structural and functional characteristics and likely regulate gene expression through shared enhancer elements. We investigated the NFkappaB family and NFAT5 in muscle inflammation, both in an in vitro model and in patients diagnosed with the inflammatory myopathies dermatomyositis (DM), polymyositis (PM), and sporadic inclusion body myositis (IBM) using quantitative PCR, immunofluorescent localization studies, and quantitative western blotting. As NFAT5 is a key osmo-regulator, we compared pro-inflammatory and hyperosmotic conditions.

In vitro, pro-inflammatory cytokines were potent inducers of both transcription factor families. Cultured primary human

differentiated myotubes treated with IFNγ and IL1β combined showed the highest increases of mRNA expression to 25-fold for RelA, 7-fold for NFkappaB1, 20.5-fold for NFkappaB2, and 4.5-fold for NFAT5. In comparison, 100 mM of added NaCl was able to increase NFAT5 expression only a moderate 2-fold. In CCL136 rhabdomyosarcoma cells, expression induction was similar, yet generally more moderate than in myotubes. In healthy skeletal muscle tissues, prominent myonuclear NFkappaB1, RelA and NFAT5 staining was observed, and sarcoplasmic staining of the Ser536 RelA and Ser1197 NFAT5 phosphorylated forms could rarely be shown. In contrast, the sarcoplasm of regenerating muscle fibers in patient tissues and of nonnecrotic invaded muscle fibers in PM/IBM tissues often was RelA and NFkappaB1 positive. Staining for phosphorylated Ser536 RelA and Ser1197 NFAT5 strongly coincided in the sarcoplasm of perifascicular atrophic fibers of DM. Relative Ser536 RelA protein levels were increased 2-fold in inflammatory myopathy patients compared to healthy controls (0.63±0.11, n=6 vs. 0.31±0.07, n=4, p=0.0007). Inflammatory cells present in DM/PM/IBM sections were invariably NFkappaB1 and RelA immuno-positive and NFAT5 negative. In PM/IBM, activated Ser536 RelA staining was more prominent in CD4+, CD8+ and CD68+ cells surrounding and invading muscle fibers, while in DM inflammatory cell staining was more homogeneous throughout the tissue.

We conclude that transcription factors of both NFkappaB and NFAT families are activated in response to muscle inflammation. Our data point to a role for both pathways in muscle fiber recovery and stress response. Intriguingly, NFAT5 expression was more powerfully induced by inflammatory cytokines than by hyperosmotic stress, putting NFAT5 forward as a more all-round stress factor. The two transcription factor families seem to function in close inter-relationship, with cytokines functioning as important go-betweens at the crossroads of the two pathways.

Keywords: myositis, polymyositis, sporadic inclusion body myositis, dermatomyositis, osmotic stress, inflammatory stress

Signalling molecules and pathways

OP-24

MACROPHAGES REQUIRE PHOSPHOSTAT3 ACTIVATION DURING EFFEROCYTOSIS TO SUPPORT AUTOPHAGY AND PHENOTYPIC CONVERSION IN STERILE INJURY

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Introduction: Sterile liver injury provides a paradigm for the cycle of injury, fibrosis and resolution in a solid organ. Phagocytosis is an evolutionary conserved, multi-step process that encompasses the recognition, engulfment and processing of phagocytic cargo, resulting in antigen presentation. Macrophages are plastic cells that acquire a variety of phenotypes depending on the surrounding microenvironment; they are necessary for damage resolution and fibrosis remodelling, switching their phenotype from pro-inflammatory to restorative. The molecular bases of this switch are still largely unknown, with phagocytosis suggested to be a key factor.

Results: In the present work we induce iterative chronic damage or acute damage in mice deficient for the step of phagocytic cargo processing (Gpnmb-) and in their wild-type (Gpnmb+) counterparts. We show that phagocytosis facilitates macrophage phenotype conversion from pro-inflammatory to restorative macrophages *in vitro* and *in vivo*. We also describe a new role for the phosphoSTAT3-IL10-IL6 axis: phosphoSTAT3 (pSTAT3) is found in the cytoplasm of macrophages minutes after the engagement of the phagocytic cargo, leading to IL10 secretion. IL10 has a pro-phagocytic role and sustains pSTAT3 activation, which in turn promotes Il6 transcription at later time points during phagocytosis. Triggering the pSTAT3-IL6 signalling further enhances macrophage phagocytosis via activation of autophagy, facilitates the phenotype switch to a restorative phenotype *in vitro* and *in vivo* and helps regeneration.

Conclusion: Our work describe a novel mechanism whereby phagocytic cargo internalisation and processing regulate macrophage function and phenotype via STAT3-IL10-IL6 signalling, directly linking mechanisms of tissue damage and repair.

Discussion: Further investigation is needed to understand the timely pSTAT3-dependent activation of autophagy during phagocytosis. Identification of downstream pSTAT3 targets may help designing therapies aimed at increasing phagocytosis in a number of clinically relevant settings characterised by deficits in apoptotic cell clearance, such as solid tumours, acute and chronic inflammation and autoimmune disorders.

Keywords: Phagocytosis, STAT3, IL6, IL10, sterile injury, macrophages

Mechanism of action of pSTAT3 as a novel pro-phagocytic pathway

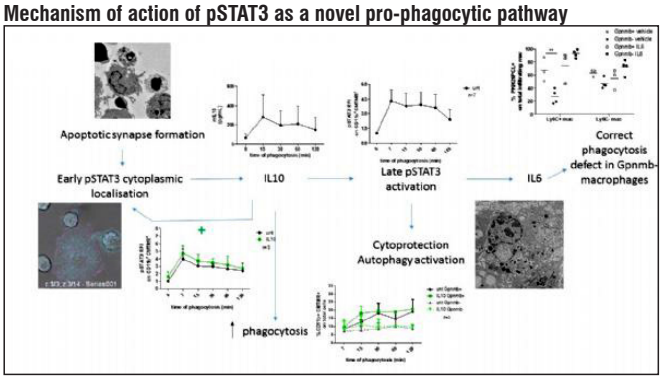

Figure 1. Schematic of pSTAT3 activation following phagocytic cargo engagement and dowstream trigger of IL10 and IL6 production. Any graph and picture reported here is part of the original work described in the present abstract.

Signalling molecules and pathways

OP-25

SCIMP IS A NOVEL TRANSMEMBRANE TLR ADAPTOR PROTEIN THAT IMPARTS CYTOKINE SPECIFICITY TO TLR4 RESPONSES IN MACROPHAGES

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Toll-like receptors (TLRs) sense pathogen-associated molecular patterns and endogenous host factors to drive pro-inflammatory responses. For example, TLR4 recognises and responds to both Gram-negative bacterial lipopolysaccharide (LPS) and host-derived high mobility group protein 1. Canonical TLR signalling, which involves Toll/Interleukin-1 receptor domain (TIR)-containing adaptor proteins such as MyD88, occurs in many cell types. However, innate immune cells are particularly potent mediators of inflammation, and TLR signalling mechanisms that provide specificity to inflammatory responses in these cells are not well understood. Here we show that SCIMP, an immune-restricted member of the transmembrane adaptor protein (TRAP) family, acts as a direct TLR4 adaptor protein to generate specificity in cytokine outputs from macrophages. We demonstrate that LPS promotes an agonist-induced association between TLR4 and SCIMP at the cell surface in macrophages, and use a variety of biochemical approaches to show that these proteins directly interact. Through gain-and loss-of-function studies, we also show that SCIMP is required for LPS-inducible IL-6 and IL-12p40 production in primary macrophages, whereas other cytokine outputs are SCIMP-independent. Specific mutations in SCIMP that abolish its association with TLR4 also abrogate SCIMP-mediated cytokine production. Mechanistically, SCIMP acts as a signalling scaffold to enable the Lyn tyrosine kinase to phosphorylate TLR4, eliciting a transient signalling code for SCIMP-dependent cytokine production. We thus reveal SCIMP as an immune-specific TLR adaptor that provides exquisite specificity to inflammatory responses emanating from macrophages to shape host defence and inflammation.

Keywords: TLRs, macrophages, cytokines, IL-6, IL-12, signalling

Neuroinflammation

OP-26

NEURONAL CUE ON MACROPHAGES TO ENHANCE NEOVASCULARIZATION; ROLES OF CGRP-RAMP1 SIGNALING

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Functional and morphological relationships between primary afferent neurons and blood vessels during cancer development have been poorly understood. However, recent evidence suggests that physical and biochemical interactions between these peripheral components are important to both tumor biology and cancer-associated pain. In addition to cancer cells, tumors consist of stromal tissues including recruited hematopoietic inflammatory cells such as macrophages, blood vessels, and adjacent primary afferent nociceptors. Cancer cells and stromal macrophages release a variety of products that either excite or sensitize the nociceptors. Nociceptor activation results in the release of neuropeptides, such as calcitonin gene-related peptide (CGRP). A sensory nerve-derived mediator, CGRP has vasodilating action, and as a result of it, the blood supply to tumor tissues may be increased. Since CGRP receptor signaling activates adenylate cyclase to elevated cAMP levels in macrophages, CGRP may enhance angiogenesis just like prostanoids. Thus, we examined whether or not endogenous CGRP acting on macrophages facilitates angiogenesis using CGRP knockout mice (CGRP-/-). Tumor growth and tumor-associated angiogenesis in CGRP-/-implanted with Lewis lung carcinoma (LLC) cells were significantly reduced compared with those in wild-type mice (WT). A CGRP antagonist, CGRP8–37 or denervation of sciatic nerves (L1–5) suppressed LLC growth in the sites of denervation compared with vehicle infusion or sham-operation. CGRP precursor mRNA levels in the dorsal root ganglion in LLC-bearing WT were increased compared with those in non-LLC bearing mice. This increase was abolished by denervation. The expression of VEGF-A in stromal macrophages was down-regulated in CGRP-/-. The same was true in the ulcer healing processes in the stomach which is rich with CGRP, and in the recovery from ischemia in a hind-limb ligation model. The mice genetically lacking a CGRP receptor, RAMP1-/-, which we developed recently, also exhibited reduced angiogenesis and lymphangiogenesis in a wound healing model. Stimulation of RAMP1 with CGRP expressing on macrophages produced VEGF-A and VEGF-C/D. RAMP1-/-bone marrow chimera mice showed the reduced wound healing. The present study addresses the significance of CGRP/RMAP1 signaling macrophages as a therapeutic target and provides the concept that the blockade of primary afferent neurons may be of benefit not only in the prevention of cancer pain but also in the inhibition of neovascularization in a macrophage-dependent manner.

Keywords: Neuropeptide, angiogenesis, lymphangiogenesis, macrophage, inflammation, cancer

Neuroinflammation

OP-27

SECRETED ECTODOMAIN OF SIALIC ACID-BINDING IG-LIKE LECTIN-9 AND MONOCYTE CHEMOATTRACTANT PROTEIN-1 SYNERGISTICALLY REGENERATE TRANSECTED RAT PERIPHERAL NERVES BY ALTERING MACROPHAGE POLARITY

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Background: We previously reported that the implantation of a collagen graft soaked with serum-free conditioned medium (CM) from the stem cells of human exfoliated deciduous teeth (SHED-CM) into the nerve gap promotes neuronal regeneration, however therapeutic mechanisms are elusive. In another study, we identified a set of M2 macrophage inducers, monocyte chemoattractant protein-1 (MCP-1) and the secreted ectodomain of sialic acid-binding Ig-like lectin-9 (sSiglec-9) in SHED-CM, and showed that they promote functional recovery after rat spinal cord injury.

Objective: We investigated the roles of MCP-1/sSiglec-9 in SHED-CM-mediated recovery from rodent facial nerve injury (FNI) and the mechanistic basis of the MCP-1/sSiglec-9-mediated regeneration of PNs.

Methods: The rat facial nerve was resected 5 mm. Next an atelocollagen sponge impregnated, MCP-1 and sSiglec-9 was placed in the nerve gap. The neurological recovery of the transected FN was assessed by examination of vibrissae movements (VMs), histological analysis with electron microscope, and gene expression analysis with real time-PCR. Human Schwann cells (SCs) were cultured for 24 h with CM from MCP-1/sSiglec-9-induced M2 macrophage (M2-CM). Effects of M2-CM for proliferation, migration and differentiation of SCs were evaluated.

Results: SHED-CM or MCP-1/sSiglec-9-treated rats exhibited markedly improved VMs that were synchronized with that of the contralateral uninjured side 5 weeks after injury. The FN of both treatment groups displayed each many myelinated axons. M2 macrophages converts pro-inflammatory circumstaces to anti-inflammatory one, and promote to proliferation, migration and differentiation of SCs.

Conclusion: We found that MCP-1 and sSiglec-9 were essential for SHED-CM-mediated functional recovery after severe PNI. The implantation of a collagen graft containing MCP-1/sSiglec-9 into the nerve gap induced anti-inflammatory M2 macrophage polarization, generated a SC bridge instead of fibrotic scar, induced axonal regrowth, and restored nerve function. Our data suggest that the unique combination of MCP-1 and sSiglec-9 may provide therapeutic benefits for severe PNI. We further propose that defined factors secreted by stem cells may provide a previously unrecognized therapeutic strategy in the field of stem cell-based regenerative medicine.

Keywords: PNI, DPSC, Macrophages, MCP-1, Siglec-9

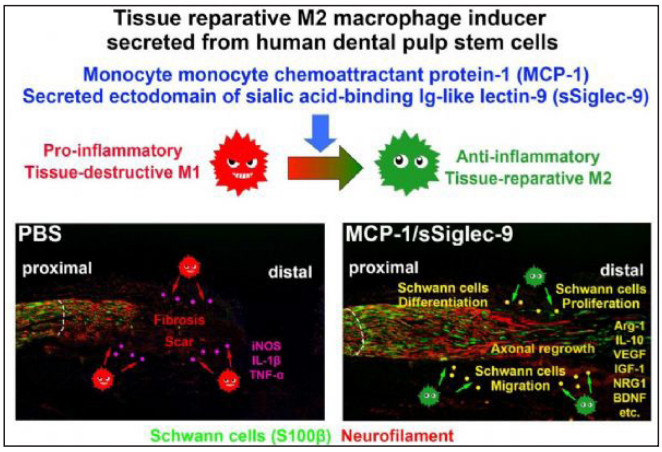

Figure 1. MCP-1/sSiglec-9 enhances the proliferation of SCs in the nerve stump and promotes formation of a SC bridge across the gap.

Neuroinflammation

OP-28

SUBTYPE-SELECTIVE $GABA_A$ RECEPTOR AGONIST REDUCES INFLAMMATION AND IMPROVES RECOVERY POST-STROKE

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Objectives: Inflammatory processes are known to contribute to tissue damage in the central nervous system (CNS) across a broad range of neuro-pathologies, including stroke. The main inhibitory neurotransmitter of the CNS, gamma amino butyric acid (GABA), has been implicated in shaping peripheral immune responses by binding to $GABA_A$ receptors on antigen-presenting cells and lymphocytes. Here, we investigated the effects of several subtype-specific $GABA_A$ receptor agonists on nuclear factor kappa B (NF-κB)-mediated inflammation *in vitro* and *in vivo*. In addition, we tested the most promising of these drug candidates in a mouse model of focal stroke.

Methods: To investigate the anti-inflammatory potential of $GABA_A$ agonists, RAW Blue macrophages were incubated with increasing doses of the drug candidates (10^{-6} 10^{-3} M) prior to the stimulation with lipopolysaccharide (LPS, 2.5 ng/mL). NF-κB-activation was determined using the Quanti-Blue assay. To test the systemic effect of the most promising $GABA_A$ agonists, mice were challenged with LPS (i. p. 0.1 mg/kg) and injected with DS2 (i. p. 0.1 m/kg) 1 hour prior or after the LPS challenge. Blood cytokine levels were analysed 2, 4, 8 and 12 hours later. For the animal model, C57BL/6J mice were subjected to focal stroke or sham surgery using the photothrombosis model of motor cortex stroke. Subsequently, mice were treated with DS2 at 0.1, 1 or 4 mg/kg or vehicle 1 and 24 hours post-stroke. Mice were tested one week prior to surgery to establish baseline behavioural measure (grid-walking/cylinder tasks) and one-week post-stroke.

Infarct sizes were assessed at the end of the behavioural testing using cresyl violet staining of post mortem tissue.

Results: We found that the $GABA_A$ receptor agonist DS2 reduced NF-κB activation over a wide concentration range *in vitro*, whereas zolpidem and bumetanide only decreased NF κB activation at high concentrations. When injected after systemic LPS administration, DS2 decreased the production of several pro-inflammatory cytokines. Treatment with DS2 *in vivo* from 1 hour post-stroke significantly reduced infarct sizes when mice were dosed at 0.1 mg/kg but not at higher doses. Motor tasks improved in mice treated with DS2 at all doses; however, the lowest dose of DS2 (0.1 mg/kg) was most beneficial.

Conclusions: Subtype-selectivity of $GABA_A$ agonists influenced their ability to inhibit activation of NF-κB. It remains to be determined if the improved recovery of stroked animals treated with DS2 depends on modulation of $GABA_A$ receptors in the CNS or on peripheral immune cells. However, as previous unpublished data shows that DS2 does not cross the blood-brain-barrier, it is likely that DS2 is mediating the degree of infarct volume via the modulation of the peripheral immune response.

Keywords: stroke, inflammation, immune response, neurotransmitter, GABA

Neuroinflammation

OP-29

NARROWBAND UVB PHOTOTHERAPY FOR CLINICALLY ISOLATED SYNDROME: DELIVERING THE BENEFITS OF ALL UVB-INDUCED MOLECULES TO EARLY MS PATIENTS

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In light of a lack of definitive outcomes in MS patients after trials of vitamin D supplementation and the ability of narrowband UVB to induce vitamin D, as well as other immune-regulatory molecules in skin, the PhoCIS trial (Phototherapy for Clinically Isolated Syndrome) was established in Perth, Australia (32 degrees S), to investigate the benefits of narrowband UVB phototherapy on MS development in individuals with CIS.

Eighteen individuals with CIS have been recruited with 44% of them given narrowband UVB phototherapy (3 sessions per week for the first 8 weeks), by a protocol similar to that given to people with psoriasis. Phototherapy is an adjunct to supplementing all 18 participants with vitamin D to 25 (OH)-vitamin D levels of approximately 80 nmol/L. MRI has been performed after 3, 6 and 12 months, and extensive blood cell phenotyping 1 week, 1, 2, 3, 6 and 12 months after recruitment. No participant was taking any disease modifying drugs at recruitment, and drugs are given in Australia only after progression to MS.

After 6 months, 7 of 9 participants (78%) who had not received phototherapy had converted to MS by the revised McDonald criteria. In contrast, 3 of 8 participants (37.5%) who received narrowband UVB phototherapy had converted to MS (P=0.09). After 12 months, 9 of 9 (100%) of those in the No phototherapy group had new lesions on their MRI. In the phototherapy group, 5 of 6 had converted after 12 months, with a further 2 participants yet to reach 12 months. The slower/delayed conversion to MS by those receiving phototherapy was supported by a tight prevention by UVB of increased levels memory B cells measured in the blood of Non-phototherapy CIS participants. UVB phototherapy prevented an increase in CD56 loCD16 hi NK cells. In the UVB phototherapy group, there was a significant increase with time of immunoprotective IgG4. Fatigue scores after 6 months were significantly reduced in those receiving narrowband UVB phototherapy.

These interim results support the use of UVB phototherapy to slow the progression of individuals with CIS to MS. The PhoCIS trial provides a fresh approach to re-defining the reported associations of 25 (OH)-vitamin D levels with MS development and progression. As all participants were supplemented to 'sufficient'vitamin D status, the outcomes suggest that molecules other than vitamin D that are produced in UVB-irradiated skin are immunomodulatory and can regulate CIS to MS progression.

Keywords: multiple sclerosis, UVB phototherapy, trial, immunomodulation, vitamin D

Neuroinflammation

OP-30

CYTOKINES AS PERIPHERAL BIOMARKERS IN NEURODEGENERATIVE DISORDERS: PLASMA AND CSF ANALYSIS OF PATIENTS WITH MILD COGNITIVE IMPAIRMENT

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Mild cognitive impairment (MCI) is a syndrome wherein a person experiences greater cognitive decline than is normal for their age. MCI often progresses to more severe conditions such as dementia and Alzheimer's disease (AD). MCI is estimated to effect 3–19% of adults over 65 and has no reliable treatments options. Neuroinflammation is a well-known feature of MCI and proinflammatory cytokines such as IL-1β and IL-6, and TNF-α are linked to progression into AD. There remains a lack of consensus as to which of these markers are up-or downregulated in cerebrospinal fluid (CSF) and blood plasma in MCI individuals. Few studies have investigated these markers in CSF and plasma from the same individuals using multiplex assays. Here, we have analysed the previously mentioned markers and seven additional proinflammatory markers (IGN-γ, IL-10, IL-12p70, IL-13, IL-2, IL-8, and IL-4) in a multiplex assay, using blood plasma and (CSF) from the same donors diagnosed with MCI and healthy controls.

CSF and blood plasma from donors diagnosed with MCI (n=10) and aged matched controls (n=10) were purchased from a biobank. Expression of cytokines, total tau and amyloid-beta (Aβ) was assessed using MesoScale Discovery systems. Donors also supplied a complete history and completed the mini-mental state examination (MMSE) and Alzheimer's disease assessment scale (ADAS).

The MCI group showed significant deficits in both the MMSE ($p<0.001$) and ADAS ($p<0.001$) compared to healthy controls.

The MCI group had a significantly higher concentration of total tau ($p<0.001$) and significantly lower concentration of Aβ ($p<0.05$) in the CSF compared to healthy controls. MCI CSF Aβ levels correlated significantly ($p<0.05$) with MCI CSF total tau. TNF-α concentration was significantly higher in the CSF for MCI compared to healthy controls ($p<0.05$) but there was no difference in TNF-α in the plasma. No significant differences were found with the other markers in the CSF or plasma. A significant correlation ($p<0.05$) was found between MCI CSF IL-1b and MCI CSF total tau but not in healthy controls.

This study is one of the few to use an optimised multiplex assay on plasma and CSF from the same MCI individuals. In line with the literature CSF total tau and Aβ were increased and decreased, respectively, confirming the progression of neurodegeneration. TNF-α has been previously reported to be upregulated in CSF of AD patients. The increase of TNF-α in the CSF in our MCI cohort suggests TNF-α plays a major role in the progression to AD. These results demonstrate the importance of using multiplex assays and using CSF and plasma from the same donors during longitudinal studies.

Keywords: cytokines, plasma, CSF, biomarker, MCI, neuroinflammation

Other

OP-31

THE ROLE OF CHEMOKINES IN THE PATHOPHYSIOLOGY OF MAJOR DEPRESSIVE DISORDER

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Major depressive disorder (MDD) is common and debilitating disorder that is a leading cause of disability worldwide. Although various possible pathophysiological mechanisms of depression has been proposed, accumulating evidence suggests association of chronic inflammation with MDD. In MDD patients serum levels of TNF-α, IL-6, C-reactive protein (CRP) and IL-1β are elevated, but some of the cytokines can also be down-regulated, suggesting that depression related alterations of the inflammatory markers could be more complex then previously assumed. Recent data has however implicated related family of immune proteins designated chemokines in many neuroimmune processes relevant to psychiatric disorders. Despite the evidence for disease specific relevance of of a number of chemokines, limited data are available for the role of chemokines in psychiatric disorders.

The purpose of this research study is to systematically examine the longitudinal change of chemokine levels in plasma of 47 patients suffering from MDD before (t0), after one (t1) and after six weeks (t6) of pharmacological treatment, using multiplex electrochemiluminescence ELISA. Depression severity was assessed with the 29-item Hamilton Depression Rating Scale at time points t0, t1, and t6. Our chemokine panel included 9 chemokines of both innate and adaptive immunity with inflammatory (Eotaxin-3, IP-10, MCP-1, MCP-4, MIP-1α, MIP-1β), and both inflammatory and homeostatic functions (Eotaxin-1, MDC, and TARC).

16 out of 47 MDD patients did not respond sufficiently to initial treatment (non-responder). Multivariate analysis showed that non-responder group presented significantly higher plasma levels of Eotaxin-1, and macrophage derived chemokine (MDC) then responder group (31 patients) after 6 weeks of antidepressive treatment. The response rate of 65.96% in our sample was comparable to other studies.

In conclusion our study provides evidence of elevated chemokine levels in non-responder MDD patients, before and after the treatment. Results from this study will hopefully identify novel biomarkers to monitor therapeutic response, and to help to develop new compounds that target inflammatory pathways.

Keywords: Chemokines, MDC, major depression, antidepressant, therapy response

Immune response

OP-32

REDOX-DEPENDENT B7-H1 DOWNREGULATION CONTRIBUTES TO LIVER FAILURE DURING MURINE POLYMICROBIAL SEPSIS

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Cytotoxic T cell (CTL) activation contributes to liver damage during sepsis. The underlying mechanism remains elusive. The PD-1 ligand, B7-H1, is a co-receptor that negatively regulates T cell function and is expressed on hepatocytes. We hypothesize that B7-H1 downregulation during sepsis provokes autoimmune CTL activation. B7-H1 expression on hepatocytes was analyzed in a murine sepsis model following cecal ligation and puncture (CLP). Because B7-H1 expression decreased because of reactive oxygen species (ROS) generation, global NADPH oxidase 4 (NOX4)-and NOX2-, as well as myeloid lineage conditional NOX2-knockout mice were used to identify the ROS source (s). Adenoviral gene transfer was employed to overexpress B7-H1 in mice and a recombinant B7-H1-Fc chimera was injected i. v. as a pharmacological approach. Downregulation of B7-H1 on hepatocytes occurred in response to bacterial components and facilitated CTL activation. Preserving hepatic B7-H1 expression by adenoviral gene transfer or exogenous administration of

recombinant B7-H1 Fc chimera attenuated liver damage. While B7-H1 expression significantly increased on hepatocytes derived from mice with a deleted NOX4 gene under control conditions, expression in both NOX2 genotypes was similar to that in wild type mice following sham operation. B7-H1 expression remained high in response to CLP only in total NOX2-knockouts. In these mice, the release of the liver damage markers aspartate and alanine transaminases was significantly reduced. Conclusion: We conclude that administering recombinant B7-H1 or inhibiting Nox activity might offer a new therapeutic approach to sepsis, preserving B7-H1 expression, and consequently maintaining tolerant CTLs.

Keywords: sepsis, liver damage, cytotoxic T cells, mouse model, autoimmune activation

Immune response

OP-33

THE EFFECT OF TITANIUM IMPLANT DERIVATIVES ON NEUTROPHIL EXTRACELLULAR TRAP FORMATION

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Introduction: Neutrophils are the major inflammatory-immune cell present in high numbers at sites of peri-implant inflammation associated with skin and mucosa penetrating devices. Neutrophil pro-inflammatory responses include the release of reactive oxygen species (ROS) and Neutrophil Extracellular Traps (NET) which are important immune responses against pathogens. Excessive neutrophil responses are associated with chronic inflammatory disorders including airway diseases, pulmonary diseases and autoimmune diseases. The contribution of dental implant material derivatives, of which titanium is most commonly used, in the activation of neutrophils, and the potential contribution of increased inflammation which may underlie the inflamed tissues characteristic of peri-implant disease, is not fully understood.

Aim: To investigate the impact of titanium implant derivatives on NET and ROS release from peripheral blood neutrophils (PBN) following stimulation.

Material-Methods: Twelve systemically healthy volunteers were recruited and donated approximately 18 ml venous blood. PBN were isolated by density centrifugation and NET release was quantified fluorometrically following stimulated with positive controls phorbol-12-myristate-13-acetate (PMA), hypochlorous acid (HOCl) and nano and micron-sized titanium (Ti) species at 25 and 250 parts per million (ppm). NET release was visualised by fluorescence microscopy and the morphology of NET structures from challenged PBNs. The capacity for NET structures to adhere to Ti implant surfaces was also investigated in the presence and absence of a physiologically representative protein adsorbed layer.

Results: Exposure to Ti implants induced significant NET formation compared to negative control ($p<0.001$), furthermore rutile and mix oxide induced significantly more NETs compared to other Ti species investigated ($p<0.02$ and $p<0.04$ respectively). NET visualisation confirmed quantification results. NET accumulation on Ti implant surfaces was significant higher in the presence of a protein adsorbed layer following stimulation with HOCl ($p<0.01$) and PMA ($p<0.002$) which was confirmed visually.

Conclusions: PBNs are key immune cells involved in protecting the host from bacterial challenge, but dysfunctional activity in PBNs may have systemic consequences. These findings demonstrate Ti implant derivatives can be potent stimulators of NETs (Figure 1). Though strongly associated with ROS production, further mechanisms sensitive to particle size and speciation also appear to be active. NETs have been demonstrated here for the first time to bind to Ti surfaces and this may impact on the peri-implant inflammatory response.

Keywords: Neutrophil extracellular traps, Reactive oxygen Species, Titanium implants, Neutrophils

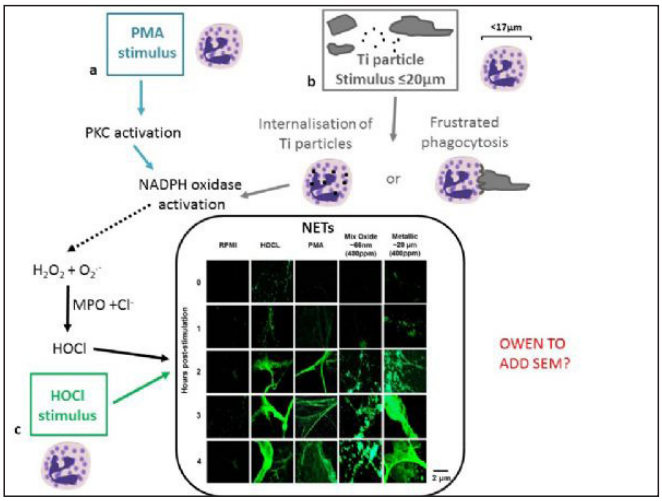

Figure 1. NET formation can occur via different pathways. PMA **(a)** directly enters the cell activating protein kinase C (PKC) which in turn activates the NADPH oxidase. Ti particles **(b)** are thought to lead to NADPH oxidase activation via entry into the cell by internalization or via frustrated phagocytosis for larger particles. Both result in the formation of HOCl which is critical for NET formation. HOCl **(c)** as a stimulus can directly initiate NET formation, reflecting its more immediate ability to induce NET formation.

Immune response

OP-34

HYDROQUINONE EXPOSURE AGGRAVATES ARTHRITIS IN RATS

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Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune and inflammatory disease. Epidemiological data show the association of smoking with RA outcome, by triggering or worsening pre-existing disease. Hydroquinone (HQ) is a phenolic compound of natural or released by anthropogenic sources, found in high concentrations in cigarette. Furthermore, HQ is a toxic benzene metabolite. Type II collagen-induced arthritis (CIA) is widely accepted as a valid RA animal model for mimicking human RA. Thus, we aimed to investigate the role of HQ

exposure on CIA development in Wistar rats and the involved mechanisms.

Methods: Animals were immunized s. c. at the tail base with 200 μl of bovine type-II collagen emulsified 1:1 with complete Freund's adjuvant (CFA). A booster injection was administered 7 days later. Rats were exposed to saline or HQ-vehicle (saline-ethanol 20:1) or HQ 25ppm, 1 hour/day, using a nebulization chamber during 35 consecutive days, which comprehended one week before collagen injection and 28 days during the disease development. At the end of the exposures periods, animals were submitted to clinical evaluation (score scale of 0–4:0 = no arthritis; 1–2 = weak arthritis, with inflamed digits; 3 = medium arthritis, with more than 2 digits and an inflamed footpad; 4 = strong arthritis, with all inflamed digits and paws), and samples were collected to hematological analysis, histological and immunofluorescence analysis of the synovium, quantification of cytokines levels and citrullinated proteins. Data were obtained in 4–6 animals per group.

Results: Data obtained showed that HQ exposure elevated scores (3–4 scores) of CIA when compared to HQ-vehicle and saline exposures (1–2 scores). HQ exposure reduced weight body, increased neutrophils influx into the synovium, enhanced the levels of citrullinated peptides in serum and levels of the cytokines IL-6 and IL-1beta in synovial fluid; caused *pannus* formation and hyperplasia of synovial cells in the synovium; increased the levels of AhR receptor, ROR, IL-17, CD90 and Ly6G in the synovium in comparison to saline or HQ-vehicle exposed animals.

Conclusion: our data show that HQ exposure aggravates systemic and local symptoms of RA, suggesting that HQ may be a determinant component of cigarette on RA development. Further experiments are being carried out to investigate this hypothesis.

Financial support: CNPQ.

Keywords: inflammation, cigarette smoking, benzene metabolite, synovium

Immune response

OP-35

IMMUNOMODULATORY EFFECT OF MINOCYCLINE IN INTESTINAL INFLAMMATION

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Introduction: Minocycline exerts immunomodulatory effects that could be beneficial in the treatment of inflammatory bowel disease (IBD). The mechanism underlying this effect is not completely understood and different actions on distinct immune populations might be implicated. The aim of this study was to evaluate the impact of minocycline on the different immune populations involved in the DSS-colitis model in mice.

Methods: Intestinal inflammation was induced in male C57BL/6J mice by administration of dextran sodium sulfate (DSS) in the drinking water (3%) for 5 days. Once the colitis process was established, one group was treated with minocycline (50 mg/kg/day) while colitic control and healthy groups received the vehicle. Mice were sacrificed after 2 and 4 days of treatment. The inflammatory status and minocycline effects were evaluated by a disease activity index (DAI), histology, gene expression and cytokine production, and leukocyte populations from colonic lamina propria and blood were analysed by multiparametric flow-cytometry.

Results: Minocycline treatment improved the recovery of colitic mice, evidenced by reduced DAI values, increasing mucosa barrier protection and ameliorating some of the inflammatory markers. However, minocycline potentiated some immune pathways at the early onset of the inflammatory process (day 2) that explain the immune changes observed at day 4. Minocycline-treated mice displayed an enhanced type-2 mucosal immune response, with increased eosinophils and Th2 populations. Dendritic cells and macrophage populations were also increased, the latter showing bias polarisation towards homeostatic intestinal macrophages. Finally, higher numbers of regulatory T cells and Th17 cells were observed in minocycline-treated animals, while the number of neutrophils was reduced. This effect was linked to an enhanced production of IL-22 and GM-CSF, especially after 2 days of treatment, and an up-regulation in Alox15 expression, suggesting a potentiation of the synthesis of pro-resolving lipid mediators.

Conclusion: Minocycline, by potentiating the immune response, accelerates the process of acute intestinal inflammation, inducing a shift towards regulatory and Th2 responses. This increases mucosal protection and activates the resolution pathways, preventing the deleterious consequences of prolonged inflammation and the associated tissue damage, resulting in an intestinal anti-inflammatory effect. These immunomodulatory properties could be of great interest to face inflammatory and immune-mediated disorders such as IBD.

Keywords: inflammatory bowel disease, mucosal inflammation, immunomodulation, resolution of inflammation, minocycline

Pain

OP-36

LOIN PAIN HAEMATURIA SYNDROME (LPHS): IN VITRO MODELLING FOR ASSESSING THE CONTRIBUTION OF INFLAMMTORY COMPONENTS FROM BLOOD

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Considered the most painful syndrome known to humankind, there is little if any research into disease mechanisms and pharmacology of Primary LPHS. Chronic flank pain occurs with spasms of catastrophic incapacitating pain associated with ureteric contractions. It is accompanied by haematuria in the absence of glomerular nephritis, renal casts, or infection. Common treatments are renal denervation or autotransplantation. We have reported a patient managed with tadalafil, a PDE5 inhibitor, reducing severity and frequency of ureteric spasms (Russell et al, 2015). We here investigate parameters by which direct muscle or sympathetic nerves stimulation of ureteric contraction may be affected by intraluminal blood (ILB) in vitro.

Rat ureters (250–300 g, male), ± ILB, were mounted (0.2 g tension, isometric Harvard transducers) in Krebs at 37C with 95% CO2/5% O2 equilibrated for 1 hr, or 4Hr in Mg-free Krebs.

Noradrenergic nerve responses are disinhibited in the absence of Mg. Ureters were stimulated by chick biventer (CBV) or platinum field electrodes (PFE). Parameters used were 10 s duration square waves 2 ms at 20 or 40Hz and 5–90V with single or twin pulses. For comparison, vas deferens were suspended at 37C at 0.5 g tension, and PFE stimulated twin pulses (75 ms) 0.1Hz and 0–10V (ADInstruments Powerlabs T26) and twitch response curves constructed. Force of contraction (FoC, g) was captured using ADInstruments Labchart V8. Data was expressed as mean ± sem.

CBV stimulation 10 s at 20Hz and 40Hz induced voltage dependent contraction peaking at 0.042±0.010 g (50V) and 0.040±0.006 g (60V) respectively. At 20Hz, tachyphylaxis was seen at 60V. Ureters did not respond to PFE. Mg-free Krebs revealed contractions to PFE, peaking at 0.0181±0.0129 (20Hz, 0–50V) and 0.0198±0.0116 (60V, 40Hz), tachyphylaxis being seen again at 20Hz 60V. Vas deferens had reduced sensitivity and FoC to PFE in Mg (Hughes et al. , 1975). Twin pulses increased the responses to 0.043±0.0119 (620Hz, 60V) and 0.025±0.004 (40 hz, 60V). The inclusion of ILB in tissues bathed in normal Krebs increased the max FoC to 0.035+0.14 (20Hz, 60V) and 0.054+0.015 g (40Hz, 60V). In Mg-free Krebs, ILB increased FoC to 0.0678±0.0314 g (20Hz 60V) and 0.042±0.024 (40Hz 60V). The local anaesthetic procaine abolished twitch (fast) vas deferens responses and stimulated slow contractions, and to the ureters. The presence of blood dramatically increased these slow responses.

Intraluminal blood in LPHS is considered an inconsequential bystander. We show ILB increases ureteric contractions. Blood constituents such as granulocytes, platelets, complement, and the kinins may contribute to the ureteric spasms in LPHS. We will investigate their roles. Procaine opens L-type Ca-channels in urinogentical smooth muscle, this being exacerbated by ILB may indicate an action of ILB on smooth muscle sensitivity to Ca channels opening.

References

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Russell A et al, 2015, BMJ Case Reports 10 (1136)

Keywords: Loin Pain Haematuria Syndrome, LPHS, blood, nerve stimulation, inflammatory mediators

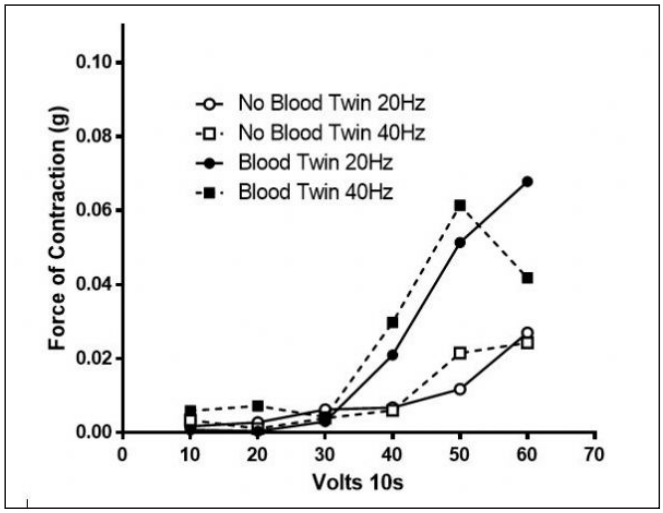

Figure 1. Voltage dependent ureteric contraction (g), 20 or 40Hz twin pulses, in the absence and presence of intraluminal blood to model LPHS.

Immune response

OP-36

T-CELL TNFA SYNTHESIS AND MONOCYTE DIFFERENTIATION TO FUNCTIONAL TNF SYNTHESISING MACROPHAGES IS INHIBITED BY SULPHATED DISACCHARIDES. AN EXPLANANTION FOR THEIR ANTI-RHEUMATIC ACTIVITY?

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We have reported to previous World Congresses of Inflammation that sulphated disaccharides (SDS) inhibit antigen induced arthritis (Jones et al 2008), whole blood TNFα synthesis, but not lps-induced synthesis by mature macrophages, MØ (Yousaf et al, 2012). SDS are heparin degradation products released by T-cell heparinase at sites of inflammation. We here investigate how SDS might inhibit whole blood TNFα synthesis and arthritis. Whole blood TNFα is derived from T-cells and monocytes. We have investigated TNFα synthesis by Jurkat T-cells, and since lps-stimulated TNFα synthesis by MØ is unaffected, the differentiation of monocytes to TNFα competent MØ has been investigated.

Jurkat T-cells were seeded in antibiotic free, FCS and glutamine supplemented RPMI-1640 into 96 well plates (10^5/well). TNFα synthesis was induced by PHA (1 μg/mL, 18 hrs). U937 cells were differentiated in 24 well plates at 10^7 cells per well to Mϕ with phorbol-myristate acetate (PMA, 80nM, 72 hrs). Differentiation to MØ was determined by washing off undifferentiated cells, washing the adherent cells, and after scraping in trypsin/EDTA, resuspending and counting be haemocytometer. MØ TNFα synthesis was stimulated with lps (1ug/mL, 5 hrs). Drugs: sucrose octasulphate (SOS, Chemika, Germany), diglucopyranosylamine octa-sulphate (diGaS, synthesised Dr M Burnet, Synovo GmbH), heparin disaccharide-IIIH (HDS-IIIH, Sigma Aldrich) or heparin disaccharide-IS (HDS-IS, Sigma Aldrich), Cell viability assessed by trypan blue exclusion.

PHA induced TNFα synthesis by Jurkat cells was inhibited by SOS, HDS1-S and diGAS in a concentration fashion from 10–11 to 10–6M. Incubation of U937 cells with the drugs for 24 hr prior to the addition of PMA reduced TNFα synthesis likewise. However, no drug administered after PMA but before lps inhibited MØ TNFα synthesis. Incubation of U937 cells with the drugs for 24 hours before PMA inhibted the differentiation of the monocytes into macrophages. Figure 1 shows that SOS, DOS and HDSIII-S potently inhibited synthesis at 10–11M, in a bell shaped fashion with the effct reducing as the conentration increased. HDS1-S had its maximum at 10–6M with increasing inhibition from 10–11M.

SDS molecules potently affect T-cell TNFα synthesis, however do not inhibit Mp TNF synthesis. However, monocyte differentiation is potently inhibited. It appears a minimum of one sulphate molecule on each saccharide is required for the activity seen of the octasaccharides. Reduction to a single sulphate group with HDS1-S changes the profile of this molecule in ita ability to inhibit monocyte differentioan to M2 TNF comptent Mp.

References

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Yousaf N et al, Inflammation Res 61 (S1): S24, 2012.

Keywords: HDS, SDS, HDS, T-cell, macrophage differentiation, TNF

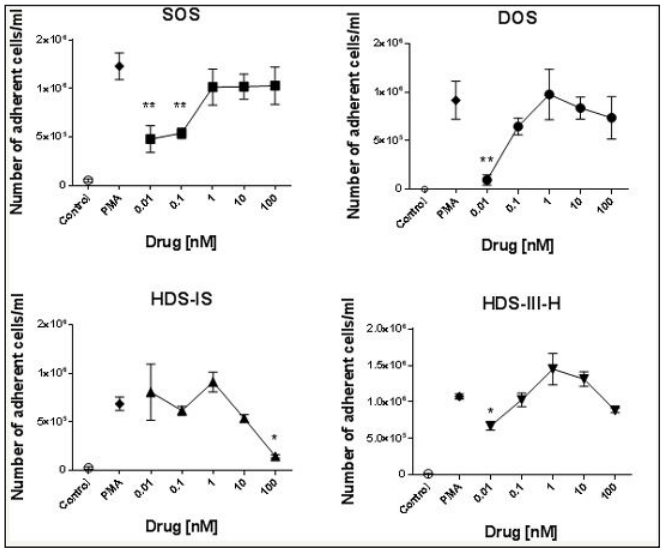

Figure 1. Preincubation of U937 cells with SDS for 24 hours prior to PMA (80nM) significantly inhibits PMA induced differentiation to adherent macrophages.

Immune response

OP-37

SELECTIVE DELETION OF T-BET IN ILCS REGULATES INTESTINAL INFLAMMATROY RESPONSES

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Background: Innate lymphoid cells (ILCs) play an important role in regulating immune responses at mucosal surfaces. The transcription factor T-bet is essential for ILC development and function. Germline deletion of T-bet results in a complete loss of NCR+ ILCs and, in the absence adaptive immunity, leads to aberrant ILC3 responses that drive microbiota-dependent intestinal inflammation.

Aim: To determine the role of T-bet in intestinal ILC responses in an intact immune system.

Methods: We have developed a model of specific deletion of T-bet in ILCs (T betfl/flxNcr1-iCreTg or T-betΔNCR+ILC mouse), in which T-bet is deleted in ILCs while the function and development of other NCR+ cells remain unaffected. ILC phenotype in the T-betΔNCR+ILC mouse was assessed at baseline and in the context of parasitic infection and inflammatory colitis.

Results: We have demonstrated that when T-bet deletion is under the control of Ncr1 expression, neither the development nor the cytokine producing ability of NCR+ ILCs is affected, suggesting that T-bet regulates NKp46 expression in ILCs rather than the development of NCR+ ILCs. In addition, specific loss of T-bet in ILCs in an immunocompetent background leads to the expansion and increased activity of ILC2 s via enhanced IL-7 signalling in a cell-intrinsic manner. Linked to enhanced ILC2 responses, T-betΔNCR+ILC mice display an accelerated worm expulsion of Trichinella spiralis. Moreover, upon DSS-colitis induction, T-betΔNCR+ILC mice develop a protective type-2 intestinal immune response, with significant eosinophilic infiltration into the colonic lamina propria and higher IL-13 and IL-5 production by ILC2 s.

Conclusions: Our work provides further insights into the role of T-bet in NCR+ ILC development and suggest a previously unappreciated role for T-bet in the regulation of ILC2 responses. T-bet expression in ILCs acts as the key molecular checkpoint in regulating pathogenic versus protective mucosal immune responses, which has significant implications for the understanding of type 2 immunity and the pathogenesis of associated inflammatory diseases.

Keywords: Intestinal Inflammation, T-bet, Innate lymphoid cells, ILC2 s, Mucosal homeostasis

Infection

OP-38

DISARMING *PSEUDOMONAS AERUGINOSA* EVASION MECHANISMS IN THE LUNG VASCULATURE WITH A BI-SPECIFIC ANTIBODY

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Introduction: *Pseudomonas aeruginosa* is an important opportunistic bacterial pathogen that causes life-threatening infections in critically ill and immunocompromised individuals. It is one of the most common causes of Gram-negative pneumonia in ventilated patients, is the principle pathogen responsible for persistent/chronic infections in the cystic fibrosis lung, and is a frequent colonizer of burn wounds, catheters and medical devices. Importantly, P. aeruginosa causes most deaths when it enters the bloodstream and disseminates. Specifically in the case of hospital-acquired pneumonia where *P. aeruginosa* has previously been reported to disrupt the lung barrier and cause bacteremia, patient prognosis is poor. Further complicating these infections is the fact that *P. aeruginosa* is often multi-drug resistant (MDR), likely contributing to its advanced innate resistance mechanisms. P. aeruginosa resistance has been reported for nearly all commonly used antibiotics.

Objective: Mechanisms of how *P. aeruginosa* avoids the innate immune system to survive in the bloodstream and disseminate to various organs is not well understood. We utilized

engineered therapeutic antibodies, designed to help host clear *P. aeruginosa*, to both decipher bacterial pathogenesis as well as understand mechanisms of therapeutic antibody efficacy.

Materials & Methods: In vivo imaging techniques such as multi-laser spinning disk intravital microscopy, as well as flow cytomtery were utilized to observe *P. aeruginosa* infections in real-time.

Results: Although *P. aeruginosa* adhered avidly to lung vasculature, patrolling neutrophils and other immune cells within the pulmonary vasculature were virtually blind to the bacteria. This cloaking phenomenon was mediated by an exopolysaccharide on *P. aeruginosa's* cell surface, Psl. An anti-Psl monoclonal antibody (mAb) blocked Psl function, activated complement, and increased neutrophil recognition of P. aeruginosa. However, neutrophil mediated clearance of the pathogen was suboptimal with anti-Psl antibody therapy due to a second, subversion mechanism, the type-3-secretion (T3S) injectisome. Indeed T3S prevented phagosome acidification and resisted killing inside these compartments. Anti-PcrV mAb inhibition of T3S did not enhance bacterial phagocytosis but did lead to enhanced killing of the few bacteria ingested by neutrophils. A bi-specific mAb targeting both Psl and PcrV enhanced neutrophil uptake of the bacteria but also greatly increased inhibition of T3S function, allowing for phagosome acidification and bacterial killing. This study highlights the need to block multiple evasion and subversion mechanisms in tandem in order to clear P. aeruginosa during blood-borne infections.

Keywords: Intravital microscopy, innate immunity, pulmonary infection

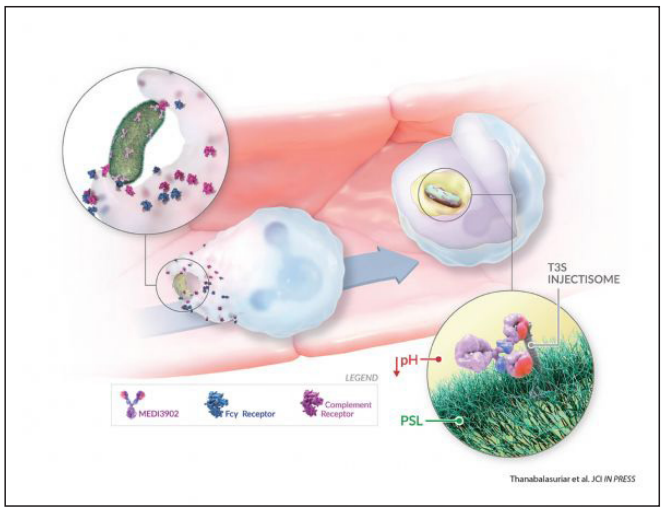

Figure 1. Schematic of pulmonary neutrophil phagocytosis of P. aeruginosa in the presence of bi-specific antibody MEDI3902.

Wild-type P. aeruginosa avoids detection/phagocytosis by neutrophils in the pulmonary capillaries. P. aeruginosa surface exopolysaccharide Psl inhibits deposition of complement C3b on its surface, cloaking itself from neutrophil recognition. Moreover, phagocytized P. aeruginosa secrete effector molecules via the T3S injectisome to hinder acidification of the neutrophil phagolysosome. Deletion of Psl expression (ΔpslA), or targeting Psl with an anti-Psl mAb increases bacterial recognition and phagocytosis by neutrophils in the lung. However, phagocytized bacteria interfere with acidification of the phagolysosomal compartment. Inhibition of the T3S injectisome, either by infecting mice with T3S injectisome deficient P. aeruginosa (ΔpcrV) or by treatment with an anti-PcrV mAb alone, does not increase recognition or phagocytosis by neutrophils, but does lead to greater acidification of phagolysosomes in the few neutrophils that engulf bacteria. In contrast, administration of bi-specific antibody MEDI3902, which simultaneously targets Psl and PcrV, leads to increased recognition, phagocytosis and killing of bacteria by neutrophils. This enhanced activity is not observed with a mixture of parental anti-Psl and anti-PcrV mAbs indicating the necessity of including both specificities on the same mAb molecule. Therefore, the anti-Psl targeting mediated by MEDI3902, facilitates transfer of anti-T3S injectisome activity (via the anti-PcrV arm) within the phagolysosome after phagocytosis resulting in enhance compartment acidification and killing of P. aeruginosa.

Infection

OP-39

SHORT CHAIN FATTY ACIDS MODULATE THE INFLAMMATORY RESPONSE DURING BACTERIAL INFECTION

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The short-chain fatty acids (SCFAs), acetic, propionic, and butyric acid, are bacterial metabolites found at high concentrations in the intestine and in places of bacterial infections. Despite their well-described role in the maintenance of intestinal homeostasis, their relevance in infectious conditions is not clear. Considering that, we investigate whether SCFAs modify the effector functions of phagocytic cells (neutrophils and macrophages) to an opportunistic pathogenic bacterium, Aggregatibacter actinomycetemcomitans (Aa). Using a subcutaneous model to generate a mono, isolated infection of Aa, we demonstrated that the presence of the SCFAs in situ did not affect leukocyte accumulation but downregulated the production of cytokines, their phagocytic capacity, and killing the bacteria. Similar effects were observed with bacteria-stimulated neutrophils and macrophages incubated with SCFAs in vitro. These effects were independent of the activation of the free-fatty acid receptor 2 (FFAR2), the main SCFA receptor expressed on neutrophils, occurring possibly through inhibition of histone deacetylases. Transcriptome analysis of neutrophils demonstrate that several pathways involved in the initial detection of microrganisms such the toll-like receptors (TLRs) and nod-like receptors (NLRs) signaling pathways and effector functions of neutrophils including proteins that participate in the internalization (CR3 and coronins) and maturation of phagossomes (Vps34 and EEA1) are inhibited by butyrate (Figure 1). Interestingly, these effects were not observed in neutrophils incubated with other SCFAs (acetic, propionic and crotonic acids). Taking together, our results suggest that in an infectious conditions, SCFAs (mainly, butyrate) may exert a detrimental effect on the host by inhibiting effector functions.

Keywords: neutrophils, infections, shot chain fatty acids, microbiota

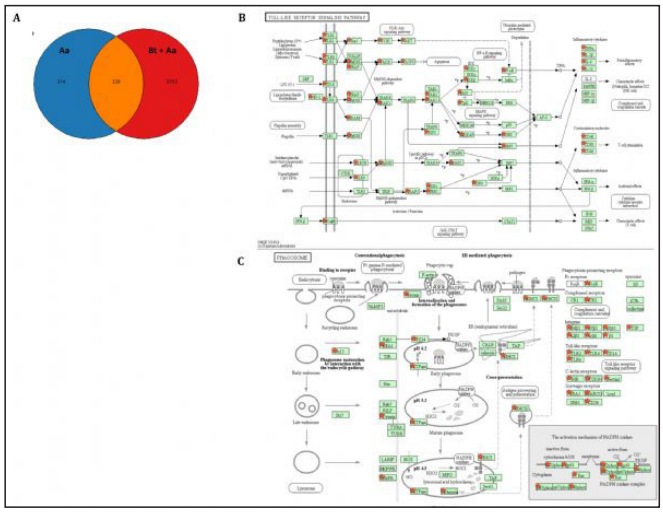

Figure 1. Legend. **A)** Total of genes differentially expressed by neutrophils stimulated with Aa in the presence or absence of butyrate in comparison to non-stimulated cells. **B)** and **C)** Examples of pathways modulated by butyrate in neutrophils. Red stars identify downregulated genes in neutrophils incubated with Aa in the presence of butyrate in comparison to the condition in which cells were stimulated with Aa in the absence of butyrate. Figures obtained using DAVID and KEGG.

Infection

OP-40

AN ECOIMMUNOLOGICAL APPROACH TO STUDY AN ANCIENT LINK BETWEEN COMPLEMENT AND HUMORAL IMMUNITY

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The proteolytic processing of many proteins involved in blood coagulation leads to the generation of peptides with antimicrobial and anti-inflammatory properties. Notably, coagulation and innate immunity are tightly interwoven and form an alliance that can be tracked back to early eukaryotic evolution. Indeed, phylogenetic studies with fibrinogen-domain-containing proteins from invertebrates and ancient chordates show that they act as pattern recognition receptors, but lack coagulant activity, suggesting that fibrinogen has evolved its clotting ability from proteins primarily involved in the innate immune response. Here we employed an ecoimmunological approach with Tissue Factor Pathway Inhibitor-1 (TFPI-1)-derived peptides from the different classes of vertebrates (mammalian, rodent, bird, reptile, and fish) and tested whether they can boost killing of various human bacterial pathogens in plasma. We found evolutionary signs of species-specific conservation and diversification in these peptides that significantly impact their antimicrobial activity. This is achieved by their interactions with immunoglobulins and subsequent activation of the classical pathway of complement. Though all TFPI-1-derived peptides executed bactericidal activity in mammalian plasma, no killing in plasma from rodents, birds, reptiles, and fish was detected, which points to a crucial role of classical pathway of complement system. We also see an interference of these peptides with the human intrinsic pathway of coagulation though unlike complement activation, this mechanism appears not to be evolutionary conserved. Together our findings decipher a novel link in innate and humoral immunity involving the coagulation and complement systems.

Keywords: TFPI-1, complement, vertebrates, antimicrobial, coagulation, evolution

Infection

OP-41

SERUM 3-NITROTYROSINE LEVELS INCREASE PRIOR TO A SEPSIS DIAGNOSIS IN INDIVIDUALS WITH POST-SURGERY SEPSIS

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Sepsis is a systemic host response to infection that is maladaptive. Sepsis involves a dysfunctional level of pro-inflammatory cytokines in the circulation followed by a later immunosuppressive stage, caused by an increase in anti-inflammatory cytokines. This inflammatory response leads to the recruitment of immune cells and the release of reactive oxygen/nitrogen species. High amounts of free radical release, e. g. $^{\bullet}NO$ and $O_2^{\bullet -}$, which react to form peroxynitrite, cause increased 3-nitrotyrosine (3NT) formation (although other mechanisms may also contribute to 3NT formation *in vivo*). 3NT can be present as a free amino acid or as a residue within the polypeptide backbone of proteins. Elevated levels of free 3NT have been observed in individuals after sepsis diagnosis. The presence of 3NT in sepsis means it could potentially be used as a biomarker for this disease.

The aim of this study was to determine if protein-associated 3NT was increased, prior to diagnosis, in patients with post-surgery sepsis, compared with patients without post-surgery sepsis. Serum samples from patients undergoing major elective surgery were collected prior to surgery and then daily, for up to 7 days, afterwards. Samples from patients that went on to develop post-surgery sepsis (26 patients) were selected for analysis, along with samples from matched, non-sepsis, control patients (25 patients). The serum was analysed using an electrochemiluminescence-based ELISA for the measurement of protein-associated 3NT.

Median levels of serum C-reactive protein (CRP) and serum 3NT, in the 51 patients, increased following surgery: CRP (mg/L)-5.00 (1.00–14.0) before surgery and 113 (48.0–179) after surgery; 3NT (fmol nitrated BSA equivalents/mg protein)-1.74 (0.59–4.79) before surgery and 3.00 (1.01–7.15) after surgery). These results confirm that surgery caused a substantial inflammatory response. The median level of serum 3NT was higher in the sepsis group, compared to the non-sepsis group, at all time points after surgery, and this was statistically significant one day before diagnosis of sepsis (median serum 3NT: 5.14 (2.38–8.32) and 1.32 (0.79–5.55) fmol nitrated BSA equivalents/mg protein,

for the sepsis group and the non-sepsis group, respectively (Mann-Whitney, p=0.02)). In the case of post-surgery sepsis the median serum 3NT showed a rise in the days following surgery, whereas the median serum 3NT of the non-sepsis group exhibited a reduction in nitrative stress (following a peak in serum 3NT observed one day post-surgery). In conclusion, these data show that sepsis patients have increased nitrative stress prior to sepsis diagnosis.

Keywords: 3-Nitrotyrosine, sepsis, oxidative stress

Infection

OP-42

MEASUREMENT OF SERUM NITRATE CONCENTRATION FOR THE DIAGNOSIS OF INFECTIVE GASTROENTERITIS

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An increased serum concentration of nitrate, a metabolic end product of nitric oxide (NO), has been reported in patients with infective gastroenteritis. Patients with infective gastroenteritis, associated with bacteria such as Campylobacter, display far greater increases in plasma nitrate concentration compared to culture negative patients admitted to hospital with diarrhoea attributable to other causes such as inflammatory bowel disease. We hypothesised that the measurement of serum nitrate concentration would be useful for the diagnosis of the cause of acute diarrhoea in patients admitted as emergency cases to hospital. To test this hypothesis, we compared the serum nitrate test with the reference method for the identification of pathogenic bacteria in diarrhoea, which is polymerase chain reaction (PCR) testing of stool samples.

Serum samples, surplus to routine diagnostic requirements, were from 94 patients admitted to hospital with diarrhoea. Patients were excluded if they had been in hospital for more than 3 days, had a bacterial infection within the last month, or were taking antibiotics. Nitrate was determined by a spectrophotometric Griess reagent-based assay. Matched faeces samples were tested for bacterial infections using PCR (BD Max automated nucleic acid extraction and thermocycling amplification platform). The median serum nitrate (μM) concentration (interquartile range) in patients (n=79) without a PCR-detectable pathogen was 19.4 (9.6–41.6). Compared with these PCR-negative patients, there was a significant increase (P<0.0001) in the median nitrate concentration in patients (n=11) with Campylobacter infections 123.9 (41.8–202.0) and patients (n=4) with Salmonella infections 430.6 (198.5–465.6) (Mann-Whitney U test). With a cut-off point of 60 μM, the assay had an area under the ROC curve of 0.92, a sensitivity of 73% and a specificity of 90%.

We suggest that the rapid and inexpensive method of serum nitrate measurement may be a useful diagnostic test for patients within the acute hospital setting. Possible mechanisms for this dramatic increase in serum nitrate in the presence of Campylobacter and Salmonella include host iNOS induction during the inflammatory response.

Keywords: nitrate, infective gastroenteritis, Campylobacter, diagnosis

Fibrosis

OP-43

DUAL INTEGRIN BLOCKADE ATTENUATES FIBROTIC AND VASCULAR ALTERATIONS IN A MURINE MODEL OF SISTEMIC SCLEROSYS

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Background and Purpose: Systemic sclerosis (SSc) is an acquired connective tissue disorder characterized by immune dysregulation and vascular damage leading to fibroblast activation and fibrosis of the skin and internal organs. It has been recently shown that alterations in cell-matrix interactions are sufficient to initiate and sustain inflammatory and pro-fibrotic processes. The aim of the study was therefore to evaluate the effect of the $\alpha v\beta 3$ and $\alpha v\beta 5$ inhibitor (cilengitide) on the development of scleroderma-specific alterations in the chronic oxidant stress murine model of systemic sclerosis.

Experimental Approach: SSc was induced in BALB/c mice by daily s. c. injections of HOCl for 6 weeks. Mice were randomized in three arms: HOCl alone (n=8), HOCl+Cilengitide (n=8) or Vehicle alone (n=8). Treatment with cilengitide 20 (mg kg-1/i. p. /day) was started four weeks after the first administration of HOCl and maintained throughout the remaining experimental period (2 weeks). Lung, skin and heart fibrosis were evaluated by histology. Kidney alterations were evaluated by PAS staining. Collagen type I, focal adhesion kinase (FAK) and α-SMA were evaluated by immunostaining; and p-FAK and TGF-β1 by western blot.

Key Results: The administration of HOCl induced multi-organ fibrosis while cilengitide treatment significantly reduced the histopathological alterations. Additionally, skin p-FAK and TGF-β1 expression were significantly modulated by the dual integrin inhibitor. Cilengitide treatment also improved kidney vascular and glomerular alterations.

Conclusions and Implications: The inhibition of integrin signaling could prove useful as future therapeutic targets for treatment of SSc.

Keywords: Systemic sclerosis, fibrosis, fibroblasts, interstitial lung disease, pulmonary

Fibrosis

OP-44

BLOCKING THE TNF SUPERFAMILY MEMBER LIGHT TO CONTROL LUNG AND SKIN FIBROSIS

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Anti-inflammatory drugs have so far failed to reduce fibrosis and tissue remodeling in the clinic. Alternatively, 5 TNF inhibitors have been FDA approved to treat different inflammatory

diseases and are safe to administer to patients. In human, LIGHT and its receptors have been linked to numerous autoimmune and inflammatory diseases with fibrotic components. We show that blocking LIGHT signaling in isolation is efficient in reducing fibrosis in the skin and lung of mice induced with bleomycin to mimic human Systemic Sclerosis and Idiopathic pulmonary fibrosis, as well as in 2 severe allergen-driven models of asthma and atopic dermatitis. Neutralizing LIGHT signaling through its receptors by genetic deletion or antibody blocking, dramatically decreased collagen and smooth muscle deposition in the lungs and skins. Moreover the specific deletion of LIGHT-receptor HVEM on keratinocytes or the therapeutic blocking of LIGHT-HVEM interaction post-disease onset, abrogated skin fibrosis induced by house dust mite in a model of atopic dermatitis. LIGHT seems to play a central role in lung and skin fibrosis since it can control the expression of major pro-fibrotic factors such as TSLP, IL-13, TGF-b and Periostin. Also LIGHT alone can induce a fibro-proliferative disorder that mimics human SSc, in the lung and skin when administered alone, by increasing the accumulation of both fibers collagen and smooth muscle actin. Lastly we observed an upregulation of LIGHT and its receptors in lung biopsies of patients suffering from asthma, scleroderma and idiopathic pulmonary fibrosis. The relevance of this work to fibrosis therapies associated with asthma, atopic dermatitis, scleroderma and idiopathic pulmonary fibrosis are tremendous

Keywords: TNFSF14, fibrosis, idiopathic pulmonary fibrosis, asthma, atopic dermatitis scleroderma

Resolution of inflammation

OP-45

FIBROBLAST INNATE MEMORY: TROPISM, DISEASE-ASSOCIATION AND CONSEQUENCES

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Memory is a defining characteristic of the adaptive immune response. However, the existence of memory in the mammalian innate immune system has also been recognized for nearly a century [1]

. For example macrophages exhibit different types of memory, from short term endotoxin tolerance to long term trained immunity [2]

. Despite their existence in all tissues and proven role in inflammation, memory in fibroblasts has received little attention.

Recently we reported that fibroblasts of both the healthy and rheumatoid joint exhibited memory, secreting significantly more interleukin 6 (IL-6) when re-challenged with tumour necrosis factor (TNF) than they did in response to the initial challenge [3]

. Expression of IL-8 was not augmented on re-challenge of synovial fibroblasts, and inflammatory memory was not displayed by healthy skin fibroblasts.

Here we extend the investigation of inflammatory memory by assessing responses of fibroblasts from different tissues to re-challenge with TNF. We found that expression of IL-6 was consistently augmented on re-stimulation of synovial, tonsil and lung fibroblasts, as well as in mouse embryonic fibroblasts. The latter finding suggests that fibroblast inflammatory memory is evolutionarily conserved. There was variation in the re-stimulation responses of other inflammatory mediators, suggesting that inflammatory memory of fibroblasts is tailored in a tissue-specific fashion. Interestingly, psoriatic skin fibroblasts displayed enhanced re-stimulation responses of several inflammatory mediators, whereas healthy skin fibroblasts did not. This points to pathological acquisition of memory in chronic inflammatory skin disease.

Finally, conditioned media were collected from synovial fibroblasts after first or second stimulation with TNF, and their effects on monocyte differentiation were tested. Conditioned media from re-stimulated fibroblasts promoted higher expression of TNF, lower expression of the M2 marker CD206 and lower phagocytic activity, consistent with skewing of monocytes towards pro-inflammatory M1 macrophage differentiation. Our data suggest fibroblast inflammatory memory to be widespread, functionally relevant, but heterogeneous in character. The discovery that psoriatic skin fibroblasts have seemingly acquired inflammatory memory may lead to new therapeutic targets in this condition, and may suggest novel links between inflammatory conditions in skin and joint [4]

Reference

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Keywords: Fibroblast, innate memory, trained immunity, rheumatoid arthritis, psoriasis

Resolution of inflammation

OP-46

ELUCIDATING THE MECHANISMS GOVERNING NEUTROPHIL REVERSE MIGRATION

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Rationale and hypothesis: There is currently no cure for respiratory inflammatory diseases including COPD, ARDS and cystic fibrosis. The excessive tissue damage characteristic of these diseases is caused by the inappropriate retention of neutrophils in the lung. The recent identification of neutrophil reverse migration as a mechanism of inflammation resolution and the ability to modulate this therapeutically has identified a potential new target to treat inflammatory disease. The pathways involved in modulating the reverse migration of neutrophils are not currently understood.

We hypothesise that neutrophil reverse migration is governed by retention signals generated by the chemokine receptor CXCR4 and its ligand CXCL12. This signalling axis has been reported to modulate neutrophil retention in bone marrow in humans, and in the caudal hematopoietic tissue of zebrafish. CXCR4/CXCL12 signalling is not thought to be involved in the recruitment of neutrophils to sites of injury or infection. Hence this work aims to identify a novel therapeutic target which will accelerate the resolution of inflammation by removing neutrophils from the lung without inhibiting their initial recruitment.

Methodology/principal findings: 3 dpf transgenic larvae were injured and those which mounted a good inflammatory response were treated with the CXCR4 antagonist AMD3100. Assays to measure inflammation resolution were performed using our *Tg (mpx: GFP) line*. CXCR4/CXCL12 expression was assessed throughout the inflammatory response using reporter zebrafish lines. We have generated a neutrophil specific CXCR4b reporter line using the GAL4/UAS system which we will use to assess receptor turnover in neutrophils at the wound site. We have an endogenous CXCL12a reporter which can be used to observe CXCL12a production throughout the inflammatory response. Neutrophil reverse migration will be measured using our *Tg (mpx: kaede)* line.

Assessment of neutrophil numbers at the wound site at 6, 8, 12 and 24 hours post injury have shown that larvae treated with AMD3100 have accelerated inflammation resolution ($n=20$ performed as 2 experimental repeats, $p<0.05$). Whole embryo neutrophil numbers were not altered in AMD3100 treated larvae compared to controls ($n=30$ performed as 3 experimental repeats). CXCL12a is found at the wound site in cells protruding out of the notochord extension, and is not detected in neutrophils at the wound. Future work aims to determine if the acceleration of inflammation resolution through the inhibition of CXCR4 is due to an increased rate of reverse migration.

Conclusions/significance: The findings of this study suggest that CXCR4/CXCL12 signalling may play an important role in neutrophil retention at the wound and interruption of this may lead to the reverse migration of neutrophils away from the wound. This identifies a potential new target for the therapeutic removal of neutrophils from the lung in chronic inflammatory disease.

Keywords: Reverse migration, retention signalling, inflammation resolution, zebrafish

Translational/Drug discovery

OP-47

TRPA1 ELICITS INFLAMMATORY EFFECTS IN CHONDROCYTES AND ITS EXPRESSION IS DOWNREGULATED BY ANTI-INFLAMMATORY DRUGS DEXAMETHASONE AND AUROTHIOMALATE

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Background: Transient receptor potential ankyrin 1 (TRPA1) is a membrane-associated cation channel, which is widely expressed in neurons and involved in nociception and neurogenic inflammation. Recently, TRPA1 has also been found in some non-neuronal cells, including keratinocytes and synoviocytes, but the functional roles of non-neuronal expression remain to be studied. TRPA1 is activated by exogenous pungent compounds but according to recent findings, also by ROS, RNS and some other factors formed endogenously in inflammatory and hypoxic conditions such as those in arthritic joints. We hypothesized that TRPA1 is expressed and functional in human chondrocytes, and that anti-inflammatory drugs regulate the expression of TRPA1.

Methods: TRPA1 expression in primary human OA chondrocytes and human T/C28a2 chondrocytes was measured by quantitative RT-PCR and Western Blot analysis. The functionality of the TRPA1 channel was assessed by Ca^{2+}-influx measurements. Production of MMP-1, MMP-3, MMP-13, IL-6 and PGE2 following TRPA1 activation were measured by immunoassay. Tissue from wild type and TRPA1 knock-out mice were used in mouse cartilage culture experiments.

Results: TRPA1 was expressed and inducible by IL-1β in primary human OA chondrocytes and human T/C28a2 chondrocytes. The TRPA1 channel was functional, as stimulation with the TRPA1 agonist AITC caused an increase in Ca^{2+}-influx, which was attenuated by the TRPA1 antagonist HC-030031. Genetic deletion of TRPA1 downregulated the production of MMP-3, IL-6 and PGE2 in murine cartilage explants. Further, pharmacological inhibition of TRPA1 with the selective antagonist HC-030031 downregulated the production of MMP-1, MMP-3, MMP-13, IL-6 and PGE2 in primary human OA chondrocytes.

Dexamethasone and aurothiomalate inhibited TRPA1 expression at the mRNA and protein level in human chondrocytes, while the other tested anti-inflammatory compounds (methotrexate, sulfasalazine, hydroxychloroquine and ibuprofen) had no effect. The downregulation was functional: TRPA1-mediated Ca^{2+}-influx was enhanced in chondrocytes which had been cultured in the presence of IL-1β while that effect was reversed in cells cultured with a combination of IL-1β and dexamethasone or aurothiomalate. NF-κB inhibitor PDTC also downregulated TRPA1 expression which, together with previous reports showing that dexamethasone and aurothiomalate inhibit NF-κB, suggests that these drugs may exert their effects on TRPA1 expression via inhibition of NF-κB activation.

Conclusions: The TRPA1 cation channel was found to be functionally expressed in primary human OA chondrocytes and immortalized human T/C28a2 chondrocytes, and to mediate inflammatory and catabolic effects. Furthermore, this study shows for the first time, that anti-inflammatory drugs dexamethasone and aurothiomalate downregulate the expression of TRPA1. These results reveal TRPA1 as a potential mediator and drug target in arthritis.

Keywords: TRPA1, chondrocyte, dexamethasone, aurothiomalate, matrix metalloproteinase, interleukin

Microbiomes

OP-48

A METABOLIC STUDY OF MICROBIOME MODULATION IN MICE

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The human gastrointestinal tract houses microbial species important for immune and metabolic homeostasis. Imbalance in composition/function of the intestinal microbiota is associated with metabolic disorders. We sought to determine the effects of microbiome modulation on body weight, blood glucose, and bone/tissue composition in a murine model of metabolic disease.

C57Bl/6NTac mice were pre-treated with antibiotic (Abx) or vehicle (veh), followed by a murine fecal microbial transplant (mFMT) or veh (Days 0 and 2). Animals were maintained on standard chow for three weeks (Days 0–21) and were weighed daily. Baseline pre-and post-prandial blood glucose and bone/tissue dual-energy x-ray absorptiometry (DEXA) measurements were taken. Animals within each group (veh+veh; Abx+veh; Abx+mFMT) were randomized by weight into sub-groups; sub-group diets were switched to high-fat (HF) or control. Body weight was observed for six weeks (Days 21–63), and pre-and post-prandial blood glucose and bone/tissue DEXA measurements were repeated.

Abx pre-treated animals had significantly ($p<0.005$) increased body weight gain when fed standard chow (Days 0–21) as compared to naïve animals regardless of mFMT treatment (veh+veh mean area under curve (AUC) 127.7; Abx+veh 295.4; Abx+mFMT 301.6). No significant differences in pre- or post-prandial blood glucose were found at the three-week time point. Significantly decreased mean bone mineral density (BMD), mean bone mineral content (BMC), and mean bone area (BA) were observed in both Abx+veh-treated animals (BMD 0.048 mg/cm2; BMC 0.384 mg/cm2; BA 7.88 cm2; $p<0.05$) and Abx+mFMT-treated animals (BMD 0.046 mg/cm2; BMC 0.360 mg/cm2; BA 7.88 cm2; $p<0.001$) as compared to naïve animals (BMD 0.051 mg/cm2; BMC 0.434 mg/cm2; BA 8.55 cm2). Abx+mFMT-treated animals had significantly ($p<0.05$) decreased mean lean tissue mass (LTM) as compared to naïve animals (15.1 g vs 14.4 g). All sub-groups fed HF diet had significantly increased body weight gain as compared to their representative control diet sub-groups, regardless of treatment. Significantly ($p<0.01$) increased body weight was observed in HF+Abx+mFMT animals as compared to HF+veh+veh animals (mean AUC 1.077 vs 755.0). No significant differences in pre-and post-prandial blood glucose were observed following HF or control diet maintenance, however a trend toward increased blood glucose was observed for animals maintained on HF diet for all treatment groups. No significant differences in BMD, BMC, BA, or LTM were observed either between treatment groups or sub-groups. All sub-groups maintained on HF diet, irrespective of treatment, demonstrated significantly increased mean fat mass and mean percent body fat compared to control diet sub-groups.

This data supports the hypothesis that modulation of the intestinal microbiome via abx administration, with or without mFMT treatment, affects body weight gain and bone/tissue composition, both when fed standard chow or HF/control diets.

Keywords: Microbiome, Metabolic Disorders, Dysbiosis, Antibiotic, FMT, DEXA

Percent Body Weight Change with or without Abx and/or mFMT

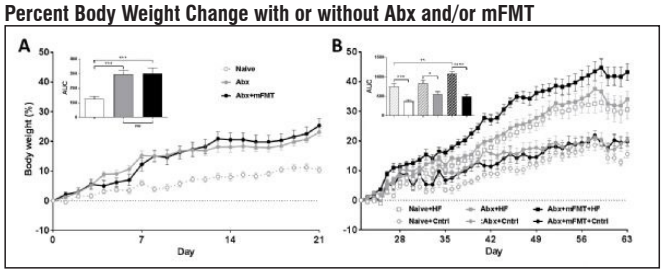

Figure 1. Animals were weighed daily throughout the study and their percent body weight relative to Day 0 **(A)** or Day 21 **(B)** was calculated. Statistical differences were assessed by one-way ANOVA with Tukey's multiple comparison test.

Microbiomes

OP-49

PROTECTIVE, ANTI-INFLAMMATORY EFFECTS OF THE MICROBIOME-ASSOCIATED METABOLITE PROPIONATE UPON THE BLOOD-BRAIN BARRIER

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Substantial epidemiological evidence indicates that consumption of a high fibre diet reduces the risk of developing neurovascular inflammatory disorders including stroke and vascular dementia, but the mechanisms underlying this association are unclear. The blood-brain barrier (BBB), the major interface between the circulation and the CNS, is critically involved in the pathogenesis of such disorders, and thus represents a key target for study. Complex carbohydrates found in a high fibre diet are primarily metabolised by gut bacteria into biologically active short-chain fatty acids (SCFAs), principally acetate, propionate and butyrate, which signal *via* the G protein coupled receptors FFAR2 and (except for acetate) FFAR3. Notably, FFAR3 is expressed on the vascular endothelium. Whilst studies have shown anti-inflammatory effects of high dose SCFAs upon leukocytes, these concentrations are usually only reached at infectious foci and it is unclear whether physiological levels of SCFAs are protective; moreover effects of SCFAs upon the BBB have not been studied.

We hypothesised that SCFAs would be protective for the BBB, limiting the response to inflammatory challenge. To test this hypothesis, we studied the effects of physiological concentrations (1 μM) of propionate upon the human immortalised cerebromicrovascular endothelial line hCMEC/D3. Preliminary experiments confirmed expression of FFAR3 upon these cells. We therefore performed an unbiased transcriptomic analysis of confluent hCMEC/D3 monolayers treated or not for 24 h with 1 μM propionate, supported by *in vitro* validation of key findings and assessment of functional endothelial permeability barrier properties.

Propionate treatment had a significant ($P_{FDR}<0.1$) effect on the expression of 1136 genes: 553 upregulated, 583 downregulated. Signalling pathway impact analysis of all differentially expressed genes identified over-representation of several inflammation-associated systems, with TLR-specific signalling, NFκB signalling and cytosolic DNA-sensing pathways all being inhibited by propionate. Additionally, gene ontology analysis with Enrichr indicated that propionate activated the Nrf2-driven protective response against oxidative stress. Functional validation of these findings confirmed the down-regulation of TLR signalling by propionate, achieved primarily through down-regulation of endothelial CD14 expression. Consequent to this, propionate prevented LPS-induced increases in paracellular permeability and loss of transendothelial electrical resistance.

Together, these data strongly suggest that the microbiome-associated SCFA propionate helps limit the vulnerability of the CNS to inflammatory damage, through down-regulation of BBB responsiveness. In addition to its well-described effects on

cholesterol metabolism, maintenance of propionate levels in the circulation may therefore be an additional mechanism whereby a high fibre diet protects against neurovascular disease.

Keywords: Blood brain barrier, SCFAs, inflammation, gut microbiota

Resolution of inflammation

OP-50

PLASMIN AND PLASMINOGEN INDUCE MACROPHAGE REPROGRAMMING AND REGULATE KEY STEPS OF INFLAMMATION RESOLUTION VIA ANNEXIN A1

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Inflammation resolution is an active process that functions to restore tissue homeostasis. Participation of the plasminogen/plasmin (Plg/Pla) system in the productive phase of inflammation is well known, but its involvement in the resolution phase remains unclear. Therefore, we aimed to investigate the potential role of Plg/Pla in key events during the resolution of acute inflammation and its underlying mechanisms. Plg/Pla injection into the pleural cavity of BALB/c mice induced a time-dependent influx of mononuclear cells that were primarily macrophages of anti-inflammatory (M2-F4/80 high Gr1-CD11bhigh) and proresolving (Mres-F4/80 med CD11blow) phenotypes, without changing the number of macrophages with a pro-inflammatory profile (M1-F4/80 low Gr1+ CD11bmed). Pleural injection of Plg/Pla also increased M2 markers (CD206 and Arginase-1) and secretory products (TGF-β and IL-6) and decreased the expression of iNOS (M1 marker). During the resolving phase of LPS-induced inflammation, when resolving macrophages predominate, we found increased Plg expression and Pla activity, further supporting a link between the Plg/Pla system and key cellular events in resolution. Indeed, Plg or Pla given at the peak of inflammation promoted resolution by decreasing neutrophil numbers and increasing neutrophil apoptosis and efferocytosis in a serine-protease inhibitor sensitive manner. Next, we confirmed the ability of Plg/Pla to both promote efferocytosis and override the pro-survival effect of LPS, via AnxA1. These findings suggest that Plg/Pla regulate several key steps in inflammation resolution, namely, neutrophil apoptosis, macrophage reprogramming and efferocytosis, which have a major impact on the establishment of an efficient resolution process.

Keywords: Resolution of inflammation, plasminogen/plasmin system, macrophage reprogramming, efferocytosis

Resolution of inflammation

OP-51

THE CANNABINOID RECEPTOR CB2 PLAYS A NON-REDUNDANT ROLE IN NEUTROPHIL RECRUITMENT IN ACUTE INFLAMMATION

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The cannabinoid receptor CB2 is a G protein coupled receptor (GPCR) activated by exogenous and endogenous cannabinoid signalling molecules that has been proposed to have an anti-inflammatory and/or immune-modulatory role in vivo. Unlike the CB1 receptor, which is predominantly expressed in the central nervous system, CB2 is expressed in peripheral tissues including B-lymphocytes, monocytes and neutrophils. CB2 specific agonists or antagonists have been used to study the role of the endocannabinoid system (ECS) in a number of pre-clinical models of disease including atherosclerosis, colitis, EAE and arthritis. Several papers have posited that CB2 agonists modulate monocyte/macrophage chemotaxis either directly or by interfering with macrophage chemotaxis to chemokines. Recently we provided compelling evidence that macrophage chemotaxis to a subset of CB2 agonists is mediated by a GPCR other than CB2 (Taylor L et al, Sci Rep. 2015; 5:10682).

In order to assess the role of CB2 in acute inflammation we studied innate immune cell mobilisation in Cnr2-/-mice in the dorsal air pouch model. Neutrophil recruitment was 4-fold higher in Cnr2-/-mice compared to littermate controls 6 hours post injection of 100 micrograms of zymosan. We repopulated lethally irradiated wild-type and Cnr2-/-mice with a 50:50 mixture of wild-type and Cnr2-/-knockout bone marrow and demonstrated that increased neutrophil CB2 recruitment was associated only with radiosensitive donor haematopoietic cells rather than radioresistant recipient stromal cells.

Our experiments reveal for the first time a non-redundant role for the CB2 cannabinoid receptor expressed on myeloid cells in limiting the magnitude and duration of the acute inflammatory response. In recent experiments we have shown that Cnr2-/-mouse bone marrow neutrophils adhere more tightly to immobilised ICAM-Fc in static adhesion assays compared to neutrophils from wild-type animals. We propose that CB2 receptor expression on neutrophils plays an important role in limiting PMN recruitment to sites of inflammation through changing the affinity of PMN integrins for cell adhesion molecules including ICAM-1.

Keywords: Inflammation, cannabinoids, CB2 receptor, neutrophils, air pouch model

Resolution of inflammation

OP-52

SRC HOMOLOGY 2 DOMAIN-CONTAINING PROTEIN TYROSINE PHOSPHATASE (SHP-1): ROLE IN POLARIZATION OF ADIPOSE TISSUE MACROPHAGES AND DEVELOPMENT OF FRET BASED ACTIVITY REPORTER

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Jamia Hamdard University

SHP-1 is a non-receptor protein tyrosine phosphatase expressed predominantly in the hematopoietic cell. SHP-1 has emerged as a promising therapeutic target in various disorders as it is a well-known regulator of many pathways e. g. cytokine signaling, macrophage function, inflammatory pathway and insulin signaling pathway. Keeping in mind, the role of SHP-1 in macrophage functions, we speculated that SHP-1 might play an important role in polarization of adipose tissue macrophages, the hallmark of obesity-induced insulin resistance. To evaluate the same, we developed obese insulin resistant c57bl/6 mice model (diet with 60% calories from fat, 20% from carbohydrates and 20% from proteins) for a period of 18 weeks and SHP-1 levels along with other metabolic and inflammatory parameters were compared with lean group (fed with normal rodent diet). The adipose tissue macrophages from high-fat diet group showed increased mRNA expression and activity of SHP-1. Further, the adipose tissue macrophages isolated from high-fat diet group displayed significantly enhanced expression of M1 macrophage surface marker (CD11 c) and intracellular markers (iNOS and STAT1) in comparison to lean group whereas the inhibition of SHP-1 with sodium stibogluconate significantly decreased CD11 c expression. This suggests that skewing of macrophage phenotype towards M1, at least in part, is mediated through SHP-1.

Further, we developed a FRET based activity reporter for SHP-1 (SHARP) that can be employed to visualize the SHP-1 activity in live cells for throughput screening of drugs targeting SHP-1. The conformational change in SHP-1 upon ligand binding provides an excellent tool to develop intramolecular FRET based reporter. SHP-1 was sandwiched between two fluorescent proteins i. e. cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP). The conformational change in purified chimeric protein (SHARP) was analyzed using phosphotyrosine peptide, LKpYLYLV, (derived from the SHP-1 binding motif of erythropoietin receptor) and a significant decrease in YFP/CFP ratio was observed suggesting activation induced conformational change in SHP-1 which was confirmed by pNPP assay. Further, RAW264.7 cells were transfected with SHARP and stimulated with LPS to mimic inflammatory milieu and FRET ratio was measured using acceptor photobleaching and spectral imaging approach. We observed a significant decrease in YFP/CFP ratio (FRET ratio) in LPS stimulated cells when compared with unstimulated cells. Additionally, we isolated adipose tissue macrophages from lean and high-fat diet group and transfected with SHARP. The significantly decreased YFP/CFP ratio demonstrated increased SHP-1 activation in high-fat diet group derived ATMs in comparison to lean group derived ATMs. Our study demonstrated an undefined function of SHP-1 in macrophage polarization and in future, SHARP may be utilized as a robust tool for the throughput screening of drugs targeting SHP-1.

Keywords: SHP-1, macrophage polarization, FRET

Graphical abstract

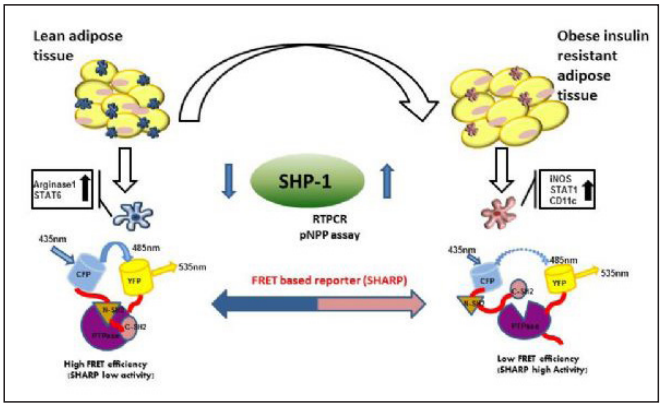

Figure 1. SHP-1, macrophage polarization and obesity induced insulin resistance

Resolution of inflammation

OP-53

NON-INVASIVE LUMINOL-BASED BIOLUMINESCENCE IMAGING AS A STANDARDIZED MEASURE OF INFLAMMATION IN ANIMAL MODELS FOR SKIN DISEASES

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Objectives: In the present study, we aimed to develop non-invasive bioluminescence imaging (BLI) as a standardized measure of inflammation using three mouse models of skin diseases. Delivery of luminol enables detection of myeloperoxidase (MPO)-mediated bioluminescence. We have tested the responses in the oxazolone (OXA)-induced delayed type hypersensitivity (DTH) model for atopic dermatitis, the imiquimod (IMQ) model for psoriasis and bleomycin (BLM) model for systemic sclerosis (SSc). DTH is an in vivo T cell-dependent immune response manifested as an inflammatory reaction that reaches peak intensity 24 to 48 hr after antigenic challenge. Imiquimod is a potent immune activator and has been shown to induce psoriasis-like symptoms. Chronic s. c. application of bleomycin to the lower back has been shown to induce skin fibrosis.

Material/Methods: In the OXA-induced dermatitis model, male CD-1 mice were sensitized by a single epicutaneous application of 50 μL of 6% OXA to the shaved abdomen and challenged (7 days later) by an application of 10 μL of 2% OXA to each side of one ear. Dexamethasone (DEX) was used as a positive reference compound (0.25%). Ear thickness and BLI (assessed with the IVIS) was measured after the challenge with OXA (at 6 h, 24 h, 48 h and 72 h). In the IMQ model, 65 mg of IMQ (5% cream) was applied to the back of female Balb/c mice. As a positive control, clobetasol (0.05%, 50 μl) was used. The severity of the inflammation was scored based on the clinical Psoriasis Area and Severity Index (PASI) and skin thickness (in vivo and by H&E staining ex vivo) and BLI were measured. In two BLM experiments, one using 5 days/week injections (0.25 mg/

mL in PBS, 100 μL) and the other by a weekly injection using a gel composed of ammonium sulfate, methylcellulose and protamine and loaded with BLM (3 mg/mL, 100 μL), BLI and collagen thickness (Masson Trichrome stain ex vivo) were measured. For BLI measurements, luminol sodium salt (50 mg/mL, 200 mg/kg) was injected intraperitoneally 20 min before imaging commenced.

Results: For DTH, peak BLI was observed 6 h after challenge, potentially reflecting early neutrophil infiltration, whereas peak ear thickness was observed 24 h after challenge. DEX reduced both ear thickness and BLI to normal. In both BLM models, BLM increased BLI and collagen thickness. IMQ induced PASI scores, increased BLI and skin thickness, an effect which was partially inhibited by clobetasol.

Conclusion: Traditionally, only in vivo ear thickness (DTH model), ex vivo epidermal thickness (BLM model) and in vivo PASI scores and ex vivo collagen thickness (IMQ model) are assessed. However, because inflammation is a multistep in vivo process, molecular imaging methods specific for different phases of inflammation allow noninvasive assessment of MPO activity as an in vivo marker of inflammation in a longitudinal manner as a valuable additional parameter.

Keywords: Bioluminescence, Bleomycin, Delayed-Type-Hypersensitivity, Imiquimod, Luminol, Myeloperoxidase

Representative images of the luminol-induced bioluminescence imaging (BLI) experiment in CD-1 mice in the IVIS 200 imaging system (Caliper Life Sciences)

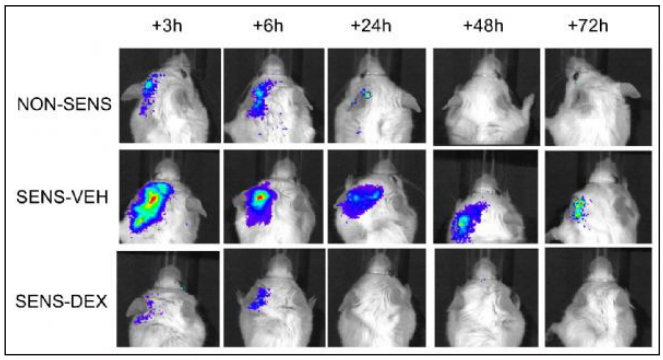

Figure 1. Effect of dexamethasone (DEX) in the model of Delayed Type Hypersensitivity (DTH) (NON-SENS non-sensitized; SENS sensitized)

Resolution of inflammation

OP-54

INHIBITION OF IκB KINASE ATTENUATES CARDIAC DYSFUNCTION CAUSED BY SEPSIS IN MICE WITH PRE-EXISTING TYPE 2 DIABETES MELLITUS

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Introduction: Patients with type 2 diabetes mellitus (T2DM) have an increased risk of developing infections. Activation of nuclear factor-κB (NF-κB) plays a crucial role in the cardiac dysfunction in both T2DM and sepsis. In this study we investigated i) if pre-existing T2DM worsens the cardiac dysfunction caused by sepsis and ii) whether pharmacological inhibition of IκB Kinase (IKK; upstream regulator of NF-κB) reduces sepsis-associated cardiac dysfunction in mice with pre-existing T2DM.

Methods: Ten-week old male C57BL/6 mice were fed with high fat diet (HFD; ≈60% energy from fat) or chow diet for 12 weeks, and then subjected to low dose lipopolysaccharide (LPS) (2 mg/kg, i. p.) and treated 1 hour later with either IKK-inhibitor (IKK-16; 1 mg/kg, i. v.) or vehicle (2% DMSO, 3 ml/kg, i. v.). At 18 hours after LPS-challenge, cardiac function (by echocardiography) and signalling events in cardiac tissue were measured.

Results: When compared to mice fed on a chow diet, mice fed on a HFD demonstrated a significant impairment in oral glucose tolerance test, indicative of the development of a diabetic phenotype. They also exhibited a small reduction in ejection fraction (EF), indicating the development of cardiac dysfunction. Cardiac tissue from mice fed on a HFD demonstrated a significantly increased phosphorylation of IKK and IκBα, translocation of p65 to the nucleus, hence activation of NF-κB, reduced Akt phosphorylation and increased iNOS expression.

When compared to mice fed on a chow diet, mice fed on a HFD and subjected to LPS-challenge, demonstrated a further significant decline in EF, increases in p65 translocation to the nucleus, and increase in iNOS expression as well as development of hepatocellular and renal injury.

The inhibition of IKK, however, resulted in a significant reduction in i) the cardiac dysfunction caused by LPS, (ii) phosphorylation of IKK and IκBα, iii) translocation of p65 to the nucleus, and iv) a significant increase in phosphorylation of Akt and reduction of iNOS expression (when compared with HFD-LPS-animals treated with vehicle).

Conclusion: Our data demonstrate that i) HFD results in cardiac inflammation and cardiac dysfunction, ii) pre-existing diabetic phenotype worsens cardiac dysfunction associated with sepsis and iii) inhibition of IKK reduces cardiac dysfunction associated with sepsis, by reducing activation of NF-κB and restoration of Akt pro-survival pathways in diabetic mice.

Keywords: IKK-16, Endotoxemia, Cardiac dysfunction, Type two diabetes mellitus

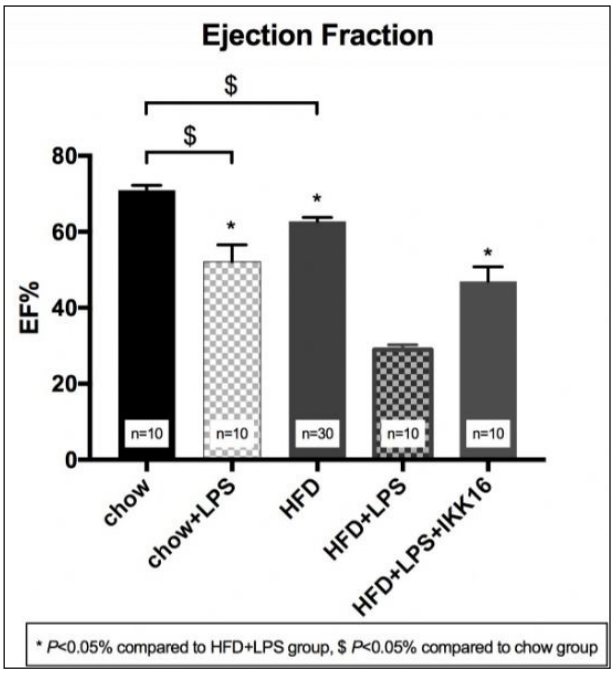

Figure 1. Effects of high fat diet, low dose LPS (2 mg/kg) administration and IKK-16 post-treatment on cardiac function in mice.

Resolution of inflammation

OP-55

ANNEXIN A1 (ANXA-1)-MIMETIC PEPTIDE CONTROLS THE INFLAMMATORY AND FIBROTIC EFFECTS INDUCED BY HOUSE DUST MITE (HDM) IN MICE

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Introduction: Asthma is an airway inflammatory response, driven by Th2 cells, marked by eosinophilic infiltration, bronchial hyper-reactivity, mucus exacerbation and peribronchiolar fibrosis. Endogenous glucocorticoid hormones are critical on their potent anti-inflammatory activity, a response partially dependent on the release of pro-resolving mediators such as AnxA1. This protein is shown to be secreted in respiratory fluid and reported to be up-regulated in asthmatic bronchial lavage fluid. In many inflammatory and cellular settings, the anti-inflammatory activity of AnxA1 is reproduced by peptides Ac2–26, derived from the N-terminal region of the protein. In this study we investigated the therapeutic properties of the N-terminal AnxA1-derived peptide Ac2–26 on experimental model of asthma induced by HDM in mice.

Methods: AnxA1 null and wild type littermate (Balb/c) mice were sensitized with intranasal instillation of house dust mite (HDM-25 μg/25 μL), every other day, during 3 weeks. In another set of experiments, wild type littermates were treated therapeutically with intranasal peptide Ac2–26 (200 μg/mouse) or budesonide (10μg/mouse), 1 h before antigen, starting on the week 2 of sensitization. Twenty four hours after the last challenge, lung function, inflammatory and fibrotic markers were measured.

Results: We found that HDM led to increased airways hyper-reactivity to methacholine and intense infiltration of leukocytes in the BALF. A marked eosinophil accumulation was noted in the peribronchial area as well as an excessive deposition of extracellular matrix. Increased tissue generation of inflammatory and fibrotic cytokines (IL-4, TGF-beta, eotaxin-1 and-2 and MCP-1) was also detected. A clear exacerbation of these pathological changes was observed in AnxA1 null mice as compared to the wild type littermate controls. Intranasal peptide inhibited HDM-induced airway hyper-reactivity and accumulation of leukocytes in the BALF. Ac2–26 also prevented other pathophysiological changes triggered by HDM in lung tissue including peribronchial eosinophil and neutrophil infiltration, subepithelial fibrosis, increased content of mucus and levels of cytokines. Treatment with budesonide was able to afford an inhibitory effect of HDM-induced lung function and morphological alterations, though being less effective that the peptide ac2–26 in some parameters.

Conclusion: Taken together, our findings show that AnxA1 null mice show an exacerbation of several aspects of asthma, indicating that AnxA1 plays a pivotal role in the negative regulation of features of severe asthma. In addition, The AnxA1-derived peptide Ac2–26 protects against several pathological changes associated with allergen provocation in wild-type mice, suggesting a pharmacological correlation that turns possible the development of a therapeutic agent for severe asthma.

Financial support: FIOCRUZ, CNPq, FAPERJ (BR) and European Community (UE FP7–2007–2013-n°HEALTH-F4–2011–281608).

Keywords: Lung, allergy, inflammation, fibrosis, Annexin-A1 peptide

Immunometabolism

OP-56

PRE-STIMULATION WITH IL-6 METABOLICALLY PREPARES T CELLS FOR THE ANTIGENIC RESPONSE

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T cells modulate their metabolism to support differentiation and activation, and to meet the bioenergetic and biosynthetic demands of their effector functions. The metabolic consequences of T cell activation have been studied in vitro and in vivo. However, activation requires integration of signals through the TCR, costimulatory receptors and cytokine receptors in the immune synapse, and the individual contributions of these signals to the metabolic phenotype of the T cell are not well understood. Indeed several such T cell activation stimuli are the targets of therapies in immune mediated inflammatory diseases. One example is interleukin-6 (IL-6), a cytokine produced by tissues in response inflammation and injury and which has important roles in CD4+ T cell differentiation and function. Blockade of IL-6 with tocilizumab is efficacious in rheumatoid arthritis. Here we demonstrate a novel role for this cytokine in metabolic priming of T cells in advance of activation through the TCR.

We showed that exposure of naïve, resting human CD4+ T cells to soluble IL-6/IL-6R potentiated their response to subsequent activation through the TCR using CD3/CD28 antibodies. Cells showed an increased capacity for proliferation, as measured by CFSE dilution and this was underpinned by an increased glycolytic response to activation, as measured by Seahorse analysis. NMR based metabolomics highlighted altered levels of several metabolites in response to IL-6 including glucose, lactate, glutamine, ATP, succinate and citrate, but this modulated metabolism was measurable only after T cell activation. This suggests that IL-6 primes metabolic change but does not directly regulate pathway usage. Whole genome array data of CD4+ T cells taken from the peripheral blood of early RA patients was stratified according to patient serum IL-6 levels at the time of cell harvest. These data highlighted MYC as the major gene, expression of which correlated with systemic IL-6. As a master regulator of metabolism, we are investigating the involvement of this pathway in metabolic priming of cells by cytokines.

Taken together, our findings suggest that the production of IL-6 in acute or chronic inflammation might modulate T cell metabolic machinery in preparation for meeting the bioenergetic and biosynthetic demands of an effective immune response.

Keywords: CD4+ T cell, metabolomics, glycolysis, interleukin-6

Immunometabolism

OP-57

HGK/MAP4K4 DEFICIENCY INDUCES TRAF2 STABILIZATION AND TH17 DIFFERENTIATION LEADING TO T-CELL-MEDIATED TYPE 2 DIABETES

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Proinflammatory cytokines play important roles in insulin resistance. Here we report that T-cell-specific conditional HGK (MAP4K4) knockout (T-HGK cKO) mice developed systemic inflammation and insulin resistance, which was ameliorated by either IL-6 or IL-17 neutralization. HGK directly phosphorylated TRAF2 at Ser-35, leading to lysosomal degradation of TRAF2 and subsequent inhibition of IL-6 production. HGK kinase activity was decreased and TRAF2 protein levels were increased under TCR signaling, suggesting that HGK maintains a resting state of T cells. Thus, HGK knockout T cells constitutively displayed overexpression of TRAF2 and overproduction of IL-6.

IL-6-overproducing HGK knockout T cells were attracted to adipose tissue by the CCL20-CCR6 axis; the accumulation of HGK knockout T cells in adipose tissue was blocked by CCR6 knockout. In adipose tissue, IL-6 secreted from HGK knockout T cells enhanced the levels of the adipokine leptin. The data derived from T-cell-specific leptin receptor/HGK double knockout mice or IL-6 KO/HGK cKO mice demonstrated that IL-6-overproducing HGK knockout T cells further differentiated into Th17 cells by a synergistic effect of leptin and IL-6. Adoptive transfer experiments further showed that these IL-6/IL-17 double-positive T cells were pathogenic cells for insulin resistance. Clinical samples from type 2 diabetes (T2D) patients were used to study the clinical relevance. Notably, HGK levels fell and IL-6 levels increased in T cells from T2D patients. The clinical significance of HGK-deficient T cells will be presented in the meeting. Taken together, HGK plays important roles in the generation of adipose-tissue Th17 cells and the pathogenesis of T-cell-mediated T2D.

Keywords: HGK/MAP4K4, TRAF2, IL-6, Th17 cells, type 2 diabetes

Immunometabolism

OP-58

SYNOVIAL FIBROBLASTS DISPLAY METABOLIC MEMORY AND BIOENERGETIC REPROGRAMMING DURING THE TRANSITION FROM RESOLVING TO PERSISTENT INFLAMMATORY DISEASE

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Synovial fibroblasts regulate healthy joint homeostasis as well as the initiation and resolution of local inflammatory processes. When activated in response to inflammation or injury fibroblasts are subject to a transient increase in bioenergetic and biosynthetic demand. However, it is poorly understood how sustained cellular activation and the associated high level of metabolic activity are maintained in diseases such as rheumatoid arthritis (RA) which are characterised by chronic, unresolving inflammation. We investigated metabolic changes which occur in fibroblasts early in RA pathogenesis and which support the transition to chronic inflammation in these patients.

Patients were recruited from the Birmingham early arthritis cohort (BEACON) and primary human fibroblasts cultured from ultrasound guided synovial biopsy tissue. Cells from patients with with a transient, resolving synovitis were compared to those with very early RA in which inflammation fails to resolve. Metabolic phenotypes were measured using 1D NMR spectroscopy based metabolomics, and glycolysis and oxidative phosphorylation were measured by Seahorse analysis. Mitochondrial fusion and fission dynamics were assessed by immunofluorescence after labeling with TOMM20.

We showed that changes in fibroblast metabolism are initiated in an inflammatory environment and reflected in their metabolomic profile after ex vivo culture; evidence for metabolic memory. We identified the importance of glucose metabolism in steady state fibroblasts and showed upregulation of glycolysis in response to acute TNFα stimulation regardless of disease status. Furthermore, we demonstrated that fibroblasts from patients with transient, resolving synovitis show increased mitochondrial dynamics and greater mitochondrial respiratory capacity in response to TNFα, when compared to cells from patients with inflammation that fails to resolve and who subsequently develop RA. This suggests that the morphology, dynamics and functionality of mitochondria may be of importance for the resolution of acute inflammation.

Our findings indicate that the modulation of mitochondrial function and restoration of fibroblast metabotype at sites of inflammation may have potential for the treatment of immune mediated inflammatory diseases such as RA.

Keywords: bioenergetics, fibroblast, rheumatoid arthritis, metabolomics, mitochondria

Other

OP-59

A NOVEL IRAK1/4 DUAL INHIBITOR PREVENTS INFLAMMATION IN RODENT MODELS OF AUTOIMMUNE DISEASE AND REVERSES LUPUS-LIKE DISEASE IN NZB/W F1 MICE

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by loss of immunological tolerance, hyperactivation of immune cells, proinflammatory cytokine production and, ultimately, end organ damage due to immune complex deposition. Toll-like receptors (TLRs), which are essential to the innate immune response to microbes and other danger signals, play a key role in the pathogenesis of SLE by recognition of self-molecules. IRAK4 is responsible for initiating MyD88-dependent signaling from most TLRs and Interleukin-1 Receptors (IL-1R), resulting in downstream production of pro-inflammatory

cytokines. Through cell-based screening, we have identified a potent small molecule IRAK1/4 kinase inhibitor that blocks TLR4- and IL-1R-induced cytokine production with potencies less than 100nM. R191 exhibits good selectivity against a broad panel of kinases. In vivo, R221, the prodrug of R191, decreases serum IL-6 in an acute mouse model of IL-1β-induced cytokine release, demonstrating excellent pharmacokinetic properties. In addition, our IRAK1/4 inhibitor suppresses inflammation in the chronic rat model of collagen-induced arthritis and in the mouse model of imiquimod-induced psoriasis. Finally, treatment of NZB/W F1 lupus-prone mice with R221 reverses the progression of lupus-like disease and the establishment of a pro-inflammatory environment, as demonstrated by decreased levels of proteinuria, blood urea nitrogen and autoantibodies, and reversal of renal pathology. The in vitro and in vivo characterization of our IRAK1/4 inhibitor is promising and confirms IRAK1/4 as an attractive therapeutic target for the management of autoimmune and inflammatory diseases.

Keywords: IRAK1/4, small molecule inhibitor, autoimmunity, inflammation, lupus

Other

OP-60

SENSORY NERVES AND MEDIATORS HAVE PROTECTIVE ROLES IN ALDARA-INDUCED SKIN INFLAMMATION AND ASSOCIATED NOCIFENSIVE BEHAVIOURS

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Psoriasis is a common chronic skin inflammation affecting 2–3% of the UK population, with itching commonly reported. Sensory nerves have been clinically associated the pathogenesis of psoriasis, as denervation has resulted in an improved skin condition (1). Using a recent murine model of psoriasis involving repeated application of Aldara cream on dorsal skin (3), we investigated the role of sensory nerves in this model. This was achieved by pharmacological and genetic ablation of sensory biomarkers/mediators, including Transient Receptor Potential Vanilloid 1 (TRPV1), Substance P (SP) and Calcitonin Gene Related Peptide (CGRP).

In vivo procedures were carried out according to the UK Home Office Animals (Scientific Procedures) Act 1986. Male WT C57BL/6J or TRPV1 knockout (KO), (n=5–7 per group, 6–8 weeks) mice were anaesthetised under 2% isoflurane during daily treatment. Sensory denervation was performed by daily injection of the ultrapotent TRPV1 agonist resiniferatoxin (RTX) (0.3 mg/kg, s. c). In separate experiments, Aprepitant (SP antagonist, 10 mg/kg, i. p.) and BIBN4096 (CGRP antagonist, 3 mg/kg, i. p.) or vehicle (5% DMSO in saline) were co-administered 30 minutes prior to topical cream application daily. Following dorsal skin hair removal, 75 mg of Aldara™ (5% imiquimod) cream (Meda Pharma, UK) or Vaseline was applied over a 2x2 cm2 dorsal skin area daily for 4 days and results were shown at 4 days. Doublefold skin thickness was used as a measure of skin inflammation. Skin erythema was assessed by blood flow measurement using the Full Field Laser Perfusion Imaging technique. Spontaneous nocifensive behaviours, measured as mouse scratching/licking movement, were observed for 30 minutes 4 hours post-skin treatment on day 4. The study was terminated by cervical dislocation.

RTX-mediated sensory denervation resulted in significantly attenuated erythema and skin thickening consistent with previous finding (2). TRPV1, SP, and α-CGRP mRNA expression in the dorsal root ganglion (DRG) were significantly reduced following RTX denervation (Table 1). The Aldara™-induced skin changes, including skin thickening, were not attenuated in the TRPV1 KO or neuropeptide antagonists-treated groups. However, sensory neuropeptides play important roles in the skin inflammation-associated nocifensive behaviours as neuropeptides antagonists and TRPV1 KO animals showed significantly reduced behaviours (Figure 1), independent of skin inflammation.

We conclude that the protective role of sensory denervation in Aldara-induced skin inflammation is independent of TRPV1 and neuropeptides. However, inhibiting neuropeptides activity is effective in reducing nocifensive behaviours in this model.

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Keywords: Aldara-induced skin inflammation, sensory nerves, Psoriasis, skin, TRP channels, neuropeptides

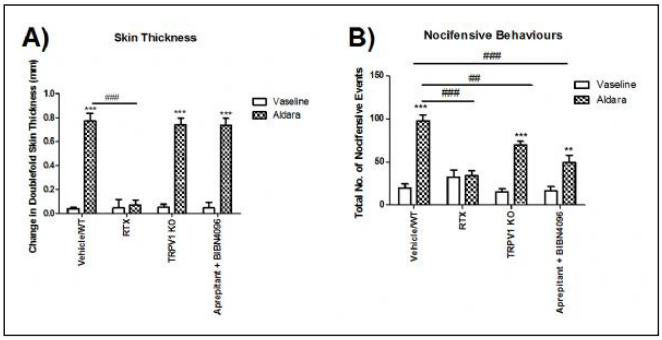

Figure 1. A) Change in skin thickness (at day 4 compared to day 0), **B)** Total number of spontaneous nocifensive behaviours (scratching, licking, and flinching) observed at 4 hours post-skin application for a total of 30 minutes. ** P<0.01, *** P<0.001 vaseline vs Aldara, ## P<0.01, ### P<0.001 between indicated groups. Mean±SEM, statistical analysis used was Two-Way ANOVA with Bonferonni's post hoc test, n=5–7 per group.

Table 1. Effect of RTX-mediated sensory denervation on TRPV1, αCGRP, and SP mRNA expression in the DRG.

	Vaseline		*Aldara*	
	Vehicle	*RTX-treated*	*Vehicle*	*RTX-treated*
TRPV1	1899.9±296.8	305.5±51.6 ###	1536.2±271.3	173.2±19.3 ###
α-CGRP	5070±441.8	2375.8±361.9 #	1579.7±221.1 ***	1364.6±197 ***
SP	8564.8±920.0	1870.6±227.5 #	6366.5±1711.7	1771.4±285.2#

*** P<0.001 Vaseline vs Aldara, # P<0.05, ### P<0.001 Vehicle vs RTX. Mean±SEM, statistical analysis used was Two-Way ANOVA with Bonferonni's post hoc test, n=5–7 per group.

Other

OP-61

DIET PROMOTED INTRA-HEPATIC INFLAMMATORY FOCI REPRESENTING A MODEL OF NONALCOHOLIC STEATOHEPATITIS (NASH)

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Introduction: The most prevalent form of chronic liver disease is non-alcoholic fatty liver disease (NAFLD) affecting one third of the adult population in developed countries. About 20% of NAFLD cases progress to chronic liver inflammation that is termed non-alcoholic steatohepatitis (NASH). A diet rich in cholesterol is a risk factor for NASH.

Objectives: Despite the fact that NASH is an inflammation driven disease, the underlying role of immune responses to diet in promoting NASH is obscure. The aim of this study was to investigate whether and how cholesterol alters the hepatic immune signature pertain to liver inflammation and NASH.

Methods: At 10 weeks old, male C57BL6 mice were given an atherogenic diet (2% cholesterol, 0.5% cholate and 33% sucrose) for a period of 12 weeks in order to induce liver inflammation. Adiposity, hepatic enzymes and metabolic parameters were investigated. The immune signature within the liver and spleen was examined using immunostaining, immuno-flow cytometry and nCounter nanostring gene expression analysis.

Results: The atherogenic diet caused histological and pathological features of NASH including hepatic steatosis, ballooning and inflammation. Higher liver/total body weight ratio and an increase in the serum ALT and AST levels ($p<0.05$) were also observed in mice fed the atherogenic diet. H&E staining showed cellular ballooning (similar to human NASH), infiltration of immune cells in the liver, and inflammatory foci formation. Nanostring mRNA expression analysis demonstrated that the atherogenic diet significantly up-regulated the hepatic expression levels of F4/80, CD11b, CD11 c, CD3 and B220 (all P value<0.001). Immunophenotyping using flow cytometry revealed a significant increase in myeloid cell populations (including inflammatory macrophages, monocytes and dendritic cells) and effector memory CD4 and CD8 T cells in the atherogenic diet group.

Conclusion: Both innate and adaptive immune responses are present in the hepatic infiltrate that arises in the context of an atherogenic diet model of NASH. This suggests a critical role for diet composition and especially dietary cholesterol in altering the hepatic immune signature leading to the development of NASH.

Keywords: Nonalcoholic Steatohepatitis (NASH), Intra-hepatic Myeloid population, Intra-hepatic Immune Foci

Other

OP-62

MELANOCORTIN 1 RECEPTOR MODULATES SKIN REPAIR BY DRIVING WOUND ANGIOGENESIS AND LYMPHANGIOGENESIS

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Wound repair is a multistep process consisting of haemostasis, inflammation, tissue regrowth and remodelling. Chronic skin wounds appear to become 'stuck'within the inflammatory phase of healing and are thus associated with a chronic inflammation, elevated oxidative stress and poor angiogenesis and lymphangiogenesis. Melanocortin 1 receptor (MC1R) is an anti-inflammatory receptor expressed in skin, however, its role in cutaneous wound repair is unknown. Here we demonstrate that the selective MC1R agonist (MC1R-Ag), BMS-470539 modestly accelerates wound closure, whilst altering the quality (alignment) of collagen deposited by dermal fibroblasts to reduce scarring. Surprisingly, wound treatment with MC1R-Ag profoundly enhances both angiogenesis and lymphangiogenesis with increased blood and lymphatic vessel density. MC1R-Ag appears to trigger a wound macrophage phenotypic shift with improved iron clearance from the wound bed, reduced wound oxidative stress and TNFalpha expression. In a model of impaired healing, induced by elevated oxidative stress, MC1R-Ag partially rescues the defective wound angiogenic response resulting in accelerated skin repair. Our data suggest that harnessing the MC1R pathway may represent a novel therapeutic strategy to improve skin repair, particularly in the context of aberrant healing responses.

Keywords: wound repair, skin, angiogenesis

Pain

OP-63

EVIDENCE FOR AN IN VIVO PROTECTIVE EFFECT OF ENDOGENOUS TRPC5 IN RHEUMATOID-AND OSTEO-ARTHRITIS

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Transient Receptor Potential Canonical 5 (TRPC5) is a non-selective Ca2+ channel member of the TRP super family. We are investigating the role of TRPC5 in murine models of arthritis using protocols that have been conducted under United Kingdom Home Office Animals (Scientific Procedures) Act 1986.

We found that the expression of TRPC5 was reduced in the synovium ($p<0.01$) in murine CFA-induced arthritis over 14 days. This was accompanied by a painful and pro-inflammatory profile in the TRPC5 knockout (KO) compared to wildtype (WT) mice with enhanced primary (weight-bearing) and secondary (mechanical) hyperalgesia. The pro-inflammatory profile was associated with increased levels of swelling, MCP-1 and TNF

and matrix metalloproteases (MMP2 and MMP3). The results were supported by use of the selective TRPC4/5 antagonist ML204. Analysis of human joint tissue with defined arthritis revealed that TRPC5 expression was reduced in osteo-arthritis (OA) as well as rheumatoid arthritis (RA); (Alawi et al. 2017).

This led us to hypothesise that TRPC5 may also be involved in the pathogenesis of osteo-arthritis (OA). To examine this, the partial meniscectomy (PMNX) model of OA was used. Pain behaviour and gait assessments were carried out weekly after recovery from surgery and significance assessed by 2-way ANOVA + Bonferroni post hoc test. TRPC5 KO PMNX mice compared to WT PMNX mice demonstrated enhanced hyperalgesia compared to during the chronic phase (days 42–56; $n=7$; $p<0.05$). Walking patterns were obtained using the automated CatWalk XT system (Noldus Information Technology, Netherlands) to provide paw print and heat maps of baseline gait parameters in WT and TRPC5 KO mice. The time-course of step cycle, a gait parameter associated with the time between two consecutive contacts of the hindpaw was reduced in TRPC5 KO compared with WT PMNX mice ($n=7$; $p<0.05$ from 42–77 days) whilst this was unchanged in sham-operated WT and TRPC5 KO mice ($n=3$–4).

This provides the first in vivo evidence of the contribution of TRPC5 to arthritic conditions. These results provide novel evidence of a mechanism by which loss or blockade of TRPC5 leads to exacerbated nociceptive symptoms in keeping with increased pain in RA and OA.

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Supported by Arthritis Research UK

Keywords: arthritis, pain, inflammation, TRP, murine models

Pain

OP-64

AMELIORATION OF EXPERIMENTAL ARTHRITIC PAIN AND DISEASE BY G-CSF RECEPTOR BLOCKADE

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Background: Granulocyte-colony stimulating factor (G-CSF or CSF-3) is a regulator of granulocyte lineage development and can play a role in inflammation due to its effects on mature lineage populations. In the current study, the efficacy of an anti-G-CSF receptor (G-CSFR) mAb was compared with that of a neutrophil-depleting mAb, anti-Ly6G, in ameliorating arthritic pain and disease was determined in both adaptive and innate immune-mediated murine arthritis models, and the ability of G-CSF itself to drive arthritic pain and disease investigated.

Methods: The T cell-dependent antigen-induced arthritis (AIA) and the innate immune-mediated zymosan-induced arthritis (ZIA) and methylated BSA (mBSA)/IL-1 arthritis models were induced in mice treated either prophylactic or therapeutically with an anti-G-CSFR mAb or a neutrophil-depleting mAb, anti-Ly6G. To determine whether G-CSF itself could drive arthritic pain and disease a new G-CSF-driven (mBSA/G-CSF-intraarticular mBSA (day 0) and subcutaneous G-CSF (days 0–2)) arthritis model was established. Pain was measured by differences in hind limb weight distribution and arthritis by histology. Cell populations were monitored by flow cytometry and joint gene expression by qPCR.

Results: Arthritic pain and disease were ameliorated in AIA, ZIA and mBSA/IL-1 arthritis by both prophylactic and therapeutic anti-G-CSFR mAb treatment, whereas only prophylactic anti-Ly6G mAb treatment was effective. Reduced pain and disease correlated with reduced joint neutrophil numbers and benefit was noted without necessarily the concomitant reduction in circulating neutrophils. The new G-CSF-driven mBSA/G-CSF arthritis model induced arthritic pain and disease, with pain being blocked by a cyclooxygenase-2 inhibitor, suggesting an indirect effect on neurons. This was supported by the fact that dorsal root ganglion (DRG) neurons cultured in G-CSF failed to respond to G-CSF in vitro and csf3r gene expression could not be detected in DRG neurons by single cell RT-PCR.

Conclusion: These data suggest that G-CSFR/G-CSF targeting, where local but not systemic neutrophil numbers are reduced, may be a safe therapeutic strategy for arthritis and other inflammatory conditions, particularly those in which pain is important.

Keywords: Inflammation, Pain, arthritis, G-CSFR

Pain

OP-65

DIMETHYL FUMARATE REDUCES TACTILE ALLODYNIA IN A HCAR2-MEDIATED MECHANISM IN TWO MODELS OF PERIPHERAL NEUROPATHIC PAIN

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Neuropathic pain is a debilitating disease and its management is currently focused only on reducing symptoms, generally by suppressing neuronal activity. However, neuropathic pain has many features of a neuroimmune disorder, and modulating the immune response may offer new opportunities for a more successful management. Dimethyl fumarate (DMF) is a fumaric acid ester that is effective in the treatment of relapsing/remitting multiple sclerosis. Its potential neuroprotective effect has been attributed to the activation of the antioxidative transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway. Beside this mechanism a possible effect mediated by the HCA2 receptor has been proposed. In this study, we have investigated the effect of DMF (administered in both acute and chronic regimen) on the

tactile allodynia, in two different genders and two models of neuropathic pain: chronic constriction injury (CCI) and spared nerve injury (SNI). We also measured the DMF capability to modulate the ascending and descending pain circuitries by recording the pro-nociceptive ON cells in the periaqueductal grey area-rostral ventromedial medulla axis (PAG-RVM-axis) through single unit extracellular recording in vivo. The results showed that DMF single injection did not alter the normal thermal threshold in both male and female mice. Moreover, both male and female mice developed alteration of mechanical threshold after CCI or SNI induction. Single injection of DMF reduced the tactile allodynia, measured in the ispilateral paw, at peak mechano-allodynia day 7. The antiallodynic effect, that lasted at least 90 minutes, was greater in female as compared to male mice. In fact, while the effective dose of DMF was 150 mpk in male, half dose (75 mpk) already reduced tactile allodynia in female mice. However, in both male and female mice the lowest dose (30 mpk) was ineffective, whereas highest dose (300 mpk) lost its efficacy. Interestingly, the effectiveness of DMF single injection in reducing allodynia was much greater in CCI model as compared to SNI. Finally, our recordings, confirmed previous evidence of pro-nociceptive changes in spontaneous and mechanical-evoked activityof ON-cells, in SNI mice, 7 days after nerve injury. A single intra-PAG microinjection or repeated oral administrations of DMF (75 and 150 mg/Kg) restored in a dose dependent manner the RVM ON-cells SNI-induced hyperexcitability in female mice. These findings, highlighted the importance of different type of immune cells in the genesis of allodynia in male and female gender, and showed the anti-neuropathic profile of DMF in an animal models of mononeuropathies. DMF, beside the immunomodulatory effect for relapsing-remitting multiple sclerosis (MS), may represent a promising target for neuropathic pain therapy.

Keywords: neuropathic pain, immune system, HCAR2

Pain

OP-66

PYRAZINE-FUSED TRITERPENOIDS BLOCK TRPA1 ION CHANNEL *IN VITRO* AND INHIBIT TRPA1-MEDIATED ACUTE INFLAMMATION *IN VIVO*

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TRPA1 is a cation channel expressed predominantly in non-myelinated nerve endings. TRPA1 has a significant role in sensing chemical and mechanical pain and according to the more recent findings, also in inflammation. We have recently shown that pharmacological blockade and genetic depletion of TRPA1 alleviates inflammation and pain in murine models of gout and (osteo) arthritis. Furthermore, TRPA1 is expressed in human articular chondrocytes and synovial cells in inflammatory conditions, and mediates inflammatory and catabolic responses in cell culture.

Triterpenoids are natural compounds, which have been shown to have anti-inflammatory and anti-cancer properties. In the present study, we synthesized derivatives of betulin, an abundant triterpenoid of the genus *Betula* and investigated their effects on TRPA1. In the initial screening based on Fluo 3-AM intracellular Ca^{2+} measurements, six of the fourteen tested triterpenoids had a statistically significant effect in blocking TRPA1 at 10 μM concentration. In further studies, the two most potent compounds had a dose-dependent, reversible and voltage-dependent antagonistic effect on TRPA1, based on whole-cell patch clamp recordings. Interestingly, the TRPA1 antagonistic activity of these two triterpenoid derivatives translated well also to *in vivo* conditions, as these compounds significantly attenuated TRPA1-mediated acute inflammatory paw edema in mice. The results introduce betulin-derived pyrazine-fused triterpenoids as effective novel blockers of TRPA1 with potential for treatment of TRPA1-mediated adverse conditions such as arthritis and arthritis-related pain.

Keywords: TRPA1, inflammation, pain, triterpenoid, arthritis

Resolution of inflammation

OP-68

ANTI-INFLAMMATORY AND ANTINOCICEPTIVE POTENTIAL OF OPUNTIA DILLENII AND ITS PURE COMPOUNDS: OPUNTIOL AND OPUNTIOSIDE VIA EICOSANOIDS AND CYTOKINES INHIBITION PATHWAYS

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Objective: Opuntia dillenii (Nagphana) is one of the edible plant, use in folklore medicine to cure inflammation. In present study the anti-inflammatory and analgesic properties of O. dillenii cladodes methanol extract, its fractions and pure α-pyrones (opuntiol and opuntioside) were evaluated.

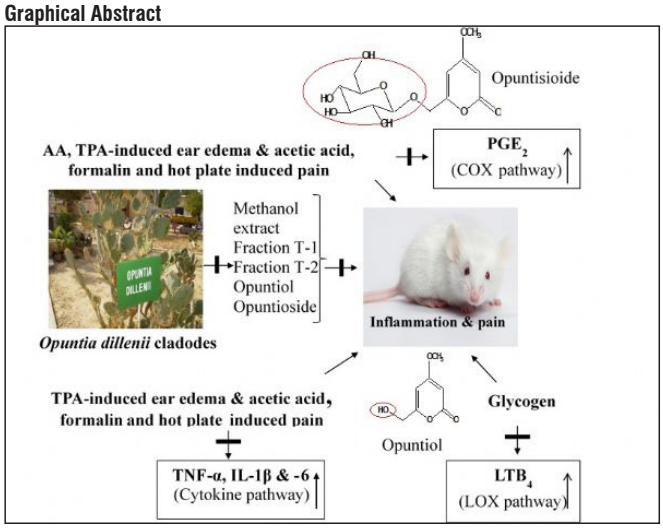
Methodology: O. dillenii derived test agents were evaluated for anti-inflammatory assay: arachidonic acid (AA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced ear edema were used for detection of prostaglandin E2 (PGE2), cytokines [tumor necrosis factor (TNF-α), interleukins IL-1 and-6] via ELISA. Glycogen-induced levels leukotriene B4 (LTB4) were measured via HPLC and reactive oxygen species (ROS) using 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA) dye. Histological studies of ear punches were also carried out. For analgesic assays acetic acid, formalin and hot plate induced writhes, paw licking and jumping responses respectively, were evaluated. To determine the participation of opioid receptors, naloxone (opioid antagonist) was used.

Result: O. dillenii methanol extract, its fractions in different solvents and pure compounds attenuated the ear edema and reduced inflammatory features induced by irritants AA and TPA. Opuntioside was 1.3~ most potent in reducing PGE2 levels than opuntiol. Opuntiol most effectively suppressed LTB4, TNF-α, IL-1β,-6 and ROS, levels by ~50% and the results are comparable to dexamethasone. Analgesic assays acetic acid-induced increase number of writhes were reduced by O. dillenii test compounds with opuntioside (IC50:26 ± 0.9 mg/kg) being most effective and comparable to diclofenac. Consistently, during early and late phases of formalin test opuntioside (IC50:28 ± 1.1 and 24 ± 1.2 mg/kg) elicited most potent effect in suppressing the paw licking response and against hot plate test producing similar effect to diclofenac and indomethacin. The extract and fractions have no effect on reversed the analgesic effects when naloxone used, whereas, opuntiol and opuntioside antagonize the analgesic effects.

Conclusion: Edible O. dillenii methanol extract, its fractions, opuntiol (aglycone) and opuntioside (glycoside) reduced acute inflammation. Opuntiol displayed as dual inhibiting properties of cyclooxygenase (COX) and lipooxygenase (LOX) pathways. It also reduced ROS and inflammatory cytokine levels. Opuntioside emerged as more pronounced effect towards COX (PGE2) pathway. It also demonstrated pain relieving effect at both peripheral and centrally level via opioid and non-opioidergic systems. The non-opioidergic analgesic effect possibly by other centrally mediated pathways such as via COX-2 pathway. Thus opuntiol and opuntioside may assist as principal compounds as new anti-inflammatory and analgesics agents in drug designing.

Keywords: Inflammation, Pain, Opuntia dillenii, Cytokines, Cyclooxygenase

Graphical Abstract



Resolution of inflammation

OP-69

HYDROALCOHOLIC CRUDE EXTRACT OF *CASEARIA SYLVESTRIS* SW. REDUCES CHRONIC POST-ISCHEMIC PAIN BY ACTIVATION OF PRO-RESOLVING PATHWAYS

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Introduction: *Casearia sylvestris* Sw. is widely used in popular medicine to treat conditions associated with pain. We have previously reported that the hydroalcoholic crude extract from this plant (HCE-CS) exert several actions on specific responses of pain and inflammation. Antinociceptive actions were evident in animal models of nociceptive, inflammatory and immune mediated pain (J Ethnopharmacol. 112 (1): 1–6, 2007); furthermore, the HCE-CS was also endowed with anti-inflammatory properties in the model of carrageenan-paw edema in mice, as well as in pleurisy model in rats reducing neutrophil recruitment and nitrite/nitrate production in pleural exsudate (J Ethnopharmacol. 147 (3): 612–7, 2013).

Aim of the study: The present study investigated the influence of HCE-CS and contribution of pro-resolving mediators on mechanical hyperalgesia in a mouse model of chronic post-ischemia pain (CPIP).

Methods-Results: Male Swiss mice were subjected to ischemia of the right hind paw (3 h), then reperfusion was allowed. At 10 min, 24 h or 48 h post-ischemia/reperfusion (I/R), different groups of animals were treated with HCE-CS (30 mg/Kg, orally [p. o]), selected agonists at the pro-resolving receptor ALX/FPR2 (natural molecules like resolvin D1 and lipoxin A4 or the synthetic compound BML-111; 0.1–1 μg/animal) or vehicle (saline, 10 mL/Kg, s. c.), in the absence or presence of the antagonist WRW4 (10 μg, s. c.). Mechanical hyperalgesia (paw withdrawal to von Frey filament) was asseseed together with histological and immunostainning analyses. In these settings, pro-resolving mediators reduced mechanical hyperalgesia and HCE-CS or BML-111 displayed anti-hyperalgesic effects which was markedly attenuated in animals treated with WRW4. ALX/FPR2 expression was raised in skeletal muscle or neutrophils after treatment with HCE-CS or BML-111.

Conclusion: These results reveal significant antihyperalgesic effect of HCE-CS and pro-resolving mediators on CPIP, mediated at least in part, by the pathway of resolution of inflammation centred on the axis modulated by ALX/FPR2.

Keywords: Casearia sylvestris, Salicaceae, Chronic post-ischemia pain, Resolution of inflammation, ALX/FPR2

Resolution of inflammation

OP-70

INHIBITION OF IL-13RA2 PROTECTS MICE FROM DSS-INDUCED MURINE IBD

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The pro-inflammatory cytokines tumor necrosis factor (TNF) α and interleukin (IL)-17 are important drivers of inflammatory bowel disease (IBD). We have previously found that TNFα and IL-17 synergistically induce expression of the IL-13 decoy receptor (IL-13Rα2), which is expressed by fibroblasts, epithelial cells, and smooth muscles in the gut. IL-13Rα2 binds IL-13 with high affinity but does not signal, thereby reducing IL-13 bioactivity. Elevated IL13RA2 transcripts have been measured in IBD patients who do not respond to anti-TNFα therapy. We hypothesize that the increased expression of IL-13Rα2 neutralizes the endogenous anti-inflammatory and wound healing activity of IL-13 signaling leading to persistent activation of pro-inflammatory cytokines and uncontrolled IBD. We used a dextran sodium sulfate (DSS)-induced model of murine colitis in IL-13Rα2-/-mice to investigate the impact of IL-13Rα2 deficiency on IBD. We found DSS increases the expression of IL-13Rα2 in colons of wild-type mice, and that DSS-induced colitis was less severe in IL-13Rα2-/-mice compared to wild-type controls. IL-13Rα2-/-mice exhibited less colon shortening, goblet cell depletion, inflammatory cell infiltration, and submucosal inflammation. Expression of pro-inflammatory genes was consistently decreased in colon tissue of IL-13Rα2-/-mice. Collectively, our data indicate that IL-13Rα2 is a critical regulator of DSS-induced murine IBD. Abrogating IL-13Rα2 shuts down the pro-inflammatory cascade during and following DSS-mediated injury likely because of increased IL-13 bioactivity. Our findings highlight the ability of IL-13 and IL-13Rα2 to control inflammation in the colon. They also suggest IL-13Rα2 is a putative therapeutic target, particularly for IBD patients who do not respond to anti-TNFα therapy.

Keywords: colitis, IBD, IL-13, gut, immunology

Signalling molecules and pathways

OP-71

CAMP ENHANCING DRUGS SALBUTAMOL AND ROLIPRAM AUGMENT THE ALTERNATIVE ACTIVATION OF MURINE MACROPHAGES THROUGH MKP-1

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Macrophages play a central role in inflammation and host defense and take part in tissue repair. They possess remarkable diversity and plasticity and respond to environmental cues with distinct functional phenotypes. These phenotypes have traditionally been described by two main functional subsets: classically (M1) and alternatively (M2) activated macrophages. M1 macrophages are characterized by expression of high levels of proinflammatory cytokines and promotion of Th1 response whereas M2 macrophages are known to be involved in resolution of inflammation and wound healing. Aberrant activation of proinflammatory M1 macrophages has been shown to be involved in the pathogenesis of autoimmune diseases such as rheumatoid arthritis. On the other hand, dysregulation of the matrix-enhancing activity of M2 macrophages may lead to excessive collagen synthesis causing fibrosis. Autoimmune diseases as well as fibrotic disorders are devastating diseases with wide-ranging negative effects on quality of life with severe unmet needs in their treatment.

In the present study we investigated the effects of cAMP enhancing drugs salbutamol and rolipram on alternative activation of macrophages. We also studied whether mitogen activated protein phosphatase 1 (MKP-1) takes part in the polarization of macrophages because we have recently shown that cAMP enhancing drugs increase also MKP-1 expression. MKP-1 has a relatively well established role in classical macrophage activation and it acts as an important negative regulator on p38 MAPK and JNK activity and therefore controls the production of many inflammatory factors.

Polarization of macrophages towards alternative activation is classically induced by Th2 cytokines IL-4 and IL-13. In cultured murine macrophages salbutamol, rolipram and their combination enhanced IL-4 and IL-13 induced expression of arginase 1, Ym-1 and MCR-1, all known markers of alternative activation. These cAMP enhancing drugs also increased MKP-1 mRNA and protein expression in untreated and IL-4 and IL-13 treated murine macrophages. To investigate whether MKP-1 mediates the effects of cAMP enhancing drugs on alternative activation, we used peritoneal macrophages derived from MKP-1 deficient and wild type mice. The enhancing effects of salbutamol, rolipram and their combination on markers of alternative activation were abolished in peritoneal macrophages from MKP-1 deficient mice indicating a role for MKP-1.

Our data suggests that cAMP enhancing drugs, such as β2 agonists and phosphodiesterase 4 inhibitors, direct macrophage polarization towards the alternative phenotype and that this effect is, at least partly, mediated by MKP-1. Our results provide new knowledge on the signaling pathways governing macrophage polarization and their functional phenotypes. Re-directing M1 macrophages towards more harmless, inflammation suppressing and tissue healing M2 macrophages affords a novel target for the development of drugs for autoimmune diseases.

Keywords: Macrophages, alternative activation, MKP-1, salbutamol, rolipram

Translational/Drug discovery

OP-72

COMPARISON OF TWO CELL-FREE ASSAYS FOR ANTI-DRUG ANTIBODY DETECTION IN BIOLOGIC THERAPY

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The introduction of biologic therapy has revolutionized modern medicine but with its wide-ranging application, assessment

of its immunogenicity has gained in importance. The immune system can recognize a biologic agent as an intruder and respond by generating anti-drug antibodies (ADA). If these ADA target the binding site of the drug they can directly neutralize its mechanism of action. In this scenario, the antibodies generated are classified as neutralizing antidrug antibodies (nADA). The presence of ADA can influence the pharmacodynamics and pharmacokinetics of the therapeutic. Patients developing ADA can suffer from an increased incidence of allergic side effects up to life-threatening anaphylactic shock and/or with a reduced drug efficacy. Detection of ADA is not only of clinical but also of preclinical relevance, since ADA can modify the toxicological properties of the biologic. We have established and validated, in accordance with the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) requirements, a multi-tiered immunogenicity testing procedure for ustekinumab (UST), a monoclonal IgG1-antibody targeting the p40-subunit of interleukin 12 and 23 which is used in patients with psoriatic arthritis (PsA). Two different approaches were used for the determination of UST, ADA specific to UST (anti-UST-ADA) and ADA neutralizing the target binding of UST (anti-UST nADA). The methods were: surface plasmon resonance (SPR) spectroscopy and Enzyme-linked Immunosorbent Assay (ELISA). Detection of UST with the ELISA was performed using anti-UST-ADA as a capture antibody and a HRP-conjugated anti-UST-antibody as a detection antibody. Anti-UST-ADA measurement was achieved by coating UST and using HRP-conjugated UST for detection. Anti-UST-nADA were detected indirectly by incubating different concentrations of nADA with UST, capturing IL-12p40 and utilizing a mouse anti-human-IgG (Fc) as a detection antibody. For SPR measurements, labeling of the binding partners was not needed. Here, differences in light reflection induced by specific binding to the chip surface were registered. UST was measured by immobilizing IL-12 to a gold layer linked to a dextran matrix (CM5 chip). Anti-UST-ADA determination was achieved by covalent binding of UST to the chip surface. For confirmation of anti-UST-nADA, UST was incubated with increasing concentrations of anti-UST-ADA leading to reduced binding of UST to IL-12. In order to reduce matrix effects, serum was diluted to 1:10 (SPR) or 1:5 (ELISA). In ELISA and SPR the intra-and inter-assay coefficient of variation (CV) of the linearity for detection of UST and ADA as well as precision and accuracy data fulfilled FDA requirements. Analytical sensitivity was high in both assays with a lower Limit of Detection (LOD) and Quantification (LOQ) with the ELISA. In conclusion, we established and validated a reliable immunogenicity detection systems that complies with the criteria of the regulatory agencies.

Keywords: antidrug antibodies, surface plasmon resonance (SPR), Ustekinumab, Food and Drug Administration (FDA)

Translational/Drug discovery

OP-73

BLOCKING IL-17C REVERSES THE DISEASE SIGNATURE IN A MOUSE MODEL OF ATOPIC DERMATITIS

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Interleukin-17C (IL-17C) has been described to play a major role in skin inflammation, such as psoriasis. Atopic dermatitis (AD) is a chronic relapsing skin disease characterized by a skin barrier dysfunction and a CD4+ T cell adaptive immune response to common environmental allergens. MOR106 is a novel potent and selective human IgG1 monoclonal anti-IL17C antibody. Here, we show that MOR106 is efficacious in the calcipotriol-induced AD-like skin inflammation mouse model and analyze the impact of MOR106 on the gene signature in skin.

Method: AD was induced by topical administration of calcipotriol on both ears of Balb/c mice for 5 consecutive days and mice were sacrificed at day 8. MOR106 or an isotype control antibody was intraperitoneally administrated at 10 mg/kg three times (3 days before the first calcipotriol application, day 1 and 5). An additional non-disease control group receiving topical ethanol administration and treated with isotype control antibody was included. Skin inflammation was assessed by daily evaluation of ear swelling by caliper measurements and on day 5 by in vivo molecular imaging using a cathepsin activatable probe. Ears were collected at day 8 for RNA extraction. Transcriptome analysis was performed using Agilent SurePrint G3. Data analysis was performed using empirical Bayes methods and linear models (limma BioConductor).

Results: Calcipotriol induced skin inflammation was demonstrated by increase of ear swelling and cathepsin activity in ears. Transcriptome analysis revealed 1455 probes were upregulated and 1468 downregulated by calcipotriol compared to ethanol, corresponding to the calcipotriol effect (False Discovery Rate <1% ; absolute log2 Fold Change >1). Pathway analysis showed calcipotriol affected genes involved in the epithelial barrier function as well as cytokines/chemokines and TLR signaling pathways. These pathways are also described to be affected in human AD patients.

MOR106 was shown to reduce skin inflammation in ears. Microarray analysis displayed 1165 probes upregulated and 1049 downregulated by MOR106 compared to isotype antibody on calcipotriol-treated samples, corresponding to the MOR106 effect. Among them, 78% were also altered by calcipotriol, indicating that MOR106 greatly reversed the calcipotriol gene signature. This was confirmed by a strong negative correlation between the calcipotriol and the MOR106 effects (Spearman R=-0.91; R^2=0.83). Nevertheless, MOR106 suppressed to some extent but not completely the calcipotriol induced effects on gene expression. Interestingly, the pathways affected by MOR106 involved several key functions such as skin barrier/epithelial integrity (Flg2), leukocyte activation/transmigration (S100a8) and cytokines/chemokines (Tslp).

Conclusion: Our data demonstrate the protective effects of an anti-IL-17C antibody in the calcipotriol-induced inflammation

model of AD. This supports the current evaluation of MOR106 in patients with AD (NCT02739009).

Keywords: Atopic dermatitis, IL-17C, transcriptome analysis

Resolution of inflammation

OP-74

CCR1 AND CCL3 IN MOUSE MODEL OF BEHCET'S DISEASE

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Behcet's disease (BD) is a immune-mediated chronic multisystemic vasculitic inflammatory rare disease. C-C chemokine receptor type 1 (CCR1) has been known as a candidate gene for BD. However, there was no evidence for protein expression in BD patients or mouse model until now. The expression of CCR1 was evaluated in inflammatory BD model mice induced by HSV inoculation. HSV is one of etiopathological factor for BD. The frequencies of CCR1+ cells on PBMC of BD mice were significantly down-regulated compared to healthy control mice by flow cytometry (7.9 ± 4.4% vs 11.1±2.5%, $p<0.001$). CCL3, a ligand of CCR1, influenced to the frequencies of CCR1+ PBMC and affected to the change of symptoms in mice. Colchicine and pentoxifylline, most frequently prescribed medicine to BD patients, improved BD symptoms of mice and up-regulated the frequencies of CCR1+ cells in BD mice (7.9 ± 4.4% vs 15.9 ± 4.5%, $p=0.01$; 7.9 ± 4.4% vs 19.1 ± 3.5%, $p<0.001$ respectively). The results showed the correlation of CCR1 and CCL3 to BD symptoms in HSV-induced inflammatory BD model mice.

Keywords: Behcet's disease, HSV, mouse model, CCR1, CCL3

Resolution of inflammation

OP-75

ACTIVATION OF ADENOSINE RECEPTOR BY PDRN IMPROVES SKIN REMODELLING IN AN EXPERIMENTAL MODEL OF PSORIASIS-LIKE DERMATITIS

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Psoriasis is an immune-mediated skin disease characterized by increased keratinocyte proliferation, epidermal hyperplasia, acanthosis, and alterations in fibroblast organization. The nucleoside adenosine is a small molecule that, stimulating A2A receptor, promotes activation of downstream targets such as PKA and Epac, involved in skin remodelling. Several studies have already demonstrated the role of A2A receptor in fibroblast activation and collagen synthesis in skin disorders.

Therefore, we investigated the effects of polydeoxyribonucleotide (PDRN), an adenosine receptor agonist, in an experimental model of psoriasis-like dermatitis.

Psoriasis-like lesions were induced by a topical application of imiquimod cream (IMQ; 62.5 mg/day) on the shaved back skin of C57BL/6 mice for 7 consecutive days. Sham psoriasis animals were challenged with vaseline cream. Sham and IMQ animals were randomized to receive PDRN (8 mg/kg/i. p.) or its vehicle (100 μl/i. p of 0.9% NaCl). Skin of IMQ animals developed erythema, scales, thickening and epidermal acanthosis starting from day 3 following IMQ application. Treatment with PDRN produced a marked reduction of inflammatory panel and blunted epidermal thickness and acanthosis. The immunohistochemical analysis of the hyperproliferative markers cytocheratin 6 and Ki67 showed that PDRN reduced cell hyperproliferation, but promoted fibroblast proliferation and skin remodelling in dermis. In fact, animals treated with PDRN showed upregulation and downregulation respectively of cyclin D1/CDK6 and its inhibitor p15, thus demonstrating an improvement of cell cycle machinery. In addition, the activation of adenosine receptor by PDRN administration determined an increase of dermal collagen deposition.

Our data suggest that PDRN reduced epidermal hyperproliferation, restoring epidermal structure and might act as a remodelling promoter in psoriasis-like dermatitis.

Keywords: adenosine receptors, PDRN, psoriasis-like dermatitis

Resolution of inflammation

OP-76

BRUTON'S TYROSINE KINASE INHIBITION REDUCES THE DEVELOPMENT OF DIABETIC NEPHROPATHY BY REDUCING NF-κB AND NLRP3 INFLAMMASOME ACTIVATION

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Introduction: The link between low-grade inflammation and insulin resistance are not completely understood. There is, however, compelling evidence that the activation of both NF-κB and the NLRP3 inflammasome play pivotal roles. Inhibitors of Bruton's tyrosine kinase (BTK) reduce the activation of both NF-κB and NLRP3 inflammasome. Here we compare the effects the novel BTK-inhibitor REDX05194 and ibrutinib on a) kinase activity and b) in a model of high fat diet (HFD) induced insulin resistance and diabetic nephropathy.

Methods: The ability of REDX05194 (1 μM) and ibrutinib (1 μM) to bind to and inhibit kinases were assessed using KINOMEscan®. Ten-week old male C57BL/6 mice were fed a normal (chow) diet or HFD diet for 12 weeks. Mice fed a HFD were administered REDX05194 (3 mg/kg, p. o.), ibrutinib (30 mg/kg, p. o) or vehicle from weeks 7–12 of dietary manipulation.

Results: At 1 μM, REDX05194 displays a higher selectivity than ibrutinib towards the panel of 456 kinases tested in the KINOMEscan assay. When compared to mice fed on a chow diet, mice fed on a HFD demonstrated an impaired tolerance to oral glucose when measured by oral glucose tolerance test (OGTT), suggesting the development of a type 2 diabetic phenotype. Mice

fed on a HFD and administered either REDX05194 or ibrutinib demonstrated an improvement in OGTT.

When compared to mice fed on a chow diet, mice fed on a HFD demonstrated an elevation in albumin-to-creatinine ratio (ACR), suggesting the development of significant proteinuria. Mice fed on a HFD and administered either REDX05194 or ibrutinib developed significantly less proteinuria.

Mice fed on a HFD demonstrate an increased phosphorylation of IKKα/β and IκB, allowing the translocation of p65 to the nucleus, and hence, activation of NF-κB (kidney/liver). Similarly, HFD evoked a significant increase in the expression of the protein complex NLRP3 inflammasome, leading to the proteolytic cleavage of pro-caspase 1 to caspase 1, in both the kidney and liver. Inhibition of BTK with either REDX05194 or ibrutinib in mice fed a HFD resulted in i) decreased phosphorylation of IKKα/β and IκB, ii) decreased translocation of p65 to the nucleus, and iii) decreased NLRP3 assembly resulting in reduced cleavage of pro-caspase 1 to caspase 1 in both the kidney and liver.

Conclusion: Our data demonstrate that REDX05194 is a more selective BTK inhibitor than ibrutinib. Feeding mice a HFD results in significant renal and hepatocellular inflammation (activation of both NF-κB and the NLRP3 inflammasome cascades), leading to severe renal proteinuria. Inhibition of BTK in mice fed a HFD results in reduced proteinuria, and this effect was associated (and possibly due to) reduction in the activation of NF-κB and the NLRP3 inflammasome activation. Most notably, REDX05194 required a 10-fold lower dose to elicit the same biological effects as ibrutinib in diabetic mice.

Keywords: Bruton's tyrosine kinase, Diabetes, Diabetic nephropathy, Inflammation

Signalling molecules and pathways

OP-77

THE ANTIMICROBIAL PEPTIDE LL37 UNDERPINS THROMBOTIC COMPLICATIONS DURING INFLAMMATORY DISEASES

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Human cationic antimicrobial peptide 18 (hCAP18) is the only cathelicidin expressed in human cells including epithelial cells and innate immune system cells such as neutrophils and monocytes. During microbial infections, the activation of immune cells releases hCAP18 to the external milieu, where it is processed by proteolytic enzymes to liberate the short, active 37 amino acid peptide LL37. LL37 acts as a powerful antimicrobial peptide against bacteria, fungi and viral particles. LL37 also modulates innate and adaptive immune responses by stimulating specific receptor-mediated [predominantly formyl peptide receptor 2 (FPR2/ALX)]

signalling within the immune cells. The binding of LL37 activates immune cells and thus exacerbates inflammatory responses to accelerate the clearance of infection. Despite detailed research on the roles of LL37 in the modulation of inflammatory responses at various pathological settings, specifically sepsis and psoriasis, the effects of LL37 on the regulation of thrombosis and platelet-mediated inflammatory responses have not been established previously. In addition to haemostasis and thrombosis, platelets play significant roles in the control of innate immunity, inflammatory responses and microbial infections. Activation of platelets during inflammatory diseases such as sepsis induces the formation of blood clots or disseminated intravascular coagulation in capillaries, or aggregation and sequestration of platelets in the lungs, instigating thrombocytopenia. Here we demonstrate the effects of LL37 in the modulation of platelet reactivity, haemostasis and thrombosis. LL37 activates a range of platelet functions and enhances thrombus formation under arterial flow conditions. Similarly, LL37 reduces bleeding time in mice indicating its significance in the modulation of haemostasis under physiological conditions. Moreover, with the aid of selective inhibitors and genetically modified mice that are deficient in formyl peptide receptors, we determined the functional dependence of LL37 on FPR1 and FPR2/ALX. Since the level of LL37 released during inflammation is significantly higher than normal, a fuller understanding of its functions on the modulation of platelet reactivity will pave the way for the determination of the fundamental mechanisms for thrombotic complications in distinctive inflammatory diseases and offer the potential for development of improved therapeutic strategies.

Keywords: platelets, LL37, formyl peptide receptor, thrombosis, inflammation, haemostasis

Translational/Drug discovery

OP-78

MODELING PSORIASIS-LIKE INFLAMMATION IN RATS

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Psoriasis is the most common chronic autoimmune skin condition, effecting more than 2% of the US population and impacting roughly 125 million people worldwide. The pathogenesis of psoriasis is assumed to be a complex interplay between environmental factors, immune dysregulation and genetic susceptibility. Data suggests that an environmental/pathogenic insult to the skin precipitates the activation of innate immune cells leading to a dysregulated pro-inflammatory response. This response triggers secretion of pathogenic cytokines IL-12 and IL-23, causing migration and differentiation of Th17 and Th1 cells. These cells release additional cytokines such as IL-22, IL-17A and IL-17F driving proliferation of keratinocytes and epidermal hyperplasia (plaque formation). With the complexity of psoriasis disease immunopathology, it has become increasingly important to model this disease, or aspects thereof, in immune competent animals. Rats are the preferred species for pharmacokinetic studies needed for IND submission, therefore it would be beneficial to answer method of action (MoA) questions in this species. To this end, MD Biosciences has optimized two psoriasis models in the Sprague Dawley rat.

The imiquimod (IMQ) induced psoriasis model has been widely used in pre-clinical drug development. IMQ mimics a pathogenic insult to the skin, eliciting a robust Th17 immune response leading to plaque formation. IMQ is applied to the shaved backs and ears of naïve rats, and disease progression and

ear (skin) thickening is monitored. Diseased animals exhibit erythema and plaque formation shortly after the start of the study and progress through termination. Corticosteroid treatment, used as a positive control, significantly inhibits all clinical symptoms.

IL-23 is the main pathogenic cytokine in the initiation of psoriasis disease progression and has become a lead target for the clinical treatment of psoriasis. To more specifically examine IL-23 driven psoriasis, exogenous IL-23 can be delivered directly to the dermis of rats to induce a downstream cascade of inflammatory biomarker upregulation, erythema and epidermal hyperplasia, similar to what is seen in human psoriatic plaques. Rats treated with corticosteroids as a positive control exhibit reduced clinical symptoms and inflammatory cytokine production.

The IMQ-and IL-23-induced psoriasis models are robust pre-clinical models to help develop therapeutics for psoriasis, as both models mimic specific disease pathologies. Additionally, these models can be used to assess efficacy of candidate drugs for general Th17, IL-23/IL-17 pathway targeting, important pathways in psoriasis pathogenesis. Both models can be performed acutely to specifically antagonize the Th-17/IL-17 pathways to screen libraries for lead drug candidates. The versatility of the models and the benefits of the rat as a host organism make these models ideal for pre-clinical drug development for psoriasis or other Th17/IL-17 dependent diseases.

Keywords: Psoriasis, IL-17, IL-23, rat model, drug discovery, drug screen

Modeling psoriasis-like inflammation with rrIL-23

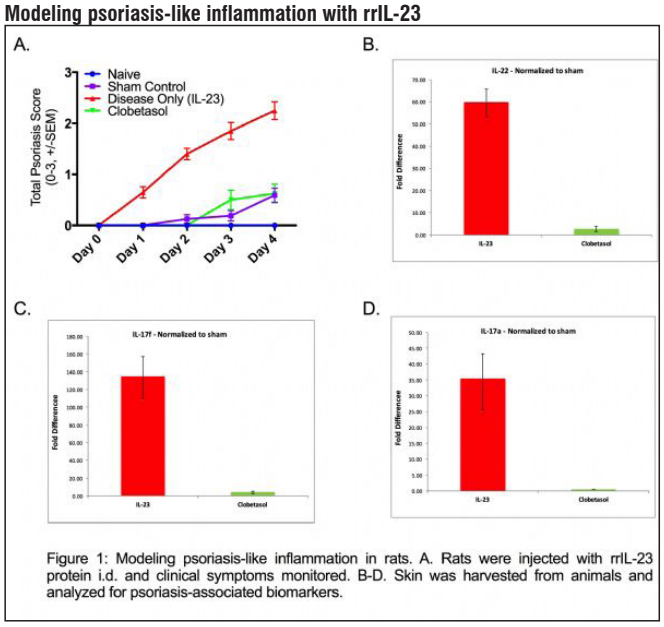

Figure 1: Modeling psoriasis-like inflammation in rats. A. Rats were injected with rrIL-23 protein i.d. and clinical symptoms monitored. B-D. Skin was harvested from animals and analyzed for psoriasis-associated biomarkers.

Vascular processes

OP-79

HOMEOSTATIC CYTOKINES INTERLEUKIN-7 (IL-7) AND IL-15: NOVEL TARGETS TO CONTROL INFLAMMATORY CD4+CD28NULL T CELLS AND REDUCE CARDIOVASCULAR RISK IN CORONARY ATHEROSCLEROSIS AND RHEUMATOID ARTHRITIS

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Background and Aim: We have previously shown that CD4+CD28null (CD28null) T cells, a unique T lymphocyte subset with strong pro-inflammatory and cell-lytic phenotype that are apoptosis resistant, expand in patients with coronary atherosclerosis (CAD). CAD patients harbouring high CD28null T cell numbers have increased risk of recurrent myocardial infarction (MI) and unfavourable prognosis. In addition to CAD, CD28null T cells also expand in other chronic inflammatory disorders and particularly in rheumatoid arthritis (RA), which associates with premature atherosclerosis and increased risk of cardiovascular disease. The mechanisms that govern CD28null T cell expansion in CAD and RA remain elusive. Both inflammatory and homeostatic cytokines (e. g. interleukin-7, IL-7 and IL-15) have crucial roles in the survival and proliferation of T cells. The inflammatory cytokines tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1β) and IL-6 have been implicated in the chronic inflammatory processes that drive atherosclerosis and RA and are targeted therapeutically. Moreover, IL-7 and IL-15 levels increase in the circulation and/or synovium of RA patients and have been suggested to have pathogenic effects in this disease. Our aim was to investigate the effect of inflammatory and homeostatic cytokines on CD28null T cells in patients with MI.

Methods: Freshly isolated cells from MI patients were treated with recombinant human cytokines (TNF-α, IL-1β, IL-6, IL-7, IL-15) and the number, activation, proliferation and function of CD28null T cells were analysed.

Results: We found that pro-inflammatory cytokines TNF-α, IL-1β and IL-6 did not have any effect on CD28null T cell number. Strikingly, homeostatic cytokines IL-7 and IL-15 triggered expansion of CD28null T cells from MI patients, which was mediated by cell proliferation. Moreover, we demonstrate for the first time that IL-7 and IL-15 activate CD28null T cells and increase their cytotoxic function.

Conclusions: We showed that IL-7 and IL-15 and not pro-inflammatory cytokines are the main drivers of CD28null T cell expansion in MI patients. Our novel findings suggest that anti-inflammatory drugs targeting TNF-α, IL-1 and IL-6 may fail to control CD28null T cell expansion in MI patients and that therapeutic strategies targeting homeostatic cytokines IL-7/IL-15 may be beneficial in these patients. Moreover, modulation of homeostatic cytokine IL-7 and IL-15 may curb the expansion of CD28null T cells in rheumatoid arthritis and reduce the incidence of premature atherosclerosis and severe cardiovascular events.

Keywords: CD4+CD28null T lymphocytes, inflammation, IL-7/IL-15, atherosclerosis, rheumatoid arthritis, anti-inflammatory therapy

Ageing

OP-80

HUMAN BONE MARROW ADIPOCYTES DISPLAY DISTINCT FUNCTIONAL IMMUNE REGULATORY PROPERTIES

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The bone marrow (BM) is a specialized primary lymphoid organ of the human immune system where T cell precursors are generated. In addition, the importance of the BM in the maintenance of antigen-experienced adaptive cells has been documented. The BM has proven to be a major reservoir of resting memory T and B cells, capable of providing protection against recurrent infections. The survival of these beneficial immune cells is mediated by cytokine and chemokine producing cells, forming specific areas known as BM niches. However, these niches involved in the production of survival factors necessary for the long-term maintenance of effector/memory T cells have been already described. The maintenance of memory T cells is supported by cytokines IL-7 and IL-15, produced by stromal cells and myeloid cell types. With increasing age, a reduction in bone formation is accompanied by the accumulation of bone marrow fat. No information is yet available on the production of memory T cell survival factors by BM fat tissue and the interaction with adaptive immune cells in the BM. In addition, whether adipocytes in the BM may have a different phenotype compared to conventional white fat adipocytes is unknown.

Using microarrays, we show that bone marrow fat significantly differs from subcutaneous fat concerning specific gene expression profiles including inflammatory response and adipogenesis. Lower expression levels of adipocyte-specific genes peroxisome proliferator-activated receptor gamma (PPARγ), fatty acid binding protein 4 (FABP4) and higher expression of effector/memory T cell survival factor IL-7 and IL-15 were found in BM adipocytes compared to subcutaneous adipocytes. Proinflammatory molecules TNF-α and IL-6, which contribute to the low-grade inflammatory background known as "inflamm-ageing", commonly observed in elderly persons, are highly produced in BM fat. Whether the expression profile of BM adipocytes or their interaction with adaptive immune cells change with age in the bone marrow and whether this type of process is influenced by body weight and the amount, as well as characteristics of fat tissue in other locations, will be considered in future studies. With our data, we can show that the unique phenotype of BM adipocytes may support the production of survival molecules for the maintenance of effector/memory T cells.

Keywords: CD36, marrow adipose tissue, immunity

POSTER PRESENTATIONS

Ageing

PP-001

AGE-ASSOCIATED CHANGES IN NATURAL KILLER CELL CYTOTOXICITY AND MIGRATION

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Background: Innate immune function changes dramatically with age including: reduced cytolytic responses towards tumour and virus infected cells by NK cells. These changes impact upon susceptibility to infections but have effects beyond this; including a possible role in the accumulation of senescent cells in tissues with age, as recent studies have shown that NK cells play a role in the killing of senescent cells. Effective elimination of senescent cells is crucial to stay free of age related diseases.

Aim: Determine if NK killing of senescent cells is reduced with age and could contribute to their accumulation in the body with age. Assess the impact of ageing on NK cell migration towards senescent cells. Elucidate the possible mechanisms that drive these pathways.

Methods: Flow cytometry was used to assess apoptosis of senescent cells after staining cells with annexin V and propidium iodide. Surface receptors for senescent chemokines CCR1, CCR2 and CCR5 were analysed as well as early activation marker CD69. Transwell model of senescent cells was set up to determine NK cell migration towards target cells. 3D constructs are created to assess separate compartments during migration. Use of Western blot and flow cytometry to analyse possible mechanistic pathways.

Results: Cytotoxicity was seen to increase in senescent cells when NK cells from young volunteers were co-cultured with fibroblasts for 1 hour at 5:1 effector target ratio, showing a significant increase of NK cytotoxicity ($p=0.002$). Early activation CD69 was also seen to have increased upon IL-12 stimulation ($p=0.01$). CCR1 and CCR5 gave a significant increase in surface expression; $p=0.03$ and $p=0.02$ respectively in young NK cells. The transwell assay showed aberrant migration of NK cells towards senescent cells with age ($p=0.0001$). 3D migration shows fewer old NK cells migrating down to the lower compartment of the 5 compartment migration construct ($p=0.003$). Comconamycin A inhibitor showed a decreased senescent cell death ($p=0.017$) at a 5:1 effector target ratio of NK cells and senescent cells respectively.

Conclusion: Young NK cells eliminated senescent cells more efficiently than aged NK cells. Reduced migratory capability from the older age group may explain why we see a reduced or delayed NK cytotoxicity effect. Surface receptors also showed a reduction in CD69 expression in the older subjects, indicating fewer NK cells are activated for the removal of senescent cells. CCR1 and CCR5 showed a significant reduction in chemokine receptors; indicating a slower response of NK cells reaching their destination for effective clearance. Perforin inhibitor concanamycin A demonstrates that granule exocytosis pathway is essential for the clearance of senescent cells. 3D migration not only confirmed a reduced NK cell migration but also demonstrated a problem with NK/endothelial interaction. Future studies will look at NK mechanism for a reduced cytotoxicity and migration.

Keywords: Natural Killer cell, Senescence, Natural Killer Cell Cytotoxicty, Migration

PP-002

EFFECT OF BAX INHIBITOR-1 (BI-1) ON AGING INDUCED ER STRESS

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Protein folding and lipid biosynthesis mainly occurs in the endoplasmic reticulum (ER). Any pathological condition that perturbs cellular machinery and disrupts ER homeostasis leads to the protein misfolding, aggregation and finally ER stress. Aging associated ER stress occurs due to declined chaperoning systems of the ER. Persistent stress in the ER induces apoptosis, reduces cellular functions, and leads to age-related diseases. BI-1 is an evolutionary conserved anti-apoptotic ER protein. In our aging study, we categorized the animals into different age groups; Young (3 months), Middle (11 months) and Old (20 months) and observed the effect of BI-1 in liver. During study, we observed that the BI-1 deficient (KO) aged mice gained body weight and higher hepatic triglyceride level when compared with wild type (WT) aged mice. BI-1 KO mice showed higher fatty liver with the accumulation of lipid droplets in the liver cells leading to mild fibrosis. This led to ROS accumulation in the liver and caused oxidative stress. Furthermore, the impaired UPR function has been observed in BI-1 KO mice with advanced age. The expression levels of ER chaperone protein and enzyme like GRP78 and PDI were significantly reduced in liver of BI-1 KO aged mice when compared with WT counterparts. Similarly, UPR components like ATF6 alpha, p-PERK, and p-eIF2 alpha were downregulated in KO aged liver. BI-1 KO aged mice showed downregulation of BCL-2 expression and increased expression of phosphorylated JNK mediated apoptosis. Another apoptotic marker, CHOP, was also upregulated in BI-1 KO aged mice liver. Collectively, these results suggest that aging induced fatty liver is regulated by BI-1 through the regulation of ER stress mediated apoptosis.

Keywords: Aging, Oxidative stress, ER stress, Apoptosis

Cancer

PP-003

ROLE OF CONTRACEPTIVE HORMONES ON COMBINATION ANTIRETROVIRAL TREATMENT INDUCED APOPTOSIS IN HUMAN CERVICAL CANCER CELLS

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Introduction: Combination of nucleoside reverse transcriptase inhibitors (NRTIs) is the predominant method for HIV treatment. Highly active antiretroviral treatment (HAART) entailing two or more NRTIs and nevirapine (NVP) has been the method of choice in most resource limiting countries. Abacavir (ABC), lamivudine (3TC), zidovudine (ZDV) and stavudine (d4T) have been in use in these settings. Some double combinations

have been of choice in patients presenting with co-infections or other clinical conditions. We previously evaluated the effect of levonorgestrel (LNG) and ethinyl estradiol (EE) [constituents of combined oral contraceptives] on ZDV+3TC+NVP induced apoptosis in human cervical epithelial cells. Here, we further evaluate the effect of (LNG) and (EE) on ABC+3TC, ZDV+3TC, ABC+ZDV+3TC and d4T+3TC+NVP induced apoptosis.

Method: HeLa cells (a human cervical epithelial cancer cell line) were treated with combination antiretroviral drugs (ARDs) at final concentrations of within the 20-25% inhibitory concentration of each drug. The cells were treated with antiretroviral drugs alone for 24hrs and co-treated with varying EE and LNG for a further 48hrs. Untreated cells saved as control. MTT cell proliferation rate was analysed by one-way ANOVA followed by Dunnett's multiple comparison test ($n=3$, $P<0.05$). Further, HeLa cells were treated with ARDs (in the presence or absence of EE or LNG) over 48hrs and apoptosis measured by four-way dot plots. Data was analysed by the one-way ANOVA followed by Turkey's test.

Results: Overall, combination ARDs increase the rate of apoptosis compared to untreated cells ($P<0.05$). Co-administration with contraceptive hormones further drastically increases the rate of combination ARD induced apoptosis compared to cells treated with ARDs only.

Conclusion: Combination ARD induced apoptosis is suggestive of decreased cervical neoplastic transformation as observed in some studies (1,2). Combination ARD co-administration with contraceptive hormones is suggestive of increased cell loss and possibly tissue damage as depicted by some epidemiological studies suggesting cervical neoplasia progression among HAART patients (3). Further prospective and retrospective clinical studies maybe of interest.

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Keywords: apoptosis, antiretroviral, contraceptives

PP-004

MACROPHAGE ANNEXIN-A1 IS CRITICAL IN THE TUMOR MICROENVIRONMENT BY PROMOTING ALTERNATIVE MACROPHAGE POLARIZATION

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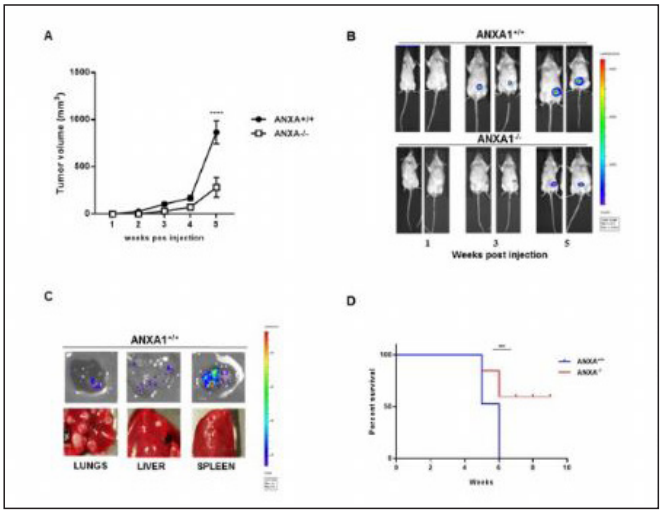
Macrophages are potent immune cells with well-established roles in the response to stress, injury, infection and inflammation. The classically activated macrophages (M1) are induced by lipopolysaccharide (LPS) and cytokines and characterized by the expression of a wide range of pro-inflammatory genes. M2 (alternatively activated macrophages) are induced by T helper type 2 (Th2) cytokines such as interleukin-4 (IL4) and express high levels of anti-inflammatory and tissue repair marker genes. The strong association between macrophages and tumor cells as well as the high incidences of leukocyte infiltration in solid tumors have contribute to tumor-associated macrophages (TAMs) to be the key to tumor progression. In this study, we investigated the effects of Annexin A1 (ANXA1), a well characterized anti-inflammatory protein on macrophages polarization and the subsequent impacts on the interaction between macrophages and breast cancer cells. Our results demonstrate that ANXA1 is required for macrophage polarization to M2-subtype, which can mediate tumor invasion and proliferation. Our *in vivo* model, shown that ANXA1 enhances tumor growth, metastasis and reduces survival. Thus, our finding provides support for ANXA1 which modulates macrophage polarization and tumor proliferation through ANXA1-CCL5-FPR2-ERK signalling. These results provide new insights into the molecular mechanisms of macrophage polarization with therapeutic potential to suppress breast cancer growth and metastasis.

Keywords: Annexin-a1, macrophages, tumour growth, signal transduction

ANXA1 regulates tumour growth and metastasis in vivo.



(A) BALBc WT and ANXA1-/- mice were injected with 4T1 murine breast cancer containing a luciferase promoter into the mammary fat pad. Tumour volumes were calculated by LxWxW/2. (B) Bioluminescence imaging for mice from control and ANXA1-/- group at week 1, 3 and 5 post-injection. (C) Representative bioluminescence images and pictures from lungs, liver and spleen at week 6 post-injection. (D) Survival curves were plotted for both groups ($n=6$ (8 per group). Data shown are mean ± SD, **** $p <= 0.001$, ANOVA.

PP-005

ANTI-CACHECTIC EFFECT OF SHENCHUJIANPI-TANG, A TRADITIONAL HERBAL REMEDY, IS ASSOCIATED WITH SUPPRESSION OF PRO-INFLAMMATORY CYTOKINE PRODUCTION AND INHIBITION OF AUTOPHAGY

Jun Yong Choi, Myungsoo Joo

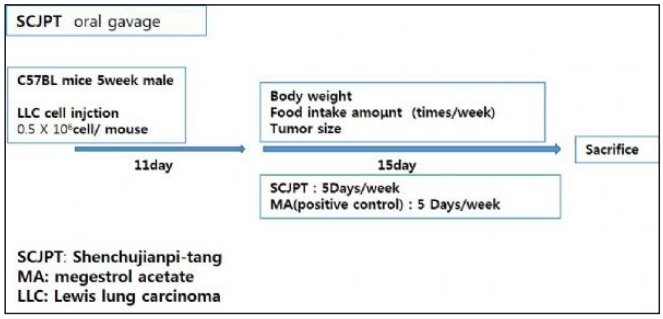
School of Korean Medicine, Pusan National University

Cancer cachexia, a complex metabolic syndrome, is characterized by systemic inflammation, weight loss, and muscle atrophy, which is closely related to mortality. Since anorexia is a major symptom of cachexia, we explored whether Shenchujianpi-tang (SCJPT), a traditional herbal prescription that has been used for various gastrointestinal conditions including anorexia, has potential to counter cachexia in a syngeneic graft mouse model. We found that SCJPT decreased soleus muscle wasting in C57BL/6 grafted with Lewis lung carcinoma (LLC), the effect of which was

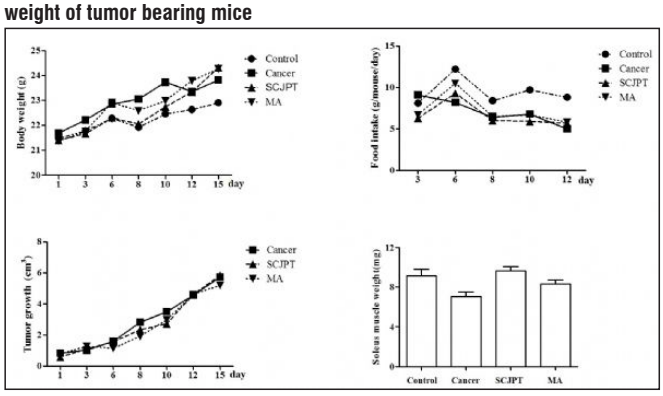
similar to megestrol acetate. Without affecting tumor growth in the mice, SCJPT relieved the atrophy of the soleus muscle, which was associated with decreased expression of pro-inflammatory cytokines, including IL-1β, IL-6, and MCP-1, in soleus muscle tissue. Similarly, SCJPT decreased the levels of TNF-α, IL-6, and MCP-1 in mouse serum. Western blot analyses showed that IL-6 or MCP-1 treatment induced the expression of LC3-II in a soleus cell line, Sol8, suggesting that elevated levels of these cytokines are related with the autophagy of the soleus muscle. Similar experiments showed that SCJPT suppressed the expression of LC3-II by restoring the activity of mTOR. Therefore, our results suggest that SCJPT has a potential to suppress cachexia, which is associated with SCJPT suppressing the production of pro-inflammatory cytokines and inhibiting the autophagy of soleus induced by those cytokines.

Keywords: cachexia, cancer, herbal medicine, autophagy

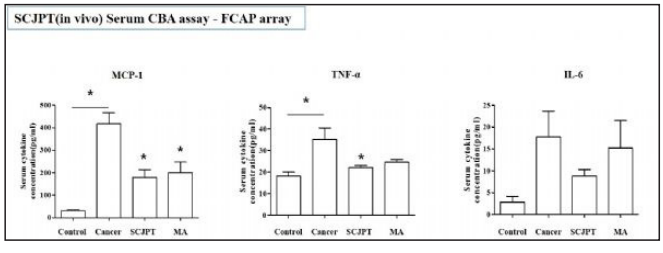
Schematic flow of the experiment



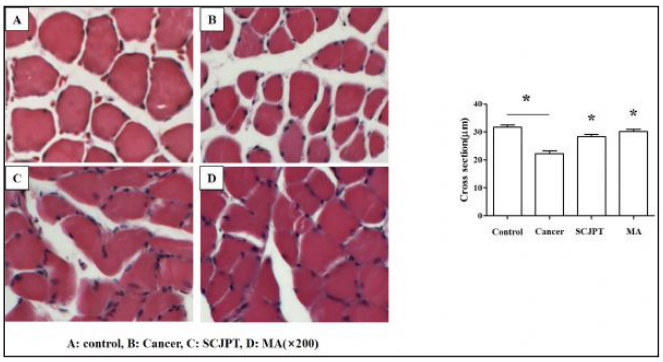
Effect of SCJPT on body weight, food intake, tumor growth and soleus muscle weight of tumor bearing mice



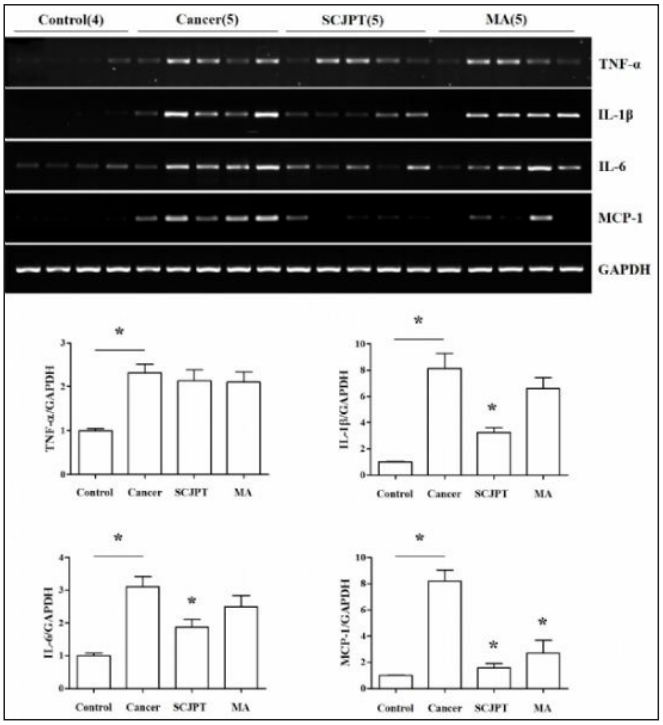
Effect of SCJPT on serum MCP-1 and TNF-a of tumor-bearing mouse



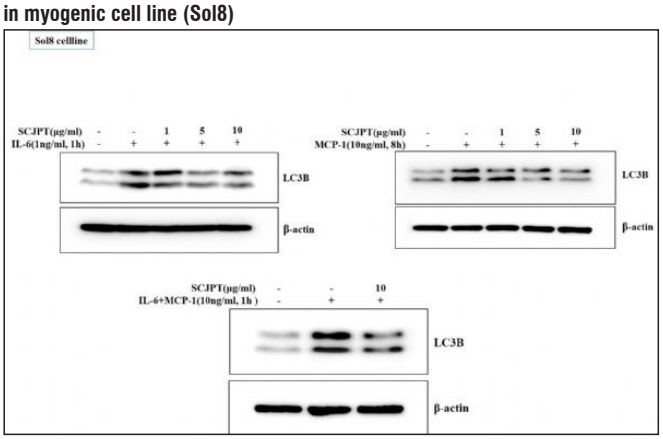
Effect of SCJPT on cross sectional diameter of soleus muscle in tumor-bearing mice



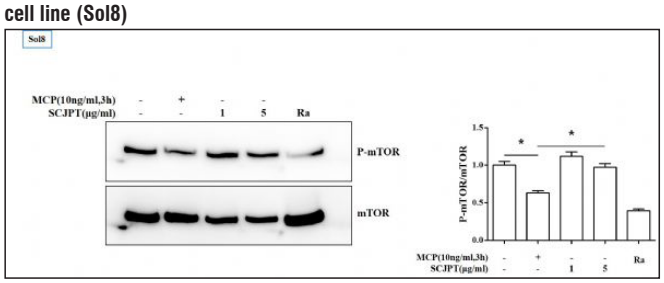
Effect of SCJPT on the expression of inflammatory cytokines in soleus muscle



Effect of SCJPT on the expression of LC3-II protein induced by IL-6 and MCP-1 in myogenic cell line (Sol8)



Restoring effect of SCJPT on mTOR protein suppressed by MCP-1 in myogenic cell line (Sol8)



PP-007

DOWNREGULATED STAT6 IN GLIOMA INCREASES HYPOXIC VIABILITY VIA MTOR/HIF-1A, BUT DECREASES STAT6-SPECIFIC GENE EXPRESSION, LEADING TO A FACILITATION OF CANCER CELL SURVIVAL

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Aberrant epigenetic alterations have emerged as common hallmarks of many cancers. In glioblastoma (GBM), the most malignant and lethal form of glioma, aberrant epigenome has also been shown for a wide variety of genes associated with tumor suppression, DNA repair, cell cycle regulation, apoptosis, invasion, and migration. Our expression profiling of STAT6 demonstrated its frequent downregulation in human GBM specimens and its downregulation results from promoter CpG methylation, which is maintained by DNMT1. Given that tumor suppressor is subject to frequent epigenetic silencing, we here investigate STAT6 to be a tumor suppressor in GBM. We show that STAT6 knockdown in GBM cells allows for survival under severe hypoxic condition, suggesting the importance of silencing STAT6 during GBM tumor progression. Furthermore we demonstrate that in hypoxic conditions STAT6 acts as a negative regulator of the synthesis of HIF-1α by repressing mammalian target of rapamycin (mTOR) - ribosomal protein S6 kinase (S6K) - S6 signaling pathway and increasing initiation factor 4E-binding potein 1 (4E-BP1) transcription, thereby facilitating translation initiation. In addition, STAT6 silencing in GBM suppresses STAT6-specific immune target gene expression, leading to a beneficial effects on cancer cell survival. Thus, our findings identify STAT6 as novel tumor suppressor in GBM. Further, given the beneficial respect of STAT6 in glioma cell survival, it could be a novel target in STAT6-downregulated glioma.

Keywords: STAT6, Glioma, HIF-1α

PP-008

LIPOXIN A4 SELECTIVELY PROGRAMS THE PROFILE OF M2 TUMOR-ASSOCIATED MACROPHAGES WHICH FAVOUR CONTROL OF TUMOR PROGRESSION

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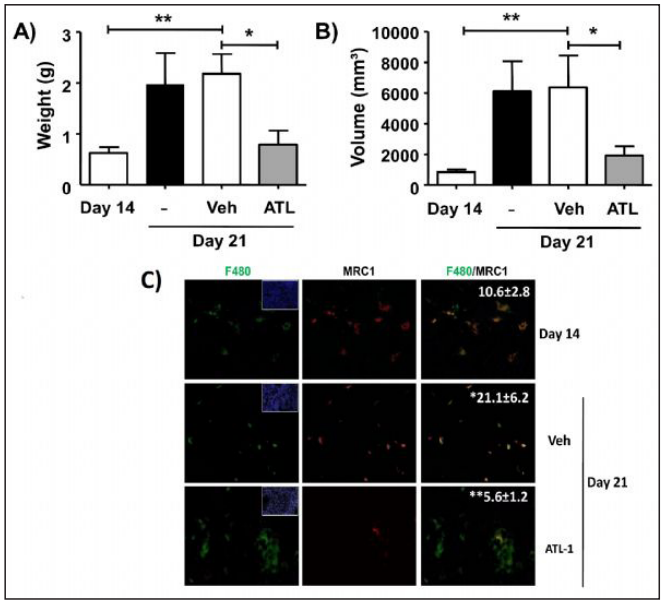
The relationship between the inflammatory response and cancer has been extensively investigated. In some types of cancer, the microenvironment is similar to that observed in inflammatory processes and tissue repair, with the presence of inflammatory cells and mediators, including chemokines, cytokines, and growth factors, as well as changes in the processes of tissue remodeling and angiogenesis. An early event in tumor progression is the recruitment of monocytes to the tumor site, where they differentiate into macrophages (MΦ). In tumor microenvironments, the macrophage population is heterogeneous, but some macrophages can acquire tumor-promoting characteristics. These tumor-associated macrophages (TAM) exhibit an M2-like profile, with deficient production of NO and ROS, characteristics of pro-inflammatory M1 cytotoxic macrophages. A number of studies have reported a positive correlation between high TAM density and poor prognosis in several human tumors. The balance between the different tumor-derived mediators may modulate the outcome of the Mϕ response and consequent tumor development. Lipoxins (LX) are specialized pro-resolving lipid mediators, produced by lipoxygenase interactions under a variety of conditions, including infection and inflammation. This study tested the hypothesis that ATL-1, a synthetic analog of 15-epi-lipoxin A4, could modulate the TAM activity. This study was performed according to guidelines of Ethical committee (CEUA/077/2012/, UERJ). Firstly, monocyte derived macrophages were differentiated into TAM after incubation with MV3 conditioned medium, a human melanoma lineage cell. Contrasting with the effects observed in other M2 subsets and M1 profile macrophages, ATL-1 selectively decreased M2 surface markers in TAM, suggesting unique behaviour of this particular M2 subset. Importantly, these results were replicated by the natural lipoxins LXA4 and the aspirin induced 15-epi-LXA4 (ATL). In parallel, ATL-1 triggered ROS production and stimulated TAM to generate NO by increasing iNOS/arginase ratio and activated NADPH oxidase. These alterations in TAM profile induced by ATL-1 increased their cytotoxic properties and led to loss of antiapoptotic effects of TAM on melanoma cells. In addition, ATL-1 inhibited endothelial cell tubulogenesis activated by TAM, a crucial step in the angiogenic process. Finally, ATL-1 inhibits tumor progression *in vivo*. We observed that treatment with a unique dose of ATL-1 at the 14th day after melanoma cells implantation is able to inhibit the tumor growth. This potent inhibition of ATL-1 was accompanied by the impaired angiogenic process and a decrease in the TAM marker in macrophages isolated from the tumor mass. Together, these results suggest that ATL-1 down-modulates the tumor progression stimulated by TAM, inducing the shift from an M2- to an M1-like profile *in vitro*

and i*n vivo*, triggering tumor cell apoptosis and the impairment of the tumor progression.

Keywords: tumor, inflammation, lipoxin, macrophage

ATL-1 inhibits tumor progression in vivo.



Groups of 6-8 mice were inoculated with B16F10 tumor cells. The tumor mass was removed from the animals on the 14th and 21st day after B16F10 injection. ATL-1 (1 μg/mouse) or ethanol (vehicle alone) was injected on day 14. A) Tumor volume and B) weight were analyzed. The excised tumors were immunostained with antibody for: C) whole macrophage (F4/80-FITC) or M2 macrophages (MRC1-PE). DAPI was used for nuclear staining shown in the upper right-hand square. An isotype IgG was used as negative control. Pictures are representative of each group..*, $P < 0.05$ when compared to 14 days; **, $P < 0.05$ when compared to ethanol 21 days.

PP-009

PRIMING ENDOTHELIAL CELLS WITH A MELANOMA-DERIVED EXTRACELLULAR MATRIX TRIGGERS THE ACTIVATION OF AVB3/VEGFR2 AXIS

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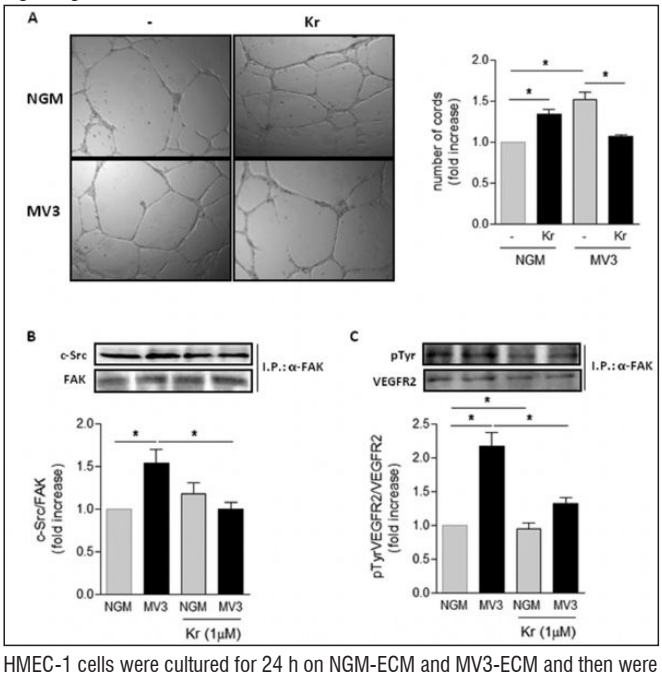
The unique composition of tumour-produced extracellular matrix (ECM) can be a determining factor in changing the profile of endothelial cells in the tumour microenvironment. ECM proteins and all molecular and cell changes creates a microenvironment that affects neighbouring cell behaviour and is involved in the formation of new blood vessels. As the main receptor for ECM proteins, integrins can activate a series of signalling pathways related to cell adhesion, migration, and differentiation of endothelial cells that interact with ECM proteins. Although it is known that certain matrix glycoproteins are up- or downregulated in tumour matrices along cancer cell transformation, evidences of the direct effect of a natural metastatic tumour-derived ECM on the activation of endothelial cells are still scarce in the literature. Here we studied the direct impact of the decellularized ECM produced by a highly metastatic human melanoma cell line (MV3) on the activation of endothelial cells and identified the intracellular signalling pathways associated with cell differentiation. Our data show that compared to the ECM derived from a human melanocyte cell line (NGM-ECM), ECM produced by a melanoma cell line (MV3-ECM) is considerably different in ultrastructural organization and composition and possesses a higher content of tenascin-C and laminin and a lower expression of fibronectin. When cultured directly on MV3-ECM, endothelial cells change morphology, displaying an increase in F-actin polymerization, and show increased adhesion, migration, proliferation, and tubulogenesis. Interaction of endothelial cells with MV3-ECM induces the activation of integrin signalling, increasing AKT and FAK phosphorylation and its association with Src. Although MV3-ECM did not altered VEGFR2 receptor expression, it could potentiate the receptor VEGFR2 response to VEGF, probably through Src activation. The blockage of $\alpha v3\beta$ integrin inhibited the FAK-Src association and VEGFR activation, thus reducing tubulogenesis (Figure). Together, our data suggest that the interaction of endothelial cells with the melanoma-ECM triggers integrin-dependent signalling, through $\alpha v\beta 3$ integrin, leading to Src pathway activation that may potentiate VEGFR2 activation and up-regulate angiogenesis.

Keywords: Melanoma, extracellular matrix, endothelial cell, integrin signaling, VEGFR

Integrin αvβ3 modulates melanoma–ECM inducing angiogenesis-associated signaling in endothelial cells.



HMEC-1 cells were cultured for 24 h on NGM-ECM and MV3-ECM and then were treated with Kistrin (1mM) for 30 min. (A) Cells were trypsinized and seeded on Matrigel for 10 h. Endothelial sprouts were quantified by counting the number of sprouts in four high-power microscopic fields (at 100x magnification) in each treatment. (B) FAK was immunoprecipitated and immunoblotted to detect Src. (C) After Kr treatment, HMEC-1 cells were stimulated with VEGF-A (30 ng/ml) for 10 min. Then, VEGFR2 was immunoprecipitated and blotted to detect pTyr. The image of pTyr blotting shows bands at 230 kDa. The results are shown as the mean fold increase relative to the NGM-ECM calculated from three individual experiments (*$P<0.05$).

PP-010

NADPH OXIDASE 4 CONTRIBUTES TO IMMUNE TOLERANCE OF ORAL CANCER BY RECRUITING REGULATORY T CELLS

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Objectives: Boosting the host immunity is a proven approach in cancer treatment. The immunosuppressive functions of regulatory T cells (Treg) have been demonstrated in multiple solid tumors. It is now recognized that recruitment of Treg by tumors could prevent the generation of anti-tumor immunity, which thereby promotes immune tolerance. Chemokine (C-C motif) ligand 2 (CCL2) is a cytokine with demonstrated ability to attract Treg. Our data reveal that NADPH oxidase 4 (NOX4) is playing a pivotal role in inducing Treg-recruiting cytokine CCL2. Thus, the objective is to investigate the feasibility to use NOX4 inhibitor to overcome the immune tolerance of oral cancer by suppressing Treg recruitment.

Methods: We investigate the role of H2O2 and NOX4 in modulating the expression and secretion of CCL2. The potential clinical use of NOX4 inhibitor GKT137831 in preventing Treg recruitment is evaluated. Clinical association of NOX4/CCL2 in our OSCC cases is revealed by QPCR analysis and immunohistochemical staining of clinical specimen.

Results: Significant increased expression of NOX4 is observed in multiple oral tongue squamous cell carcinoma. A high correlation between NOX4 and CCL2 expression level is observed in oral cancer. QPCR analysis and gel images show that knockdown NOX4 expression using NOX4-specific shRNA could reduce CCL2 transcript level in oral cancer cells. ELISA results reveal that suppressing NOX4 expression could significantly inhibit CCL2 secretion into the culture medium. H2O2 treatment increases CCL2 production with increased concentration. NOX4 inhibitor GKT137831 could prevent Treg recruitment towards OSCC cells.

Conclusions: Our work reveals that NOX4 inhibitor GKT137831 could overcome the immune tolerance of oral cancer by suppressing Treg recruitment. GKT137831 is now an orphan drug recognized by EMA and FDA for treatment of idiopathic pulmonary fibrosis. Blocking the NOX4 by GKT137831 sheds light on oral cancer immunotherapy and opens the doors to innovative oral cancer treatment.

Keywords: regulatory T cells, NADPH oxidase 4, oral cancer

PP-011

REGULATION OF NATURAL KILLER CELLS BY LONG PENTRAXIN-3 IN ORAL CANCER

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Natural killer (NK) cell is a cytotoxic effector in human innate system with the capability to kill tumor cells. Evasion from NK cells surveillance enables cancer to proliferate and spread at early stage. Oral cancer is susceptible to NK cell mediated lysis in vitro. In clinical settings, however, the ability of NK cells to recognize and eliminate cancerous tissues is severely hampered. NK cells can recognize cancer with high surface MICA expression. However, cancer can stunt the tumor rejection functions of NK cells by shedding the ectodomains of membrane-bound MICA. The soluble MICA release into the tumor microenvironment can integrate to NKG2D receptor on NK cell surface and promote degradation of NKG2D which thereby inactivate NK cells and compromise immunity. Our preliminary results showed that PTX3 is a member of pentraxin has the capability to induce MICA sheddases expression in oral cancers. Expression changes of the 3 classes of MICA sheddases including disulfide isomerases (GRP78 & PDIA6), ADAM proteases (ADAM-9, -10, -17) and MMPs are observed in PTX3-treated oral cancer cell lines. With the majority of the oral cancer patients exhibiting high level of infiltrating natural killer cells, targeting the potent inducer of MICA sheddases shall harness the host immunity to clean the tumor load.

Keywords: Natural killer cells, Oral Cancer, PTX3, MICA

PP-185

ACHILLEA FRAGRANTISSIMA EXTRACT INDUCED DIFFERENTIATION AND DEATH OF CHRONIC MYELOID LEUKEMIA K562 CELLS

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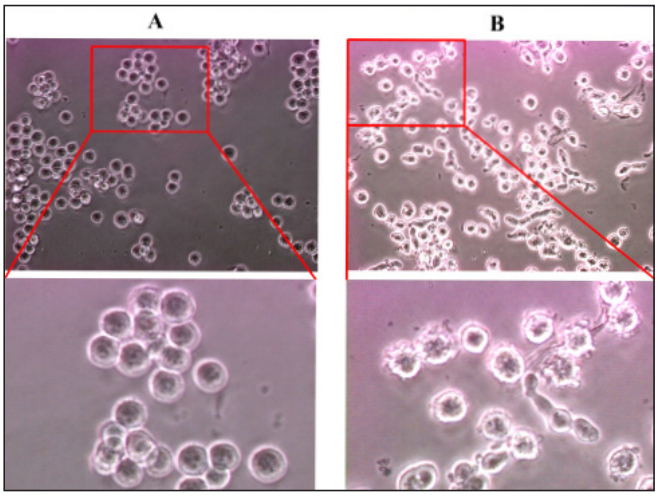
King saud uniersity, College of science, Chemistry and Natural product department

Herbs with medicinal properties have regained worldwide attention due to their therapeutic effect in the treatment of many chronic disease conditions. The advantages of herbs are due to their non-toxic nature and specificity to aberrantly expressed molecules. Most of these beneficial effects arise from the antioxidant components of herbs. Achillea fragrantissima has been traditionally used to treat viral fever, arthritis and diabetes in the Middle East. In this study, anticancer properties of extracts from A. fragrantissima plant were tested using chronic myeloid leukemia (CML) cell line K562. A. fragrantissima extract induced differentiation, inhibited growth and brought about death of K562 cells in a time and concentration dependent manner. Differentiation of K562 cells was associated with clearly identifiable morphological change from a round to a spindle shape. A. fragrantissima extract also caused disintegration of K562 cell membrane. Induction of cellular differentiation was followed by terminal maturation and eventual death. A. fragrantissima extract may serve as a therapeutic in the treatment of CML due to the prospect of chemotherapies eventually failing due to drug resistance and merits further examination as a general antitumoural agent.

Keywords: Chronic Myeloid Leukemia, Differentiation, K562, Achillea fragrantissima

K562 cell membrane disintegration. Close-up images of K562 cells undergoing blebbing and membrane disintegration following incubation with A. fragrantissima extract. K562 cells in RPMI medium (A); K562 cells in RPMI + A. fragrantissima extract (1mg/m)



A. fragrantissima extract induced morphological alterations in K562 cells. K562 cells in RPMI medium were incubated overnight (16 h) without (A) or with (B) crude extract from A. fragrantissima (0.5 mg/mL). Pictures of cells in flasks were taken using an inverted light microscope. K562 cell membrane disintegration. Close-up images of K562 cells undergoing blebbing and membrane disintegration following incubation with A. fragrantissima extract. K562 cells in RPMI medium (A); K562 cells in RPMI + A. fragrantissima extract (1mg/mL).

Fibrosis

PP-012

CARDIAC MELANOCYTES INFLUENCE ATRIAL REACTIVE OXYGEN SPECIES INVOLVED WITH ELECTRICAL AND STRUCTURAL REMODELING IN MICE

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Cardiac melanocyte-like cells (CMLCs) contribute to atrial arrhythmias when missing the melanin synthesis enzyme dopachrome tautomerase (Dct). While scavenging reactive oxygen species (ROS) in Dct-null mice partially suppressed atrial arrhythmias, it remains unclear if CMLCs influence atrial ROS and structure or if the electrical response of CMLCs to ROS differs from that of atrial myocytes. This study is designed to determine if CMLCs contribute to overall atrial oxidative stress or structural remodeling, and if ROS affects the electrophysiology of CMLCs differently than atrial myocytes. Immunohistochemical analysis showed higher expression of the oxidative marker 8-hydroxy-2'-deoxyguanosine in Dct-null atria versus Dct-heterozygous (Dct-het) atria. Exposing isolated CMLCs from Dct-het and Dct-null mice to hydrogen peroxide increased superoxide anion more in Dct-null CMLCs. Trichrome staining showed increased fibrosis in Dct-null atria, and treating Dct-null mice with the ROS scavenger Tempol reduced atrial fibrosis. Action potential recordings from atrial myocytes and isolated Dct-het and Dct-null CMLCs in response to hydrogen peroxide showed that the EC50 for action potential duration (APD) prolongation of Dct-null CMLCs was 8.2 ± 1.7 μmol/L versus 16.8 ± 2.0 μmol/L for Dct-het CMLCs, 19.9 ± 2.1 μmol/L for Dct-null atrial myocytes, and 20.5 ± 1.9 μmol/L for Dct-het atrial myocytes. However, APD90 was longer in CMLCs versus atrial myocytes in response to hydrogen peroxide. Hydrogen peroxide also induced more afterdepolarizations in CMLCs compared to atrial myocytes. These studies suggest that Dct within CMLCs contributes to atrial ROS balance and remodeling. ROS prolongs APD to a greater extent and induces afterdepolarizations more frequently in CMLCs than in atrial myocytes.

Keywords: Cardiac fibrosis, ROS, dopachrome tautomerase (Dct)

PP-013

THE ROLE OF THE G-ACTIN SEQUESTERING PEPTIDE, THYMOSIN-BETA4, IN MACROPHAGE FUNCTION

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Background: Irrespective of the underlying aetiology, kidney disease is often accompanied by an inflammatory response characterised by leukocyte infiltration in the kidney and subsequent fibrosis, which correlates with declining renal function. Thymosin-beta4 (TB4) is a G-actin-sequestering peptide with anti-inflammatory and anti-fibrotic properties that is highly expressed in macrophages. Using TB4 knockout mice, we found that lack of endogenous TB4 exacerbates kidney disease progression and increases macrophage accumulation and fibrosis. We thus hypothesise that TB4 may directly modulate macrophage function.

Aim: The aim of this project is to investigate the role of TB4 in macrophage function.

Methods: RAW264.7 mouse macrophage cells were treated with IFNγ (5 ng/ml) and LPS (50 ng/ml) to stimulate a pro-inflammatory phenotype. The cells were treated with control or TB4 siRNA (10nM) to silence endogenous TB4 expression. To test the effects of excess TB4 availability the cells were treated with recombinant TB4 (100 ng/ml) or vehicle alone. The mRNA levels of pro and anti-inflammatory markers were assessed by real time PCR. Cell migration was assessed using the transwell migration assay. To assess phagocytic ability, cells were incubated with fluorescently labelled Zymosan bioparticles and visualised by fluorescent microscopy.

Results: Transfection of RAW264.7 cells with TB4 siRNA resulted in >80% knockdown of endogenous thymosin-beta4 levels ($p<0.001$, $n=4$). Silencing endogenous TB4 resulted in a 5-fold upregulation of Arginase 1 under pro-inflammatory conditions ($p<0.001$, $n=4$), compared with control siRNA. There were no statistically significant changes in the expression of Mcp-1, CD86 or CD206. Treatment with recombinant TB4 did not alter the expression of these genes. Silencing endogenous TB4 did not affect cell migration. Both lack of endogenous TB4 and treatment with recombinant TB4 impaired phagocytic ability as indicated by a reduced percentage of cells with engulfed zymosan bioparticles ($p<0.05$, $n=6$ for both).

Conclusion: Lack of TB4 may induce a pro-fibrotic phenotype in macrophages as suggested by the upregulation of Arginase 1, an

enzyme that has been associated with enhanced collagen synthesis and fibrosis. Changes in the levels of TB4 impair the phagocytic ability of macrophages. Further work will assess whether this is due to remodelling of the actin cytoskeleton. Overall, our data shows that TB4 is a novel modulator of macrophage function.

Keywords: macrophage, kidney, thymosin-beta4

PP-016

INFLUENCE OF *VSIG4* GENE POLYMORPHISM ON THE CLINICAL EXPRESSION OF RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is a systemic inflammatory auto immune disease associated to excessive activation of the complement system by immune complexes. CRIg is a member of the complement receptors family encoded by the *VSIG4* gene located on chromosome Xp12. Its role on inflammatory diseases, including RA, was already proposed, however, the functional impact of *VSIG4* polymorphisms and association with diseases is yet to be explored. In the present study we aimed to investigate the influence of *VSIG4* gene polymorphisms on the susceptibility and clinical expression of RA. A total of 127 RA women patients and 150 controls were genotyped by sequencing for six single nucleotide polymorphism (SNPs) of *VSIG4* gene: at promoter region (g.-1431T>A, rs2284705), in exon 1 (g.-53G>A, rs41305393) and in exon 8 (g.17684C>T, p.T383I, rs41307375); (g.17710G>T, p.S397I, rs35553694); (g.18073C>A, rs17276275) and (g.18115C>T, rs1044165). The T allele of SNP g.18115C>T (rs1044165) as well as genotypes heterozygous or homozygous for this variant (TT or CT) were associated in females patients with more severe functional classes (classes III/IV) when compared to those of mild class (class I) (T: p=0.039, OR 4.11; TT or CT: p=0.008, OR 9.09). Results were adjusted for demographic factors, smoking habit and disease duration by logistic regression when possible. Our results suggest the g.18115T allele of *VSIG4* gene as a new genetic risk factor and potential biomarker for RA severity in women patients.

Keywords: VSIG4, Polymorphisms, Rheumatoid Arthritis, Complement System, CRIg

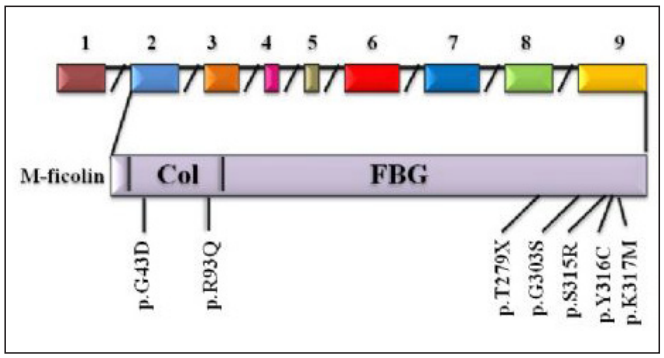

Figure 1. Complement Syste

PP-017

MASP2 INTRONIC POLYMORPHISMS INCREASE THE SUSCEPTIBILITY TO ENDEMIC PEMPHIGUS FOLIACEOUS

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Pemphigus foliaceous (PF) is an autoimmune disorder endemic in Central Western Brazil. PF patients present autoantibodies directed against desmoglein 1, loss of intercellular keratinocyte adhesion (acantholysis) and painful epidermal blisters. Exposed neoantigens may activate the lectin pathway of complement, which relies on cleavage of the serine protease MASP-2, found in complexes with mannose-binding lectin or ficolins. In a former study using TRIFMA, we found a trend for lower MASP-2 levels in 114 patients with PF (compared with 82 controls), which may indicate higher complement consumption in these individuals. In this work, we investigated *MASP2* polymorphisms in 247 PF patients and 262 controls using multiplex sequence-specific PCR: rs2273344 in intron 4 (g.7164A>G), rs9430347 in intron 5 (g.7441G>A), rs17409276 in intron 9 (g.21081C>T) and rs12711521 (p.Y371D) and rs2273346 (p.V377A) in exon 10, and measured MASP-2 levels in additional 76 controls, using ELISA. Confirming the trend formerly found, MASP-2 levels were lower in patients than in controls (p=0.0002). The distribution of haplotype frequencies did not differ between patients and controls, but patient genotype frequencies were not at Hardy and Weinberg equilibrium. In fact, GA/GA homozygotes presented higher susceptibility to PF (OR=4.59, p=0.002). We also found an association between CYV/TDV heterozygotes and the disease (p=0.04). Besides, we found 5 patients with GACYV/GATDV, but this genotype was not found in controls (p=0.02). The GA/GA genotype and the TDV haplotype were associated with higher MASP-2 levels in controls (p=0.005 and p<0.0001). Our results lead us to suggest that individuals with intronic *MASP2* polymorphisms associated with increased MASP-2 levels are at higher risk for the disease, probably due to increased complement-driven epithelial tissue injury.

Keywords: Pemphigus Foliaceous, MASP2, MASP-2, Complement System, Lectin Pathway

PP-018

ASSOCIATION BETWEEN COMPLEMENT RECEPTOR 1 POLYMORPHISMS AND LEPROSY IN BRAZIL

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Leprosy is a chronic infectious disease caused by the obligate intracellular pathogens *Mycobacterium leprae* and *M. lepromatosis*, which invade macrophages and Schwann cells. Complement receptor 1 (CR1) binds to C3b/C4b fragments and to mannose binding lectin (MBL) deposited on opsonized bacteria, facilitating bacterial entrance into phagocytes. Here, we developed a multiplex PCR sequence-specific assay and genotyped nine single nucleotide polymorphisms (SNPs) in 213 leprosy patients and 297 controls: rs6656401 (intron 4), rs3849266 (intron 21), rs2274567 (exon 22), rs3737002 (exon 26), rs11118131 (intron 26), rs11118167 (intron 28), rs17047660 (exon 29), rs4844610 (intron 37) and rs12034383 (intron 37). We measured the mRNA and soluble CR1 levels in a subset of up to 80 samples. We identified 18 haplotypes, whose frequencies differed between ethnic groups ($p<0.000001$). Afro-Brazilians with A alleles from polymorphism rs6656401 and rs4844610 presented almost four times increased susceptibility to leprosy (OR=3.89, $p=0.003$). Euro-Brazilians with the intronic rs3849266T presented higher susceptibility to leprosy (OR=1.63, $p=0.028$). Carriers of the rs11118167C presented higher CR1 mRNA expression in comparison to T/T homozygotes ($p=0.036$). Euro-Brazilians with the variant rs12034383A exhibited higher sCR1 levels compared to G/G homozygotes ($p=0.0175$). A negative correlation between sCR1 and MBL levels was also observed ($r= -0.52$; $p=0.007$). The results lead us to suggest that CR1 polymorphisms modulate gene expression and sCR1 levels, as well as susceptibility to leprosy, with the different effects in distict ethnic groups.

Keywords: CR1, Leprosy, Complement System, Polymorphisms

PP-019

FCN1 POLYMORPHISMS AND RHEUMATIC FEVER IN BRAZILIAN PATIENTS

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Rheumatic fever (RF) and chronic rheumatic heart disease (RHD), its most severe sequel, are chronic inflammations that follow oropharynx infection by β-hemolytic Streptococcus group A. The disease occurs in genetically predisposed children and teenagers (aged 3-19 years) affecting the heart, joints, nervous system and skin. Ficolin-1 (FCN-1) is the only membrane-bound activator molecule of the lectin pathway of complement. It also occurs in soluble form and recognizes acetylated residues on a great variety of pathogens including Streptococcus group A. Promoter polymorphisms of *FCN1* gene are associated with FCN-1 plasma concentration. In this work, we investigated a possible association between *FCN1* polymorphisms with the susceptibility to RF and RHD. We genotiped five *FCN1* polymorphisms in the promoter region by three sequence-specific PCR reactions in 193 patients with history of RF (92 with RHD, 42 without cardiac lesion and 59 unclassified) and 193 healthy blood donors, as controls. Genotypic distribution was in accordance with the Hardy-Weinberg model, excepting two SNPs in patients (-542 and -144). All surveyed SNPs were associated with the disease. Minor -399 A allele of SNP rs17039495 was associated with disease susceptibility ($P=0.006$, OR 4.95). The other minor alleles were associated with disease protection: rs2989727 (-1981 A, $P<0.001$, OR=0.57), rs10120023 (-542 A, $P<0.001$, OR=0.48), rs10117466 (-144 A, $P<0.001$, OR=0.38), rs10858293 (+33 T, $P=0.004$, OR=0.59). Based on these results, we suggest that *FCN1* polymorphisms may play a role in differential susceptibility to RF and RHD, emphasizing the importance of the lectin pathway in this condition.

Keywords: Rheumatic Fever, FCN1, Ficolin-1, Complement System, Lectin Pathway

PP-020

PEMPHIGUS FOLIACEUS AND THE LECTIN PATHWAY OF COMPLEMENT: EVIDENCE FOR AN ASSOCIATION WITH *MASP1* POLYMORPHISMS

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Pemphigus Foliaceus (PF) is an autoimmune endemic disease characterized by autoantibodies against desmosome glycoproteins and deposition of complement components on painful epidermal blisters. This deposition may occur through the lectin and alternative antibody-independent pathways, both of which are regulated by the mannose-binding lectin associated serine protease MASP-3. The *MASP1* gene encodes MASP-1, MASP-3 and the truncated MAp44 protein through alternative mRNA processing. In this study, we genotyped *MASP1* single nucleotide polymorphisms (SNPs) in intron1 (g.4099G>A - rs7609662 and g.4780C>T - rs13064994), common to all three mRNAs, and exon 12 (g.57882C>G - rs72549262, g.58208C>T - rs1109452 and g.6224G>A -rs850314), unique to the 3'UTR of MASP-3, in 188 PF patients and 190 controls (blood donors). In controls, MASP-1, MASP-3 and MAp44 serum levels was also measureed using TRIFMA. Genotype distribution of controls presented Hardy and Weinberg equilibrium. There was an association of the intron 1 GC and exon 12 CCA haplotypes with susceptibility to PF (OR=1.4, $P=0.031$ and OR=1.7, $P=0.005$, respectively). The CCA haplotype was also associated with higher MASP-3 levels ($P=0.0006$), probably due to disruption of the miRNA hs-miR-3181 recognition site. Thus, MASP1 polymorphisms leading to higher MASP-3 levels may increase susceptibility to PF. This may occur by increasing alternative complement activation, as well as by blocking the lectin pathway. Functional studies will clarify these not mutually exclusive hypotheses and might benefit the therapeutics of PF patients with the use of appropriate complement regulators.

Keywords: Pemphigus Foliaceus, MASP1, MASP-1, Complement System, polymorphisms

PP-021

ROLE OF LECTIN PATHWAY COMPONENTS IN SUSCETIBILITY TO HIV, AIDS AND HEPATITIS COINFECTION

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The complement system is a key effector against invading pathogens and plays an important role in neutralizing viruses and eliminating virus-infected cells. Mannose binding lectin (MBL) and ficolins (FCN-1, FCN-2 or FCN-3) in complex with MBL-serine proteases (MASP-1 and MASP-2) can initiate the complement cascade through lectin pathway activation. In this work, we evaluated the association between functional polymorphisms of the *FCN2*, *FCN3*, *MASP1* and *MASP2* genes and serum concentrations of FCN-2, FCN-3, MASP-2 and MASP-3 with the susceptibility and progression of HIV infection. A retrospective analytical study was performed in 94 controls and 126 Euro-Brazilian HIV patients, of which 71 had AIDS (CDC criteria). A total of 74 HIV-infected patients had HBV and/or HCV coinfections, of whom 57 had a past HBV infection, 13 were coinfected with HIV/HBV/HCV and 4 with HIV/HCV. We genotyped 22 SNPs using multiplex sequence-specific PCR, and analyzed the association of the serum levels and polymorphisms on HIV infection by logistic regression, correcting the results, if necessary, by sex and age using STATA (version 9.1) and R (version 3.3.3). Variables that presented a significance level of $P<0.20$ were selected for multiple analysis and a reduced model was obtained employing a $P<0.05$ threshold. Higher MASP-2 levels were associated with resistance against HIV infection (summing all patients: OR=0.02, P=0.001), independently of MASP-3 levels. MASP-3 is an inhibitor of the lectin pathway and activator of the alternative pathway, and was associated with susceptibility to HIV infection (OR=12.6, P=0.014). In contrast, *MASP2* genotypes containing low-expression alleles were associated with protection against AIDS (OR=0.19, P=0.028), which may be explained by its proinflammatory role. *MASP2*CDV* (g.1961795C, p.371D and p.377V) and *FCN3*CInsA* (g.27373182C, g.27371297-27371298insTATTTGGCC and g.27370346A) haplotypes were associated with susceptibility to HIV itself (OR=5.1, P=0.013 and OR=2.7, P=0.016, respectively). Higher FCN-2 levels predisposed HIV-infected individuals to HIV/HBV coinfection (OR=9.8, P=0.048), in contrast to the *FCN2*AGA* (g.680489A, g.680873G and g.681471A) haplotype, which protected against the same coinfection (OR=0.11, P=0.018). Finally, *MASP1*58267T* (rs1109452), located in the untranslated region of exon 12 and associated with decreased serum MASP-3 levels, was found as a protective factor against both coinfections, HBV and/or HCV (OR=0.17, P=0.001). The results lead us to suggest a key role of the lectin pathway in the susceptibility not only to HIV itself, but also to HBV/HCV coinfection and AIDS progression. Although activation of this pathway seems to be important in getting rid of HIV/HBV/HCV coinfection, exacerbated inflammation may predispose infected individuals to AIDS.

Keywords: HIV, Hepatitis, Lectin Pathway, Complement System, Polymorphisms

PP-022

FICOLIN-3 ASSOCIATION WITH VIRAL HEPATITIS IN HIV INFECTED PATIENTS

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The human immunodeficiency virus (HIV), hepatitis B (HBV) and C viruses (HCV) are the leading causes of morbidity and mortality among chronic viral infections. The lectin pathway of complement initiates by the recognition of sugar moieties or acetylated residues on these pathogens, culminating with their destruction and phagocytic removal. Several gene polymorphisms modify function and serum levels of lectin pathway components and some were already associated with these diseases. Ficolin-3 is the most abundant pattern recognition molecule of the lectin pathway in serum. In this study, we investigated a possible association between *FCN3* gene polymorphisms, ficolin-3 serum levels and the susceptibility to HIV/HBV/HCV coinfection. To this end, we genotyped the exon 5 polymorphism associated with primary immunodeficiency (rs532781899 or g.27373182delC, p.Leu106SerfsTer65) and two polymorphisms located in a regulatory intronic region (rs28362807 or g.27371297-27371298insTATTTGGCC and rs4494157 or g.27370346C>A), with multiplex PCR-SSP in 136 HIV patients and 96 blood donors. We also measured ficolin-3 serum levels with a commercial enzyme-linked immunosorbent assay (ELISA) in 37 controls and 108 patients. Genotype distribution was in Hardy-Weinberg equilibrium. The *CDelC* haplotype protected against HBV coinfection (OR=0.28, p=0.027). Lower ficolin-3 levels were found in HIV+ patients, comparing with controls (P=0.02). On the other hand, HIV patients with past or present HBV and/or HCV infection had higher ficolin-3 levels, compared with HIV patients without it (p=0.0014). There was no association between ficolin-3 levels and *FCN3* genotypes, CD4+ cell counts and viral load. Thus, higher ficolin-3 concentrations seems to provide protection against HIV infection and also to increase susceptibility to viral hepatitis, but does not play a role in disease progression. Nevertheless, they may also be a consequence of the disease or of antiretroviral therapy, since haplotypes did not associate with protein levels. The *CDelC* haplotype has a dominant protective effect against HBV infection. These results lead us to suggest that ficolin-3 plays a role in the response to viral hepatitis coinfection in HIV+ patients.

Keywords: Ficolin-3, FCN3, HIV, Lectin Pathway, Complement System

[PP-023]

ASSOCIATION BETWEEN *FCN3* INTRONIC POLYMORPHISMS AND PEMPHIGUS FOLIACEOUS

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Pemphigus foliaceous (PF) is an autoimmune disease endemic in Brazil, also known as "Fogo Selvagem" (in Portuguese, wild fire) due to burning sensation and heat caused by the disease. PF is characterized by the presence of specific autoantibodies against epitopes of the desmoglein 1, protein of the desmosomes. This is followed by the loss of epidermal cell adhesion (acantholysis), consequently leading to painful skin blisters. The ficolin-3 protein recognizes acetylated residues, possibly exposed in the acantholytic process, and activates the lectin pathway of complement. In this study, 105 patients and 105 endemic controls were genotyped by sequence-specific PCR for two *FCN3* polymorphisms: rs28362807 (g.27371297-27371298insTATTTGGCC in intron 5) and rs4494157 (g.27370346C>A in intron 7). Three haplotypes were identified: delC, insA, insC. Carriers of the insC haplotype presented resistance to disease (OR=0.28, p=0.031), as well as heterozygotes delC/insC (OR=0.18, p=0.032). This is the first study to report an association of *FCN3* intronic variants with PF. The association can be due to alternative pre mRNA-splicing, caused by the intronic variants flanking exon 4. This hypothesis will be further investigated using real-time RT-PCR.

Keywords: Pemphigus Foliaceous, FCN3, Polymorphisms, Lectin Pathway, Complement System

PP-024

GENETIC POLYMORPHISMS OF THE COMPLEMENT SYSTEM INFLUENCE SUSCEPTIBILITY TO PEMPHIGUS FOLIACEOUS

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Pemphigus foliaceus (PF) is a bullous disease characterized by autoantibodies against desmosomal antigens, acantholysis and formation of painful epidermal blisters. Among all autoimmune diseases, PF is unique to be endemic. It occurs in a region of Central-Western Brazil, coincident with the distribution of hematophagous Simulidae insects. This led to the proposal that PF may be unleashed by molecular mimicry with a component of the insect saliva or of an infectious agent carried by Simulidae vectors. Autoantigen recognition activates the complement system (CS), whose deregulation probably collaborates with the acantholytic process and tissue injury. In this study, we genotyped 16 polymorphisms of two complement regulator genes (membrane-attack complex inhibitor *CD59* and complement receptor *CR1*) and two initiator molecules of the lectin pathway of complement (MBL-associated serine protease *MASP2* and ficolin *FCN3*) in up to 238 PF patients and 268 controls, using multiplex sequence-specific PCR. We also measured CD59 and CR1 mRNA levels with real-time RT-PCR, as well as sCR1, FCN3 and MASP-2 protein levels with ELISA, correcting all possible associations for demographic factors. The *CD59* haplotype *GC* (rs861256*G and rs831625*C), associated with higher CD59 mRNA levels, increased susceptibility [OR=2.62, p=0.009], whereas the *CR1* allele rs3737002*T (p.1408M), found within a haplotype associated with reduced CR1 mRNA levels, protected against the disease [OR=0.647, p=0.027]. These associations were independent of each other and imply that protection relies on lower complement inhibition. Similarly, *FCN3* genotypes *InsC+* and *DelC/InsC* (*FCN3* alleles: rs28362807*indel and rs4494157*C), associated with protection against PF [respectively: OR=0.273, p=0.033; OR=0.193, p=0.042]. There was genetic interaction between polymorphisms of *FCN3* and *CD59* or *CR1* (p=0.004 – 0.027). On the other hand, *MASP2* genotypes *CV/TV* and *TV+*, associated with higher MASP-2 levels, increased susceptibility to the disease (*MASP2* SNPs: rs17409276 and rs2273346) [respectively: OR=2.002, p=0.031; OR=1.944, p=0.032], which may indicate a proinflammatory role in the disease. These results lead us to suggest that complement activation and regulation plays an important role in the susceptibility to PF. PF patients may possibly benefit from therapeutic strategies aiming to control this immunological proteolytic cascade.

Keywords: Pemphigus Foliaceous, Polymorphisms, Complement System, CD59, CR1

PP-025

TMEM203: A PUTATIVE CO-REGULATOR OF STING-MEDIATED INNATE IMMUNITY

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Background: The ER-resident innate immune adaptor protein STING (Stimulator of Interferon Genes) mediates the crucial type I interferon signalling downstream of double-stranded DNA-pathogen infection. From immunofluorescence and co-immunoprecipitation studies, we discovered that another ER transmembrane protein named Transmembrane Protein 203 (TMEM203) co-localises and interacts with STING, suggesting it is a putative co-regulator of STING.

Hypothesis and Aims: Hereby, we propose that TMEM203 regulates STING-dependent innate immune signalling. We aim to characterise the role of TMEM203 in STING-mediated expression of type I interferons and chemokines in response to STING ligand, and to investigate their mechanism of molecular interaction using protein complementary assay (PCA).

Methods-Results: We developed a robust siRNA-based knockdown system to suppress ~70% TMEM203 expression in human monocyte-derived macrophages (MDMs). In the presence of STING ligand 2'-3' cGAMP, TMEM203 knockdown MDMs showed a ~50% reduction of IFN-β and IL-8 expression in comparison to the control cells. To study the interaction between TMEM203 and STING, we created C-terminal

truncation mutants of STING tagged with half GFP signal and co-transfected these with wildtype TMEM203, tagged with the complementary GFP, into HEK 293 cells. Flow cytometry showed that wildtype TMEM203 and STING interact via STING's C-terminal domain. Truncation of the TBK1/IRF3 interacting domain in the C-terminus of STING enhanced the STING-TMEM203 PCA interaction, suggesting TMEM203 binding may influence STING-TBK1 or –IRF3 binding.

Conclusion: TMEM203 regulates STING-mediated type I interferon and chemokine expression in human macrophages. Interaction between TMEM203 and STING is dependent upon their respective C-terminal domains.

Keywords: TMEM203, STING, Innate Immunity, Type I interferons

PP-026

EFFECT OF DECREASED BCAA SYNTHESIS THROUGH DISRUPTION OF ILVC GENE ON THE VIRULENCE OF *STREPTOCOCCUS PNEUMONIAE*

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Streptococcus pneumoniae (pneumococcus) is responsible for significant morbidity and mortality worldwide. It causes a variety of life-threatening infections such as pneumonia, bacteremia, and meningitis. In bacterial physiology, the metabolic pathway of branched-chain amino acids (BCAAs) plays an important role in virulence. Nonetheless, the function of IlvC, one of the enzymes involved in the biosynthesis of BCAAs, in S. pneumoniae remains unclear. Here, we demonstrated that downregulation of BCAA biosynthesis by ilvC ablation can diminish BCAA concentration and expression of pneumolysin (Ply) and LytA, and subsequently attenuate virulence. Infection with an ilvC mutant showed significantly reduced mortality and colonization in comparison with strain D39 (serotype 2, wild type), suggesting that ilvC can potentiate S. pneumoniae virulence due to adequate BCAA synthesis. Taken together, these results suggest that the function of ilvC in BCAA synthesis may be a potential virulence factor and could play an important role in the pathogenesis of respiratory infections.

Keywords: Streptococcus pneumoniae, ilvC, BCAA, virulence, colonization

PP-027

INDUCTION OF ATOPIC DERMATITIS (AD)-LIKE SYMPTOMS IN NC/NGA MUTANT MICE WITH REPEATED TOPICAL APPLICATIONS OF THE HAPTEN, OXAZOLONE

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Background: Among the different atopic dermatitis (AD) models in laboratory animals, those induced by epicutaneous application of allergens and haptens are more common due to their controlled induction. In this study, we examined induction of AD-like symptoms in NC/Nga mutant mice with repeated applications of the hapten, oxazolone.

Methods: Intended skin area was shaved and baseline skin thickness and redness were measured before start of the first sensitizing or priming application of oxazolone. Animals were sensitized on Day 0 by application of 0.3% oxazolone on the shaved skin area. On Days 5, 8, 12, and 15, challenges on the same skin site were performed by application of 0.3% oxazolone solution. Control animals received sensitization and challenge with the vehicle 80% acetone and 20% olive oil. Skin thickness, redness and scaling were scored on each challenge days. The first cohorts of mice were sacrificed on Day 12 following two challenges and the second cohorts were sacrificed on Day 17 following four challenges. Treated back skin sections were collected at necropsy for histopathological evaluation.

Results: Repeated oxazolone applications induced AD-like skin symptoms in mice compared to naive or vehicle treated mice. There were oxazolone challenge-dependent increases in skin thickening, reddening and scaling in the mice. Skin thickness increased following the first challenge that gradually intensified with the follow up oxazolone challenges and peaked on Day 17. Skin redness and scales started to appear from Day 15. In histopathological evaluation, hallmarks of skin inflammation such as hyper-keratosis, acanthosis and infiltration of inflammatory cells in the dermis/epidermis were observed on Day 12 as well as on Day 17 with overall higher scores observed at the later time point. Additionally, increased serine protease activity was observed in the upper epidermis of the mouse skin after treatment with oxazolone as measured by in-situ zymography, consistent with the literature report that serine protease activity also increases in the epidermis of atopic dermatitis patients.

Conclusion: The current results show that AD-like symptoms can be induced in NC/Nga mutant mice with repeated oxazolone applications on the skin. Based on these results, four oxazolone challenges every three days following sensitization were deemed necessary for full induction of AD-like symptoms in NC/Nga strain of mice.

Keywords: Atopic dermatitis, Oxazolone, Topical, Mice

PP-028

ACTIVITY OF INFLAMMATION AND VARIOUS PARTS OF IMMUNE RESPONSE DEPENDING ON THE DURATION OF DISEASE AND FREQUENCY OF EXACERBATIONS IN CHRONIC INFLAMMATORY DISEASES

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The levels of interleukins 1β, 2 and 10, and C-reactive protein (CRP) in the blood during exacerbation of chronic inflammatory diseases depending on the duration of disease and frequency of exacerbations are analyzed. It is established that in chronic bronchitis the level of IL-1β remains practically the same in durations of the disease 3 years, 4-6 years, 7 years or more; the level of IL-2 decreases; the content of IL-10 increases in duration of the disease 4-6 years versus up to three years, and then (in duration of the disease more than 7 years) decreases. These data indicate that with increasing duration of the disease cellular

adaptive immunity weakens, humoral adaptive immunity temporarily reinforces and further reduces too. This secondary immunodeficiency in turn probably contributes to the progression of chronic inflammation. The content of CRP tends to increase with duration of the disease 4-6 years compared with the first three years of the disease and to reduce in the future (in duration of the disease more than 7 years), which suggests increased and then reduced activity of inflammation and is consistent with manifestations of immune system dysfunction.

In patients with chronic pyelonephritis with duration of the disease 4-6 years, the level of IL-1β increases compared with the first three years, and then (7 years and more) decreases. The content of IL-2 in duration of the disease 4-6 years and 7 years and more is reduced compared to the first three years. Levels of IL-10 and CRP have the same tendency as IL-1β. Thus, with increasing duration of the disease, during subsequent exacerbations, an inflammatory process is less expressed, which could indicate a more profound immunological disorder in the long history of the disease.

In chronic gastroduodenitis with increasing relapse rate (1 per year or less; 2 times a year; three or more times a year) there is a further increase in blood IL-1β, while the level of IL-2 is practically unchanged and levels of IL-10 and CRP decrease slightly, indicating a rise of immunological disorders and reduction of inflammatory activity.

Thus, in chronic inflammatory diseases, with increasing disease duration and frequency of exacerbations, activity of inflammation in the first 6 years increases and further reduces. This is caused by weakening of cellular adaptive immunity, temporary enhancement of humoral adaptive immunity, which then reduces too.

Keywords: Chronic inflammatory diseases, activity of inflammation, immune response, duration of disease, frequency of exacerbations

PP-030

CASE OF ANGIOLYMPHOID HYPERPLASIA WITH EOSINOPHILIA ASSOCIATED WITH ANTI-TNF INHIBITOR

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Angiolymphoid hyperplasia with eosinophilia (ALHE) is a benign and rare condition consisting of papular or nodular lesions of the dermis, subcutaneous tissue, and adjacent lymph nodes, typically at the head and neck. While the etiology is unknown, case reports have so far supported an idiopathic origin. This is a case of ALHE occurring as an adverse effect of anti-tumor necrosis factor-alpha (TNF-alpha) inhibitor use, a situation that has not previously been reported. An 81-year-old man with a history of rheumatoid arthritis had poor symptomatic relief from salsalate, methotrexate, and prednisone. He also developed dizziness from hydroxychloroquine. Three years after his diagnosis, he was started on adalimumab, and anti-TNF-alpha inhibitor. While he had resolution of left hand joint pains, he also developed a new dry, itchy rash at his lower back, face, and scalp. Adalimumab was replaced with a prednisone taper, while hydroxyzine and fluocinonide cream were started to manage the rash. Skin biopsies from the left lower back confirmed the presence of ALHE (histopathological figure included). Four months later, after the prednisone taper had ended and the rash had resolved, adalimumab was resumed and the patient's rash recurred at the patient's back, buttocks, and left leg. Adalimumab was again discontinued, this time in favor of etanercept, another anti-TNF-alpha inhibitor. After this change, the patient's rash resolved and did not recur again. The pathologic confirmation of ALHE and the temporal relationship between the development and resolution of the patient's rash and the use of adalimumab indicates that ALHE can occur as an adverse reaction to certain anti-TNF-alpha inhibitors. The fact that ALHE developed in the setting of adalimumab and etanercept, which have slightly different mechanisms of action to inhibit anti-TNF-alpha, may shed light on the exact pathogenesis of ALHE.

Keywords: Angiolymphoid hyperplasia with eosinophilia, ALHE, Tumor necrosis factor-alpha inhibitor, TNF-alpha, Adalimumab, Etanercept

ALHE Histopathology

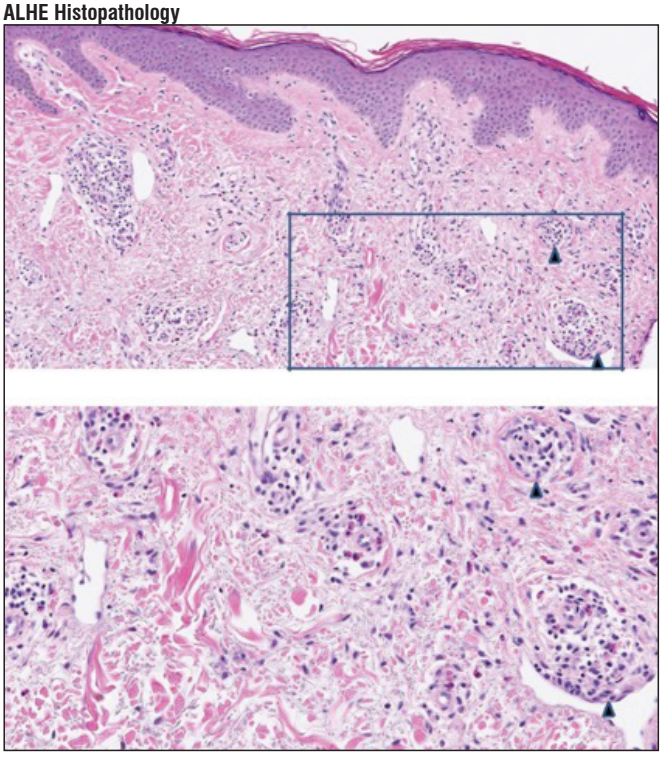

Figure 1. Histologic examination from a punch biopsy of skin affected by the patient's rash revealed a circumscribed dermal collection of vessels with plump endothelial cells. The vessels were surrounded by an inflammatory infiltrate consisting of mononuclear cells, eosinophils, and rare mast cells.

PP-031

MIRNA REGULATION OF CXCL12B DURING INFLAMMATION

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Background: Inflammation play important role in infectious and non-infectious diseases. MiRNA is also reported to play role in inflammation and associated cancers. Chemokine CXCL12 is also know to play role in inflammation and various cancers. We looked at the regulation of CXCL12β by miRNA in UC colitis.

Prolonged inflammation of colon in UC patient increases the risk of developing colorectal cancer. We looked at the expression differences of CXCl12β and its targeting miRNA in cancer susceptible area of colon of UC patients. Aim: Aim of this study was to find out the expression regulation of CXCL12β by miRNA in inflammation.

Materials and Methodes: Biopsy samples and blood samples were collected from UC patients and non-IBD controls. mRNA expression was analyzed using microarray and real time PCR. CXCL12β targeting miRNA were looked by using online target prediction tools. Expression of CXCL12β in blood samples and cell line supernatant was analyzed using ELISA. miRNA target was validated using dual luciferase assay. Results and Conclusion: We found miR-200a regulate the expression of CXCL12β in UC. Expression of CXCL12β was increased in cancer susceptible part of colon and expression of its targeting miRNA was decreased in the same part of colon. miR-200a regulate CXCL12β expression in inflammation and may be an important therapeutic target in inflammation associated cancer.

Keywords: Inflammation, miRNA, CXCL12β, regulation

PP-032

ABERRANT EXPRESSION OF INTERLEUKIN-10 AND ACTIVATION-INDUCED CYTIDINE DEAMINASE IN B CELLS FROM PATIENTS WITH BEHÇET'S DISEASE

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Background & Objective: Behçet's disease (BD) is a systemic auto-inflammatory disorder characterized mainly by recurrent oral aphthous ulcers, genital ulcers, and ocular inflammation. Despite extensive studies, the pathogenesis of BD is still not clear. In particular, B cells in patients with BD have not been elucidated. Many reports have continued to emerge regarding the contributions of abnormalities in B cells such as CD43, activation-induced cytidine deaminase (AID)- and interleukin (IL)-10-producing B cells to the progression of autoimmune disease. CD43 antigen is expressed on most leukocytes; in particular, CD43 antigen is expressed on activated B cells and plasma cells but not on resting B cells (naïve B cells). AID is a critical enzyme for immunoglobulin heavy chain class switching and somatic hypermutation in B cells. B10 cells, IL-10-secreting subset of regulatory B cells, function to downregulation of autoimmunity, inflammation, and both innate and adaptive immune responses. Thus, in this study, we investigated the relevance of B cells in patients with BD.

Materials & Methods: 16 Korean patients with BD (11 women and 5 men) and 16 age- and sex-matched healthy controls (HCs) were recruited at Konyang University Hospital. Fresh peripheral blood was obtained from each patient or HCs by venipuncture. The plasma levels of IL-10 and IgA and the proportion of CD43+ B cells, excluding naïve B cells, were measured in patients with BD and HCs. IL-10 and AID mRNA levels were assessed in B cells.

Results: Plasma levels of IL-10 in patients with BD did not differ from those in HCs. Similarly, there were no significant differences in plasma levels of IgA, although a slight increase was observed in patients with BD compared with that in HCs. There were no differences in CD43+ CD19+ B cell numbers between patients with BD and HCs. However, IL-10 mRNA levels were significantly reduced, whereas AID mRNA levels were dramatically increased in B cells from patients with BD compared with HCs.

Conclusion: Correlations between disease severity and AID expression can also be evaluated, and AID may be found to have application in the diagnostic or treatment of this common disease. Moreover, based on our findings of decreased IL-10 mRNA expression in B cells in vivo, we propose that further studies should be performed to analyze B10 cells, which downregulate immune responses, in order to further elucidate the immunological mechanisms of Behçet's disease. Our results provide insights into the role of B cells in patients with BD.

Keywords: Behçet's disease, B cells, activation-induced cytidine deaminase, interleukin-10, CD43

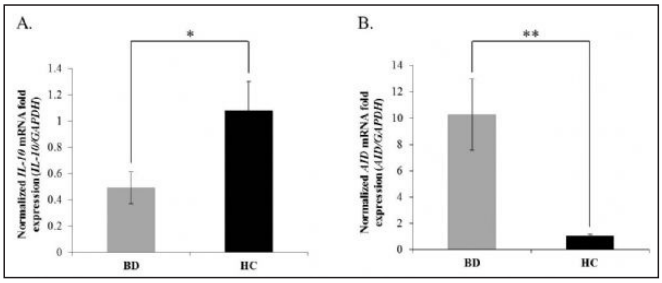

Figure 1. Changes in expression of **(A)** IL-10 and **(B)** activation-induced cytidine deaminase (AID) mRNAs in B cells

RNAs were isolated from purified CD19+ B cells of patients with BD (n = 11) and HCs (n = 11). Levels of IL-10 and AID mRNA were measured using qRT-PCR. All results are represented as fold changes using GAPDH as a control. *P<0.05, **P<0.01

PP-033

M1-POLARIZED MACROPHAGES CONTROL MYCOBACTERIAL SURVIVAL BY INDUCTION OF ENDOPLASMIC RETICULUM STRESS-MEDIATED APOPTOSIS

Yun Ji Lim, Ji Ae Choi, Junghwan Lee, Soo Na Cho, Seon Hwa Kim, Dam Go, Chang Hwa Song

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Macrophages are the most critical immune cells in the combat of mycobacterial infection. The functional phenotypes of macrophages can be classified as M1 or M2 macrophages. But, there have been few reports on about the alteration of macrophage function during *Mycobacterium tuberculosis* (Mtb) infection. Previously, we reported that ER stress-induced apoptosis plays an important roles in regulation of Mtb survival. However, the relationship between ER stress-mediated apoptosis and macrophage polarization is still not completely understood.

We confirmed that the functional alterations of macrophages play an important role to regulate survival of mycobacteria. Attenuated H37Ra infected macrophages induced the expression

of M1-related molecules, whereas M2 phenotype was dominant in virulent H37Rv infected macrophages. Mtb-secreted protein ESAT-6 is a key factor to skew macrophage function toward M2 types for survival within macrophages. Further, we found that TLR2/MyD88-dependent signaling pathway is important to modulate macrophage polarization to M1 phenotype against Mtb infection. Interestingly, ER stress response was significantly increased in M1 macrophages during M tb infection. Additionally, M1 macrophages were effective to elimination of Mtb via apoptotic cell death.

Collectively, these data suggest that M1 macrophage polarization contributes to the defense against mycobacterial infection though ER stress-induced apoptosis. To manipulate the environment for survival in host, virulent mycobacteria can skew the macrophage function toward the M2 phenotype, particularly through the secretion of ESAT-6 Ag. Our studies provide new knowledge about how ER stress-mediated apoptosis controls bacterial survival in polarized macrophages, but also insight into new therapeutic strategies targeting macrophage polarization against TB.

Keywords: Mycobacterium tuberculosis, Macrophage polarization, ER stress, Apoptosis

PP-034

DOWNREGULATION OF TUMOR NECROSIS FACTOR AND INTERLEUKIN-1 BY NANOPROTEIN ENCAPSULATED RESVERATOL IN MURINE AND HUMAN MONOCYTE CELL LINE

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Background/Objectives: Resveratol (RSV) is a phenolic constituent in fruits and nuts, such as raspberries, strawberries, walnuts, mango kernel and pomegranate. It was documented that RSV shows anti-fibrotic, anti-inflammatory activity in vivo model of bowel inflammation and lung fibrosis, however the precise mechanism of signal inhibition was not extensively investigated. The objectives of this study are first, to elucidate the anti-inflammatory effect of nanoprotein encapsulated RSV (nano-RSV) in RAW 264.7 murine macrophage cell line and THP-1 human monocytic leukemia cell line by NF-kB pathway in vitro, and second, to compare it with effect of other immuno-modulating agent, tacrolimus and colchicine.

Design/Method: We determined the cytotoxic effect of nano-RSV using MTT assay. The effects of nano-RSV on tumor necrosis facor (TNF)-alpha induced mRNA and protein expression were investigated using quantitative real-time PCR and western blot. In addition, we examined the effects of pharmaceutical drugs with anti-inflammatory properties on expression of TNF-alpha. Immunofluorescence analysis for NF-kBp65 was performed.

Results: Nano-RSV significantly suppressed the expression of interleukin – 1 beta and TNF-alpha in a dose dependent manner. Nano-RSV suppressed TNF-alpha induced inflammatory genes expression by inhibiting the phosphorylation of JNK and Akt, whereas had no significant effect on p38 activation. In addition, the inhibitory effect of nano-RSV on TNF-alpha induced inflammatory genes expression was regulated by suppression of IkB-alpha phosphorylation and NF-kB translocation. The expression of TNF-alpha mRNA level was reduced comparably treated with nano-RSV and other anti-inflammatory agents (ascorbic acid, dexamethasone and colchicine).

Conclusions: These findings suggest that nano-RSV suppresses inflammatory genes expression through phosphorylation of JNK/Akt and regulation of NF-kB signal pathway in TNF-alpha induced inflammation. Further investigations regarding effects of nano-RSV on autoimmune, inflammatory diseases are scheduled.

Keywords: resveratol, anti-inflammatory, NF-kB

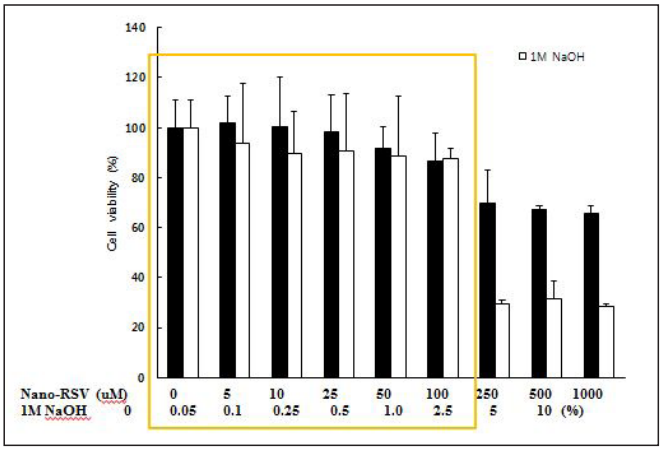

Figure 1. Effects of Nano-RSV on cell viability

We first investigated the effects of Nano-RSV on the viability of Raw 264.7 and U937 cells. Cells were treated with Nano-RSV in amount ranging from 0 to 1000 μM and incubated for 24 hr at 37°C. These results showed that the effects of ellagic acid (EA) on cells were not due to a reduction in cell viability at concentration for up to 100 μM. Based on these results Nano-RSV in amount ranging from 0 to 100 μM were used in experimental.

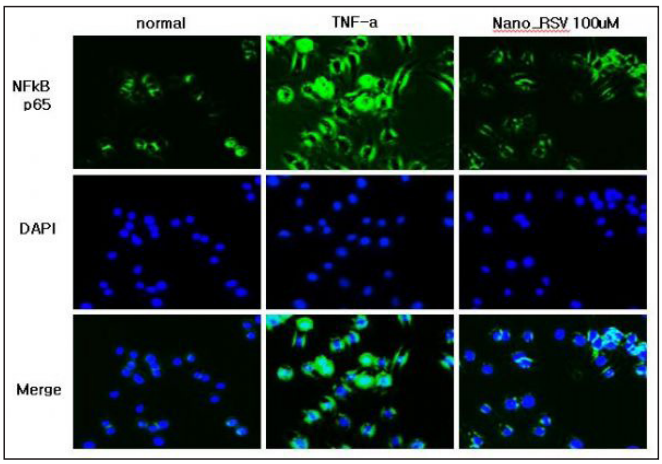

Figure 2. Immunocytochemistry in RAW264.7 cell

To investigate the inhibitory effect of Nano-RSV on TNF-a induced NF-kB phosphorylation levels, immunofluorescence staining was performed. RAW264.7 cells were pre-treated with Nano-RSV for 24 h, followed by incubation with TNF-a for 24 h. The results suggested that stimulation to TNF-a caused increased NF-kB p65 expression in nucleus, but Nano-RSV inhibited translocation of NF-kB p65 into the nucleus.

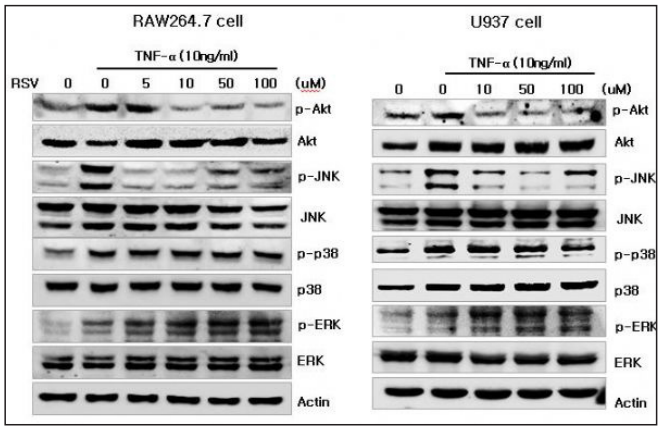

Figure 3. Nano-RSV inhibits TNF-α induced IkB-a/NF-kB signaling pathway.

The effects of Nano-RSV on the phosphorylation level of the IkB-a, NF-kB and IL-1β expression were determined via western blot analysis. Raw 264.7 and U937 cells were pretreated with Nano-RSV for 24 h and stimulated with TNF-a (10 ng/ml) for 24 h. As shown in Figure, Nano-RSV significantly inhibited TNF-a induced phosphorylation of IkB-a, NF-kB and IL-1b expression in Raw 264.7 and U937 cells. It has been reported that NF-kB activation is mediated by IkB regulation. Thus, our results indicate that Nano-RSV inhibits NF-kB activation through inhibition IkB-a phosphorylation. Therefore, we concluded that Nano-RSV inhibits TNF-a induced phosphorylation of IkB-a, NF-kB and IL-1β expression in monocytic cells.

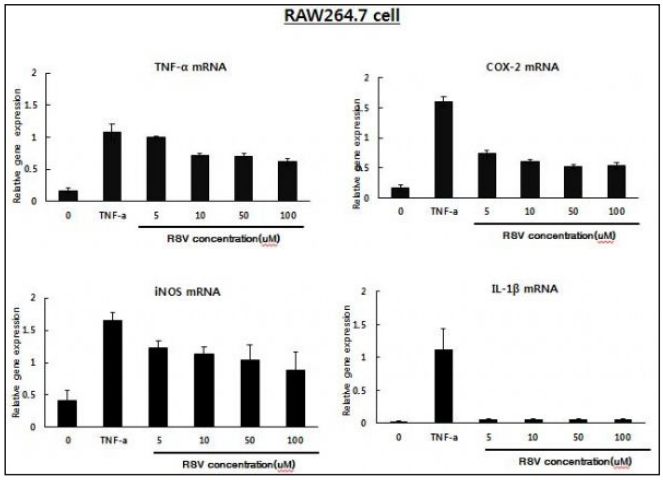

Figure 4. Nano-RSV inhibits TNF-a induced mRNA expression.

To investigate the inhibitory effects of Nano-RSV in TNF-a induced RAW264.7 and U937 cells, real-time PCR was analysis was performed to determine the cox-2, iNOS and pro-inflammatory cytokines. Cells were pre-treated with Nano-RSV for 24h at various dosages, followed by incubation with 10 ng/ml TNF-a for 6h. Expression of TNF-a, cox-2 and iNOS were inhibited following ellagic acid. Also, IL-1β was markedly inhibited following Nano-RSV (100 μM) in RAW264.7 and U937 cells.

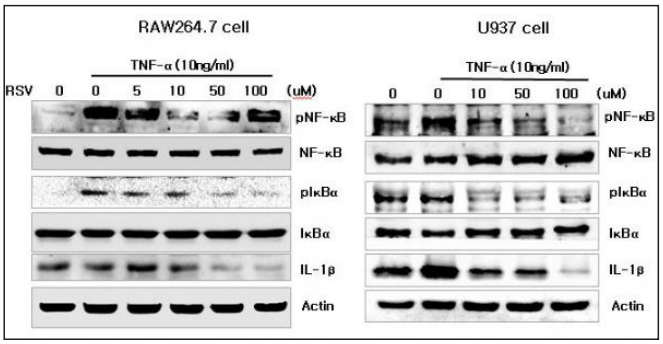

Figure 5. TNF-a-induced NF-kB activation and IL-1β expression in RAW 264.7 cells and U937.

We investigated the activity of IL-1β, and the signaling pathways related to NF-kB activation in RAW 264.7 cells and U937 using the western blot analysis after treatment with 10 ng/ml of TNF-a for 1, 6 and 24 h. The results showed that, phosphorylation of IkB-α and NF-kB protein peaked at 24 h after TNF-a treatment. And IL-1β expression increased with TNF-a treatment over 24 h in a time-dependent manner.

PP-035

INVOLVEMENT OF PROSTAGLANDIN TERMINAL SYNTHASES PGIS AND MPGES-1 IN CONTACT HYPERSENSITIVITY

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Prostacyclin (PGI_2) synthase (PGIS) and microsomal prostaglandin (PG) E synthase-1 (mPGES-1) are PG terminal synthases that function downstream of inducible cyclooxygenase (COX-2) in the PGI_2 and PGE_2 biosynthetic pathway, respectively. Both PGI_2 and PGE_2 are produced in the skin and suggested to play a role in the regulation of cutaneous immune homeostasis and responses. However, involvement of these PG terminal syntheses, PGIS and mPGES-1, in acquired cutaneous immune responses remain unclear. In the present study, to reveal the pathophysiological roles of PGIS and mPGES-1 in cutaneous immune responses, we applied PGIS- or mPGES-1-deficient mice to contact hypersensitivity (CHS) as a model of acquired immune response. 5 days after the sensitization of DNFB (dinitrofluorobenzene), the ears were challenged with DNFB application and then ear thickness was sequentially measured. As the results, we found that both PGIS- and mPGES-1-deficient mice exhibited a significantly decreased severity of ear swelling. Histological examination of the ears revealed that leukocyte infiltration and edema in the dermis were suppressed in both genotypes. PGE_2 level was increased in the ears of wild-type and PGIS-deficient mice by DNFB treatment, but the increment in PGE_2 was reduced in mPGES-1-deficient mice. 6-ketoPGF$_{1\alpha}$ (a PGI_2 metabolite) was not detected in the ears of PGIS-deficient mice. These results indicate that PGIS-derived PGI_2 and mPGES-1-derived PGE_2 cooperatively promote DNFB-induced CHS. Furthermore, our quantitative real-time PCR analysis of ear skin revealed that the expression level of interferon γ was increased by DNFB treatment in wild-type and mPGES-1-defient mice, but its expression was not induced in PGIS-deficient mice.

PGIS-derived PGI_2 might promote Th1 differentiation and thereby initiate acquired cutaneous immune responses.

Keywords: contact hypersensitivity, dermatitis, prostacyclin, prostaglandin, PGIS, mPGES-1

PP-036

PERIOSTIN DEFICIENCY EXACERBATES JOINT INFLAMMATION AND BONE DESTRUCTION IN MOUSE MODELS OF RHEUMATOID ARTHRITIS

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Background: Periostin (POSTN), a matricellular protein, is involved in many fundamental biological processes such as bone metabolism, cell proliferation, cell invasion, and angiogenesis. Also, POSTN has been shown to be involved in many aspects of inflammation, wound fibrosis and recruitment several immune cells. Although POSTN expression has been reported to promote migration and invasion of fibroblast-like synoviocyte (FLS) in vitro, there is no study to investigate the role of POSTN in mouse models of rheumatoid arthritis (RA).

Objective: This study was performed to assess the function of POSTN in 3 mouse models of arthritis, K/BxN serum transfer arthritis (STA), collagen-induced arthritis (CIA) and collagen-antibody induced arthritis (CAIA).

Methods: Periostin level in synovial fluid from RA and osteoarthritis (OA) patients were measured by ELISA. The expression of periostin FLS was stained by immunohistochemistry. STA, CIA and CAIA was induced in POSTN-/- and POSTN+/+ mice. Arthritis was monitored in 3 mouse models of arthritis using defined criteria (clinical and histologic). Osteoclastogenesis was assessed using bone marrow monocytes (BMM) cultures from POSTN-/- and POSTN+/+ mice.

Results: POSTN level in synoviocyte tissue were increased in patient with RA compare to OA patient. In STA studies, the clinical score and hind paw thickness were significantly increased in POSTN-/- mice compared with POSTN+/+ mice. Mean histologic severity scores including synovial inflammation, bone erosion and cartilage damage were increased in diseased joints from POSTN-/- mice compared with those from POSTN+/+ mice. The IL-1β was increased in the serum, and TNF-α, IL-1β, and MMPs were increased in the ankle of POSTN-/- mice than wild type control. BMMs from POSTN-/- mice showed increased osteoclast formation compared with BMMs from POSTN+/+ mice. Similarly, in CAIA and CIA model, both mean clinical severity scores and ankle joint swelling were significantly increased in POSTN-/- mice compared with POSTN+/+ mice.

Conclusions: This study suggests that POSTN contributes to pathogenesis of RA and might have a potential protective role in RA.

Keywords: Inflammation, Periostin, Rheumatoid Arthritis

PP-037

PNEUMOCOCCAL *PEP27* MUTANT IMMUNIZATION CONFERS A WIDE RANGE OF PROTECTION WITH LONG-TERM IMMUNITY VIA ACTIVATION OF ADAPTIVE IMMUNE RESPONSE

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Streptococcus pneumoniae is comprised of more than 90 serotypes and is the major causative agent of pneumonia, which results in over 1 million deaths worldwide every year. However, currently available injectable vaccines can protect against only 13-23 serotypes, and none of them protect against initial colonization in the nasopharynx against various serotypes. Thus, development of a vaccine conveying broader protection at the colonization stage is required. This study examined whether the Δpep27 could provide protection at the nasopharynx against a broad range of serotypes. Δpep27 immunization stimulated secretion of IL-4, IL-10, INF-γ, IL-17 in the splenocytes, as well as significantly increased proliferation of Tfh that might lead to differentiation of plasma cells. Colonization and opsonophagocytosis assays demonstrated that Δpep27 immunization could protect against many heterologous infections, including non-typeable strains, at the nasopharynx, and prompted efficient killing of heterologous strains, suggesting that Δpep27 immunization provides a wide range of cross-protection. Furthermore, Δpep27 immunization significantly increased both the survival rate and the level of IgG 3 months post-immunization with activation of memory T cell response, demonstrating long-lasting immunity. Thus, Δpep27 could serve as a highly feasible mucosal vaccine.

Keywords: Streptococcus pneumoniae, Mucosal vaccine, Broad protection

PP-038

ENHANCING EFFECTS OF KOREAN RED GINSENG ON PNEUMOCOCCAL *PEP27* MUTANT IMMUNIZATION

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Streptococcus pneumoniae comprises of more than 90 serotypes and is regarded as the etiologic agent of pneumonia, otitis media, meningitis, sepsis etc with significant morbidity and mortality throughout the World. Current available pneumococcal vaccines cannot protect against all the serotypes, thus serotype-independent vaccines or preventive measures need to be explored urgently. Previously, the efficacy of pneumococcal *pep27* mutant immunization was demonstrated to show comprehensive protection even against the non-typeable strains with long term immunity. However, Δ*pep27* could only show its effectiveness as a vaccine after at least 3rd immunization. Therefore, any treatment that can enhance the efficiency of Δ*pep27* immunization is needed. *Panax ginseng*, one of the most renowned medicinal plants, has already been known to show many pharmacological effects. In this study, whether the ginseng can enhance the efficacy of *pep27* mutant immunization was investigated. KRG was administrated (100 mg/kg) orally to C57BL/6 mice for 15 days and immunized them with Δ*pep27* only one time prior to infection with *S. pneumoniae* strain D39. Mice treated independently with Δ*pep27* and KRG extracts were also monitored. It was evident that followed by D39 challenge,

the mice pre-treated with the combination of Δ*pep27* and KRG showed significantly higher survival rate and as well as rapid bacterial clearance compared to other groups. Taken together, combination of single dose of *pep27* mutant immunization and 100 mg/kg of KRG seemed to protect hosts from lethal pneumococcal infection by augmenting bacterial clearance.

Keywords: Streptococcus pneumoniae, Korean Red Ginseng, Δpep27

PP-039

NEUTROPHIL PHENOTYPE AND FUNCTION IN OCULAR INFLAMMATORY DISORDERS

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University of Birmingham

The polymorphonuclear leukocytes are the most abundant cellular constituents of the immune response that allow a successful immune cell cross-talk between different immune cells. Neutrophils govern the responses induced by the innate and adaptive immune response, through secretion of a diverse range of pro-inflammatory cytokines and express presentation of antigen to activate lymphocytes.

Ocular inflammatory diseases are the result of breakdown of protective ocular barriers. Behçet's disease (BD) and ocular mucus membrane pemphigoid (OcMMP) are chronic inflammatory eye disorders in which neutrophils have been implicated in pathogenesis. The aim of this study is to investigate whether neutrophils contribute towards the pathogenesis of ocular inflammatory disorders (BD and OcMMP). It is hypothesised that constant mucosal ulceration leads to activation of neutrophils, and as a result neutrophils enter immune privileged sites and cause the breakdown of the immune tolerance.

Patients with BD (n=21) OcMMP (n=23) and healthy aged matched (n=28) controls were obtained from Birmingham and Midland Eye Centre (BMEC) after ethical approval. Phagocytic capacity of neutrophils in healthy individuals (n=28) in comparison to individuals diagnosed with BD and OcMMP was measured using PhagoTest kit (Glycotope Biotechnology). The production of reactive oxygen species was investigated in healthy individuals in comparison to individuals diagnosed with BD and OcMMP by using a PhagoBurst kit (Glycotope Biotechnology). Activated neutrophils, newly released, reverse migrated, low density neutrophils and granulocytic myeloid depressor cells were analysed in diseased patients in comparison to healthy controls by flow cytometry.

The results demonstrate a significantly reduced (<0.05) phagocytic capacity and ROS production (< 0.05) in patients with BD, and OcMMP in comparison to healthy controls (age matched). In addition, the results show a significantly (<0.05) reduced ROS production after stimulating neutrophils with E.coli in BD and OcMMP patients in comparison to healthy controls. Flow cytometry showed that a significant (<0.05) high percentage of CD11B bright, CD16 bright, CD66B bright, CD15 bright, CD54 bright were observed in OcMMP and BD patients in comparison to healthy controls.

The results suggest that neutrophils from OcMMP and BD patients have an altered function and are fully activated in disease severity. Neutrophils display a heterogeneous phenotype and may have the ability to supress T cell function (adaptive immunity) in BD and OcMMP.

Keywords: Polymorphonuclear leukocytes, OcMMP, BD

PP-040

IMMUNE RESPONSE PROFILE IN NATURAL DENTINE REPAIR

Vitor De Carvalho Moreno Das Neves, Paul Thomas Sharpe
King's College London

Regenerative dentistry aims to understand the responses of dental tissues to injury with the hope of creating treatments that promote tissue repair and regeneration whilst preserving tooth vitality. The inflammatory process of dental repair is assumed to be crucial for pulp healing and cell differentiation. However, the inflammatory cells present in the reparative process and their time profile has never been shown.

Current dentistry uses synthetic materials based on mineral ions exchange such as MTA and Biodentine, both have been show to aid the formation of tertiary dentine. In addition, our group recently found that GSK-3 inhibiting drugs, such as Tideglusib, promotes natural dentine repair.

In order to assay the immune response profile, we have developed a reproducible system to damage mouse molars. This consists on using a dental burr to cut the dentine and a needle to expose the dental pulp, and then we seal the exposed pulp with the current gold standard materials in dentistry or our Wnt agonist drug.

We show that the dental pulp is abundant in macrophages and absence of neutrophils when in homeostasis, and soon after injury there is invasion of Ly6g+ neutrophils in the dental pulp and F4/80+ macrophages are present at the injury site. Moreover, super activation of local Wnt signalling with Tideglusib at the injury site leads to increased chemotaxis of Ly6g+neutrophils at 1 and 3 days after injury. This provides a basal knowledge of the immune response cells profile throughout the reparative process, and the effect of Wnt signalling at the injury site.

Keywords: Regenerative dentistry, Macrophages, Neutrophils, Wnt signalling

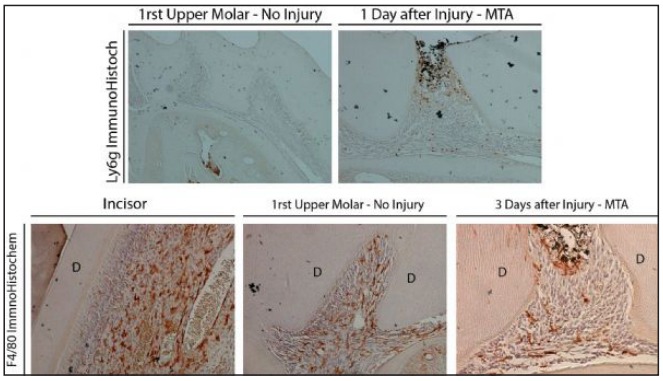

Figure 1. Neutrophils and Macrophages in mouse teeth

PP-041

PROPHYLACTIC AND THERAPEUTIC EFFECTS OF THREE DIFFERENT PHARMACOLOGICAL AGENTS IN THE KEY-HOLE LIMPET HEMOCYANIN (KLH)-INDUCED DELAYED-TYPE HYPERSENSITIVITY (DTH) MODEL IN MICE

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Background: Delayed-type or type IV hypersensitivity reactions are cell-mediated immune reactions involving activation of T cells and are known to play roles in autoimmune diseases and organ transplantation. In the present study, using two different dosing protocols, effects of clinically used anti-inflammatory, immunosuppressive agents were evaluated in the antigen, KLH-induced DTH model in mice.

Methods: Animals were sensitized on Day 1 by subcutaneous injection with KLH and then challenged with KLH by intra-dermal injection in the ear to induce the model. Once daily (QD) oral gavage dosing (PO) with budesonide (0.01, 0.1 and 1 mg/kg), cyclosporine A (0.6, 6 and 60 mg/kg) and dexamethasone (0.03, 0.3 and 3 mg/kg) were done from Day 1-10 for the prophylactic effects and Day 7-10 for therapeutic effects. Ear thicknesses were measured on Day 7 before challenge and on Days 8, 9 and 10 at approximately 24h, 48h and 72h post challenge. Animals were sacrificed on Day 10 following the final ear thickness measurements.

Results: On Days 8, 9 and 10, there was significant ear swelling in animals that received KLH subcutaneously for the sensitization on Day 1 and intra-dermally for challenge in the ear on Day 7 compared to animals that received KLH challenge only. The extents of ear swelling were almost the same level on all three days. For measuring overall effects of the test agents, data were converted into area under the curve (AUC) for the ear thickness from Day 7 to Day 10. For budesonide, significant effects were observed at 1.0 mg/kg both in prophylactic and therapeutic mode although some milder and significant effects were also observed at 0.1 mg/kg in the therapeutic mode only. Percent inhibition at 1.0 mg/kg was slightly better in prophylactic mode compared with therapeutic mode (15% in therapeutic vs 20% prophylactic). For both Cyclosporine A and Dexamethasone, statistically significant effects were observed only at the highest dose level i.e. at 60 mg/kg for Cyclosporine A and at 3 mg/kg for Dexamethasone. Percent inhibition in prophylactic mode was greater than in therapeutic mode for both Cyclosporine A (26% in therapeutic vs 35% in prophylactic) and Dexamethasone (20% in therapeutic vs 43% in prophylactic).

Conclusion: The current results show significant induction of delayed-type hypersensitivity by the antigen KLH in mice for up to 3 days post challenge. There was slightly better effect with prophylactic dosing than with therapeutic dosing for all three test agents. Overall, cyclosporine A and dexamethasone showed greater efficacy compared with budesonide. Thus, cyclosporine A and dexamethasone have been chosen as the optimal positive control agents for studies in this model.

Keywords: delayed type hyper-sensitivity, keyhole limpet hemocyanin, prophylactic, therapeutic, mice

PP-042

PLATELET-MEDIATED ACTIVATION OF NEUTROPHILS RESULTS IN COAGULOPATHY AND CONTRIBUTES TO LUNG PATHOGENESIS ASSOCIATED WITH VIRUS INFECTION

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Influenza A virus (IAV) is a common cause of respiratory tract infection in humans with approximately 10% of the population infected by the virus each year. The hallmark of severe IAV infection is excessive inflammation and tissue pathology in the lungs. Recent studies have suggested that platelets and neutrophils are key players in the inflammatory responses associated with IAV infection and strongly involved in the pathogenesis of IAV, but specific mechanisms have not yet been clarified. Using multi-color confocal intravital microscopy in mouse models of lung viral infection, we observed profound platelet aggregation, NET production and thrombin activation in vivo. Mice were either treated twice with the viral analogue, polyinosinic–polycytidylic acid [poly(I:C)] intratracheally before 48, 24 hours or were infected with a mouse-adapted influenza virus (A/Puerto Rico/8/34 H1N1) intranasally before 5 days prior to imaging. Both approaches resulted in profound neutrophil and platelet recruitment to the lung resulting in a significant increase in the number and size of platelet aggregates within the vasculature. We hypothesized that blockade of platelet activation or platelet-neutrophil interactions may ameliorate the neutrophil accumulation and neutrophil extracellular traps (NETs) formation during IAV infection, and thrombin may act as a key regulator of these responses. Blockade of CD18 or CD41 reduced virus-induced NET production in the lung. In addition to cell recruitment, we also observed marked thrombin activation following viral challenge and pharmacological inhibition of protease-activated receptor 4 (PAR4) reduced platelet aggregation and NET production. Importantly, PAR4 inhibition, or treatments that prevent NET production, protected the mice from virus-induced lung tissue damage and edema. Together, these data show that platelets play critical role in activation/recruitment of peripheral blood neutrophils, and contribute to viral pathogenesis in the lung.

Keywords: Platelet, Neutrophil, Neutrophil extracellular trap, Thrombin, Influenza

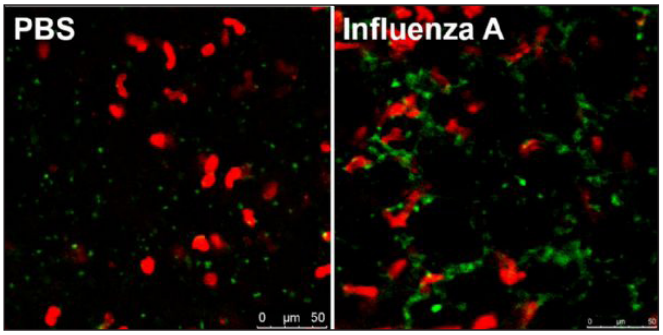

Figure 1. Intravital visualization of platelet aggregation within the lungs of virally challenged mice

Intravital visualization of platelet aggregation within the lungs of virally challenged mice. Platelets in PBS-treated lungs do not aggregated and appear as individual platelets located within the lung vasculature. After 5 days of intranasal influenza virus infection, platelets form large, dynamic aggregates in the lung vasculature (neutrophils, Alexa Fluor 647-conjugated anti-Ly6G [red]; platelets, PE-conjugated anti-CD49b [green])

PP-043

A MULTIFUNCTIONAL APPROACH TOWARDS TESTING IMMUNOMODULATORY EFFECTS OF NEW DRUGS

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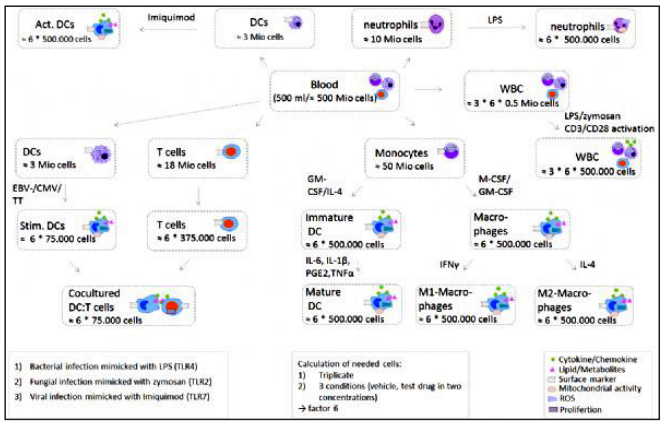
Fraunhofer IME-TMP

With the current extensive interest in therapeutic and adverse immunological effects of drugs, there is an increasing need to test new compounds for their immunomodulatory effects. Moreover, development of antibiotics with both antibacterial and immunomodulatory effects are likely to be therapeutically more effective. Biologicals, on the other hand should exert selective actions without inducing an immune response. To test the immunomodulatory effects of new drugs we propose a multifunctional approach. The effects of the drugs should be tested in a cell type and functionally specific way and natural bacterial, fungal or viral challenges should be adequately mimicked. This means investigating the possible interactions of the drugs with important inflammatory processes such as the differentiation of monocytes to macrophages or to dendritic cells and their subsequent activation. Furthermore, the influence of the drugs on T cell activation and proliferation and the activation of neutrophils should be analyzed. The effects of the drugs on these cellular processes can be investigated by the determination of surface markers, reactive oxygen production, proliferation and mRNA and protein levels of cytokines, chemokines and inflammatory mediators. The analysis of all these parameters together allows a suitable characterisation of the immunomodulatory effects of new drugs.

Keywords: biologicals, immunmodulatory effects, inflammation

Case



Conclusion

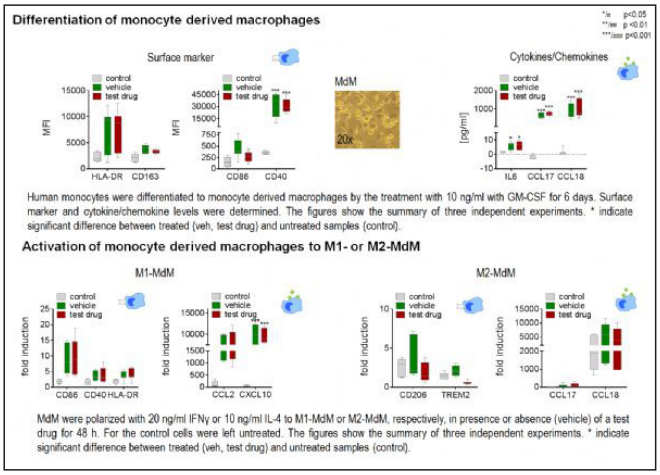
Marker/ Cell type	Surface marker	Cytokine/ Chemokine	ROS	Mitochondrial activity	Lipids
MdM	CD40, CD86	IL6, IL10, CCL17, CCL18	NO	nd	---
MdM1	CD40, CD86, HLA-DR	CCL2, CXCL10	---	nd	---
MdM2	CD206, TREM2	CCL17, CCL18	nd	nd	nd
Im. MdDC	CD1a, Cd1c, CD40, CD54, CD83, CD86, CD206, CD209, HLA-DR	IL6, IL1β,IL12p40, IL10	nd	nd	nd
Mat. MdDC	CD40, CD80, CD83, CD86, HLA-DR, HLA-ABC	IL8, IL12p40, IL23	nd	nd	nd
Act. DC	CD80, CD83, CD86, CD123, CD163, C197, HLA-DR, HLA-ABC	nd	nd	nd	nd
Cocult. T cell	CD25, CD45RO, CD69, CD134, CD154	IL2, IL6, IL8, IL9, IL10, CCL4, IL12p40, CXCL10, TNFα, IFNγ	nd	nd	nd
Cocult. DC	CD40, CD86, HLA-DR	IL6, IL8	nd	nd	nd
Neutrophils	nd	nd	H_2O_2	nd	nd
WBC	nd		nd	nd	nd
M1-Φ	CD80	TNFα, RelB, SOCS3, SOCS1, CCR7	nd	nd	nd
M2a-Φ	CD206, CD200R	MRC1, CCL18, CCL22	nd	nd	nd
M2b-Φ	---	---	nd	nd	nd

nd= not determined; --- = no regulated parameter detected

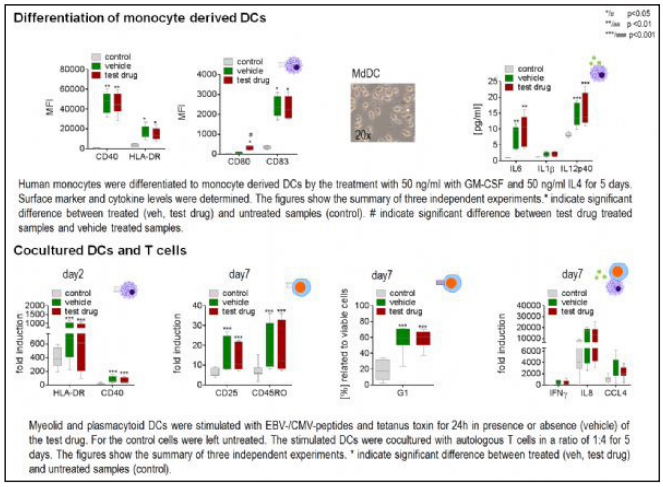
Objective

Cell types	Cellular function	Techniques
Macrophages	Antigen presentation Activation	Surface marker
Dendritic cells	Antigen presentation Activation	Cytokines, chemokines
T cells	Proliferation Activation	Inflammtory mediators
Neutrohpils	Activation	Proliferation
		Mitochondrial activity
		ROS production

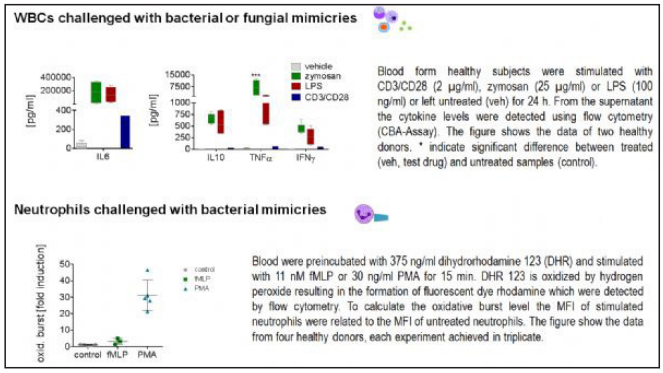
Results-1



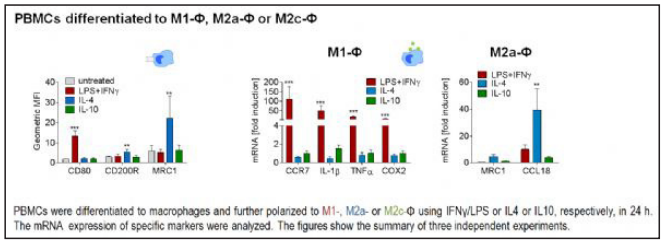
Results-2



Results-3



Results-4



PP-044

ROLES OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN CARTILAGE TISSUE ENGINEERING

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To obtain stable outcomes in regenerative medicine, understanding and controlling immunological responses in transplanted tissues are of great importance. In our previous study, auricular chondrocytes in tissue-engineered cartilage transplanted in mice were shown to express immunological factors, including macrophage migration inhibitory factor (MIF). Since MIF exerts pleiotropic functions, in this study, we examined the roles of MIF in cartilage regenerative medicine. We made tissue-engineered cartilage consisting of auricular chondrocytes of C57BL/6J mouse, atellocollagen gel and PLLA scaffold, and transplanted the construct subcutaneously in a syngeneic manner. Localization of MIF was prominent in cartilage areas of tissue-engineered cartilage at 2 weeks after transplantation, though it became less apparent by 8 weeks. Co-culture with RAW264 significantly increased the expression of MIF in chondrocytes, suggesting that the transplanted chondrocytes in tissue-engineered cartilage could enhance the expression of MIF by stimulation of surrounding macrophages. When MIF was added in the culture of chondrocytes, the expression of type II collagen was increased, indicating that MIF could promote the maturation of chondrocytes. Meanwhile, toluidine blue staining of constructs containing wild type (*Mif*+/+) chondrocytes showed increased metachromasia compared to MIF-knockout (*Mif*-/-) constructs at 2 weeks. However, this tendency was reversed by 8 weeks, suggesting that the initial increase in cartilage maturation in *Mif*+/+ constructs deteriorated by 8 weeks. Since the *Mif*+/+ constructs included more iNOS-positive inflammatory macrophages at 2 weeks, MIF might induce an M1 macrophage-polarized environment, which may eventually worsen the maturation of tissue-engineered cartilage in the long term.

Keywords: Macrophage migration inhibitory factor (MIF), Cartilage tissue engineering, Transplantation

PP-045

INTERRELATIONSHIPS BETWEEN INFLAMMATION AND HUMORAL IMMUNE RESPONSE TO ENTEROBACTERIAL LIPOPOLYSACCHARIDES IN PATIENTS WITH RHEUMATOID ARTHRITIS

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The main reason of death in rheumatoid arthritis is cardiovascular complications caused by chronic low-intensity inflammation in vascular intima. It is known that in patients suffering from rheumatoid arthritis the wide range of gastrointestinal disorders leading to intestinal barrier injury and bacterial translocation improvement. In this regard, one of the most important factors of chronic low-intensity inflammation maintaining in rheumatoid arthritis potentially could be enterobacterial lipopolysaccharides. Dysfunctions of enterobacterial lipopolysaccharides' immune mechanisms of neutralization and clearance contribute their both proinflammatory potential implementation and cardiovascular risk rise.

The aim of the investigation was to study interrelationships between humoral immune response to enterobacterial lipopolysaccharides and low-intensity inflammation in rheumatoid arthritis. 58 women of average age 51,6±1,3 suffered from rheumatoid arthritis of I-II stage from 1 to 20 years were investigated. All patients were undergone standard treatment including disease-modifying drugs (methotrexate 7,5-20mg per week combining with folic acid) and symptomatic treatment. The levels of serum antibodies to enterobacterial lipopolysaccharides (anti-LPS-Ab), general concentration of the given immunoglobulins and concentration of serum C-reactive protein (CRP) were determined with the enzyme- linked immunosorbent assay (ELISA).

Performed study showed that the majority of patients with rheumatoid arthritis (about 75%) demonstrated reliable decline of anti-LPS-Ab classes G and M and normal levels of anti-LPS-Ab class A. At the same time, about 25% of patients with rheumatoid arthritis revealed reliable rise of anti-LPS-Ab class A against the background of normal levels of anti-LPS-Ab classes G and M. The named changes were connected neither with the duration of the disease nor with the general B-cellular immune mechanisms. Cluster analysis determined that the rise of inflammatory activity in patients with rheumatoid arthritis was tightly associated with the deeper drop of levels of anti-LPS-Ab classes G and M.

The applied data proved that compromised humoral immunity against enterobacterial lipopolysaccharides in patients with rheumatoid arthritis potentially causes the increase of serum endotoxins' concentration leading to both the persistence of chronic low-intensity inflammation and higher cardiovascular risk.

Keywords: Rheumatoid arthritis, lipopolysaccharides, immune response

PP-046

REDUCING VIRULENCE FACTORS AND INCREASING INFLAMMATORY RESPONSES BY PNEUMOCOCCAL ΔPEP27

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Streptococcus pneumoniae is the most common gram-positive commensal bacteria residing in host nasopharynx and upper airway. *S.pneumoniae* causes life-threatening diseases such as pneumonia, bacteremia, and meningitis. It comprised of more than 90 serotypes yielding a range of colonization and virulence factors, including the polysaccharide capsule, surface proteins and enzymes, and the toxin pneumolysin. These virulence factors protect bacteria by avoiding host immune response. Pep27, one of virulence factor, is known to mediate LytA-dependent and independent lysis modulator that highly contribute to the pathogenesis of *S. pneumoniae*. Previous report suggested that loss of Pep27 shows significantly protect against *S.pneumoniae* by reducing the virulence factors. However, there are no clearly demonstrated which virulence factors of *S.pneumoniae* were modulated and whether inflammatory responses triggered by pneumococcal pep27 mutant. Therefore, in this study, established virulence factors of wild type (serotype 2) and Δ*pep27* was determined and compared by microarray analysis. And through *in vivo* study, Δ*pep27* was immunized to mice once a week three times, and then serum was collected from the mice and determined by ELISA analysis. Here we report our findings that pneumococcal Δ*pep27* has lowering effects on the virulence of pneumolysin, Cps, Ply, PspA, IgA, NanA as compared to wild type. In addition, pneumococcal Δ*pep27* triggered 10 fold more destructive inflammatory responses by increasing pro-inflammatory cytokines such as IL-1β and TNF-α. However, how pneumococcal Δ*pep27* modulate virulence genes and pathways in the host remain unanswered. This understanding and identification of novel virulence determinants of the role and pathogenesis of Δ*pep27* can be used to identify possible points of intervention for treatment or vaccination.

Keywords: Streptococcus pneumoniae, Virulence factors, pneumococcal Δpep27, Inflammatory response

PP-047

LEUKOTRIENE (LT) B_4 METABOLITES INHIBIT THE LTB_4-MEDIATED FUNCTIONS OF HUMAN NEUTROPHILS

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Context: Neutrophils play an important role in host defense. They eliminate pathogens by phagocytosis and by the liberation of antimicrobial peptides such as α-defensins. Many mediators are implicated in the recruitment of neutrophils to infection sites, notably leukotriene (LT) B_4. Mainly known as a chemoattractant

for neutrophils, LTB_4 is also recognized as an excellent promoter of host defense. Indeed, through the activation of the BLT_1receptor, LTB_4 induces phagocyte recruitment, the release of antimicrobial peptides and potentiates the ingestion and the killing of pathogens. In humans, LTB_4 is rapidly metabolized into 12-oxo-LTB_4 by monocytes/macrophages, or into 20-OH- and 20-COOH-LTB4 by neutrophils. Although those LTB_4 metabolites bind to the BLT_1 receptor with high affinity, they poorly activate neutrophils.

Hypothesis: We postulated that LTB_4 metabolites act as inhibitors of BLT_1-mediated responses, therefore limiting the impact of LTB_4 on human neutrophil functions and that inhibiting LTB_4 degradation would enhance its effects on neutrophils.

Results: 20-OH-, 20-COOH-, and 12-oxo-LTB_4 inhibited all LTB_4-mediated responses of neutrophils we tested (migration, degranulation, leukotriene biosynthesis). 20-OH-LTB_4 was as efficient as the BLT_1 antagonist CP 105,696 and all LTB_4 metabolites were more potent than the anti-inflammatory and pro-resolving lipids resolvin E1 and 5(S),6(R)-lipoxin A_4. In contrast, the fMLP- and IL-8-mediated responses were not affected by LTB_4metabolites. Inhibiting LTB_4 degradation into 20-OH-LTB_4 in neutrophils notably amplified the LTB_4-induced kinase phosphorylation and diminished the chemokinetic effect of LTB_4.

Conclusion: Our data indicate that LTB_4 metabolites act as natural inhibitors of LTB_4-mediated responses and that preventing LTB_4 metabolism can modulate some functions of neutrophils. These results support the concept that preventing LTB_4 degradation might enhance host defense by boosting innate immunity.

Keywords: Leukotriene B4, neutrophils, host defense

PP-049

FICOLIN-1 SERUM LEVELS IN PATIENTS WITH HIV INFECTION FROM BRAZIL

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HIV infection is a chronic disease that if left untreated leads to immunodeficiency syndrome (AIDS) and death. The complement system is one of the key components of innate immune system, responsible for surveillance of the body against infectious agents. Once activated by three different pathways, complement mediates cells lysis, opsonization, signalizing pathogens for phagocytosis, and, induces the adaptive immune response. The lectin pathway comprises several soluble and membrane bound proteins, also called pattern recognition molecules (PRM) such as mannose binding lectin (MBL), ficolins 1, 2 e 3 and collectin 11. These PRMs recognize pathogens molecular patterns (PAMPs) such as residues carbohydrates, N- linked glycans, LPS, sialic acid in the surface of pathogens, leading to complement activation. The HIV viral envelope contains mannose-rich glycans, the glycoprotein Gp120 and Gp41, that are essential in the infection process and may be target by complement PRMs, such as MBL and ficolins. The complement is known to be involved in the pathogenesis of HIV infection, contributing to the control and viral replication and clearance; among others. Although many reports have shown that MBL and ficolin-2 play a critical role in early HIV infection, studies on ficolin-1 are scarce. In this context, our study investigated the serum levels of ficolin-1 in 96 HIV-infected patients from Southern Brazil using ELISA commercial kit. Demographical, laboratorial, and clinical findings of the patients were obtained from medical records. Moreover, 86 Southern Brazilian with negative HIV serology and without clinical complaints were used as control subjects. All parameters were analyzed with adjustment for age, ancestry and sex using logistic regression analysis. Formal written consent was obtained from each individual and this study was approved by local ethics committee. Our results demonstrated lower levels of ficolin-1 in HIV-infected patients when compared to controls ($p<0.0001$ Median: 677 ng/ml vs. 1,189 ng/ml, respectively), and no significant difference between HIV and HIV/AIDS patients ($p=0.94$ Median 762 ng/ml vs. 682 ng/ml, respectively). Ficolin-1 levels were not correlated to the time of HIV infection, viral load or CD4 count, or the presence of opportunistic disease. In addition, men presented lower ficolin-1 levels than women for both HIV-infected patients ($p=0.03$ Median 601 ng/ml vs. 861 ng/ml, respectively) and controls ($p=0.0003$ Median 1,079 ng/ml vs. 1,457 ng/ml, respectively). These findings suggest that low levels of ficolin-1 might contribute to virus-host interactions, defining HIV infection.

Keywords: HIV, Ficolin-1, Complement System, Lectin pathway

PP-050

MANNOSE BINDING LECTIN (MBL) AND PENTRAXIN 3 PLASMA LEVELS AS BIOMARKERS OF DIABETIC RETINOPATHY

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Introdution: Mannose binding lectin (MBL) is a protein of complement system and Pentraxin-3 (PTX3) is an acute phase protein. In this study, we evaluated whether MBL and PTX3 plasma levels were associated to development of DR and if are able to differentiate mild from severe Diabetic retinopathy (DR).

Material-Methods: Patients included were divided in three groups: diabetic without DR; with mild DR and severe DR. Measurement of PTX3 and MBL was performed with ELISA kits.

Results: A total of 74 patients were included. A significant association of MBL high levels with severe DR was observed, which 47% patients with severe DR had high levels of MBL, while 12% of the diabetic patients without DR (P=0.008;OR: 6.06;CI: 1.4-25.0). High levels of MBL were more frequent in patients with severe disease (47%) when compared to mild RD (20%), P=0.04(OR:3.46;CI:1.0-11.8). PTX3 levels distribution was similar among all groups and were neither related to the development nor severity of DR.

Conclusion: We found a significant association between high levels of MBL plasma levels with DR development and with severe DR. We observed no relation of PTX3 plasma levels with development nor severity of DR.

Keywords: MBL, Pentraxin 3, Diabetic Retinopathy, Complement System

PP-051

ABLATING IFITM GENES POTENTIATES INFLAMMATORY RESPONSES IN THE MOUSE

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Ifitm genes are a group of interferon inducible genes that are transcriptional activated upon interferon stimulation and play important roles in interferon meditated antiviral functions. However, the biochemical properties and other biological functions of these proteins are not fully understood. Previously IfitmDel mutant mice lacking 5 Ifitm genes were generated to investigate the biological functions of Ifitm genes. We recently observed age dependent low-grade inflammation phenotype in IfitmDel mice. Mutants at 4-month-old of age started to develop increased plasma levels of pro-inflammatory cytokines including TNF-α and IL-1β, with elevated levels of monocyte activation markers such as CD86 and MHCII in peripheral blood of those mutants. Obesity and hyperphagia have been reported in IfitmDel mutants. The expression levels of hypothalamic nos2 and cytokine genes tnf and il6 are upregulated in the mutants, indicating that hypothalamic inflammation is also developed. To test whether inflammation is caused by malfunctioned mutant myeloid cells or by excessive inflammatory signals, we further generate bone marrow derived macrophages from both IfitmDel mutants and wild type controls. Increased activation status and expression levels of pro-inflammatory cytokines are developed spontaneously in the differentiated mutant macrophages without stimulation. Upon LPS stimulation, mutant macrophages are drastically activated and the activation status is significant higher than that of wild type macrophages. The molecular mechanisms underlying this inflammatory phenotype and its relationship with the metabolic and neurological phenotype are further studied.

Keywords: Ifitm, inflammation, macrophages

PP-052

SYNTHETIC TRIPEPTIDE WOL074-029 SHOWS POTENT SYSTEMIC AND LOCAL ANTI-INFLAMMATORY CAPACITIES

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KdPT is a well-known anti-inflammatory tripeptide with proven effectiveness in animal models of intestinal inflammation and psoriasis. Moreover, in a phase II trial KdPT was shown to significantly accelerate disease remission in patients with mild to moderate active ulcerative colitis. Since phys.-chem. properties of KdPT do not allow topical application and, moreover, since oral availability can be improved, we designed and synthesized several chemical analogues of KdPT with optimized characteristics.

In vitro studies demonstrated that three tripeptides (WOL074-009 (9), WOL074-019 (19), and WOL074-029 (29)) exhibit strong anti-inflammatory effects (significant reduction of inflammatory markers in murine as well as human T-cells and HaCaT cells).

WOL074-029 was thus selected for further in vivo analysis in mouse models of imiquimod-induced psoriasis-like skin inflammation and dextrane sodium sulphate (DSS)-induced colitis.

Interestingly, topical application with 29 (at days 4, 6 and 8 using a cream containing 100 μg of the tripeptide in 50 mg cream or vehicle cream) resulted in a significant reduction of epidermal thickness, a decreased activation of effector cells and downregulated expression of pro-inflammatory cytokines (IL-1β, IL-6 or TNF-α) in lesional skin. Worth mentioning, topical treatment was as effective as the systemic (intravenous, i.v.) application of the compound or KdPT.

Besides reducing imiquimod-induced psoriasis-like skin inflammation the tripeptide also ameliorated ongoing DSS-induced colitis. Either intraperitoneal injection of the tripeptide (5 μg per mouse and day) at days 4 to 7 or oral application (100 μg per mouse and day) at days 4 to 7 protected mice from weight loss, diarrhea and rectal bleeding. Of note, at the end of the experiment (day 8) mice that received tripeptide 29 showed an improved clinical score compared to i.v. KdPT treatment and a clinical score comparable to betamethasone-treated controls. Mechanistically, the anti-inflammatory effect of the tripeptide 29 and KdPT was mediated by decreasing the expression of IL-6, IL-1β, TNF-α and IFN-γ (as shown by qPCR and immunofluorescence staining).

In summary, the synthetic tripeptide 29, with improved phys.-chem. properties compared to the original tripeptide KdPT, demonstrated broad anti-inflammatory effects comparable to the ones observed for KdPT. Finally, due to the improved chemical structure can be formulated for topical AND oral treatment.

Keywords: synthetic tripeptide, psoriasis, colitis

PP-053

IMPACT OF TOLL-LIKE RECEPTOR 4-MEDIATED SIGNALLING IN THE MODULATION OF PLATELET ACTIVATION, HAEMOSTASIS, AND THROMBOSIS

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Platelets are small, circulating blood cells that play a vital role in haemostasis and, under pathological conditions, thrombus formation. Moreover, platelets are also involved in the progression of innate immune responses through interactions with pathogen-associated molecular patterns and leukocytes via multiple receptors including toll-like receptor 4 (TLR4) that binds to bacterial lipopolysaccharides (LPS). Unlike other TLRs, TLR4 can signal through both the MyD88-dependent and -independent pathways. TLR4 has been previously shown to be expressed on platelets and, is functional with implications on atherosclerosis, sepsis, and cancer metastasis. As both the MyD88-dependent and -independent pathways couple to transcription factors (and platelets lack a nucleus), we hypothesise that these transcription factors have non-genomic roles in the modulation of platelet activation, haemostasis, and thrombosis. Recently, LPS was found to be capable of differentially modulating responses of nucleated cells, via preferential activation of one of the two pathways depending on the bacterial source. Hence, in this study, we examined whether the LPS obtained from different bacteria species may exert diverse effects in the modulation of platelet function. Here, we demonstrate that *Escherichia coli* (*E. coli*) LPS increases the aggregation response of platelet-rich plasma

(PRP) to CRP-XL whereas equivalent concentrations of LPS from either *Salmonella minnesota* (*S. minnesota*) or *Rhodobacter sphaeroides* (*R. sphaeroides*) provoked a decrease in the magnitude of the aggregation response elicited by CRP-XL. Additional experiments using flow cytometry suggest that treatment of PRP with *E. coli* LPS increases fibrinogen binding in platelets whereas equivalent concentrations of *S. minnesota* LPS or *R. sphaeroides* LPS decreased fibrinogen binding. We then investigated whether the effects of different LPS chemotypes were due to differences in the intracellular signalling cascades induced downstream of TLR4 by biased signalling. To determine this, selective inhibitors of either IKKβ (IMD0354) or IKKε (BX795) were used to prevent signalling via the MyD88-dependent pathway or the MyD88-independent pathway respectively. The effects of *E. coli* LPS were partially reduced following the addition of IMD0354 and the effects of PRP treatment with *S. minnesota* LPS were susceptible to partial reversal in the presence of BX795. *R. sphaeroides* LPS, a competitive inhibitor of TLR4, was unaffected by either of the IKK inhibitors. These data suggest that different LPS chemotypes preferentially activate one TLR4 signalling pathway over another in platelets, to promote pro- or anti-thrombotic/inflammatory effects.

Keywords: platelet, Toll-like receptor 4, IKKbeta, IKKepsilon

PP-054

CD39 CONTRIBUTES SEPSIS-INDUCED IMMUNOSUPPRESSION BY EXPANDING THE REGULATORY T CELL POPULATION

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Introduction and Aim: It is well established that sepsis predisposes to the development of long-term immunosuppression by expanding Treg cells. In our previous work, we demonstrated that M2 macrophages induce Treg cells expansion in sepsis-surviving mice. However, the mechanism underlying the expansion of Treg cells by M2 macrophages remains unclear. Adenosine, a purine nucleoside, has been shown to increase the numbers of Treg cells and further promotes their immunoregulatory activity. In this study, we investigate the role of ectonucleotidase CD39, the main pathway for extracellular adenosine production, in the induction of FOXP3$^+$ Treg cells and development of sepsis-induced immunosuppression.

Methods: Mice were subjected to severe sepsis by cecal ligation and puncture (CLP) model and treated with antibiotic (ertapenem). Surviving C57BL/6 and CD39$^{-/-}$mice undergoing CLP and antibiotic treatment were challenged with *Aspergillus fumigatus* or sacrificed 15 days after CLP. In another experiments, surviving C57BL/6 undergoing CLP and antibiotic treatment were also treated with CD39 inhibitor [ARL671516] beginning 3 days after CLP and then everyday up to day 12.

Results: We found that the surface expression and activity of CD39 are up regulated in macrophages of sepsis-surviving mice. In parallel to the increased expression of CD39, we also found high levels of adenosine concentration in the serum of sepsis-surviving mice, suggesting that this pathway may be implicated in the development of sepsis-induced immunosuppression. Moreover, genetic deficiency or pharmacologic inhibition of CD39 reduced the expansion of Foxp3$^+$ Treg cells observed in the spleen of sepsis-surviving mice. To investigate the potential role of ectonucleotidase CD39 in the establishment of sepsis-induced immunosuppression, we used a second challenge induced by *Aspergillus fumigatus*, as readout of immunosuppression state. At 15th day after CLP, sepsis-surviving mice showed high susceptibility to a secondary infection induced by *A. fumigatus*, resulting in 80% mortality. Notably, genetic deficiency or pharmacologic inhibition of CD39 improved fungal clearance and rendered sepsis-surviving mice more resistant to a secondary challenge with *A. fumigatus* infection.

Conclusion: Our results suggest that extracellular production of adenosine by CD39-expressing in macrophages establishes an immunosuppressive microenvironment that promotes of Treg cells expansion and mediates immunosuppression in sepsis-surviving mice.

Financial support: FAPESP and CNRS.

Keywords: Sepsis, Adenosine, Immunosuppression, CD39, regulatory T cells

PP-055

IMMATURE SINGLE AND DOUBLE POSITIVE THYMOCYTES CONTRIBUTE TO SEPTIC THYMUS INVOLUTION

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Thymocyte development is a strictly controlled process. Crucial for their differentiation and survival is an inductive intra-thymic environment. Thymocytes are differentiated on the basis of CD4 and CD8 surface expression. When entering the thymus, cells are double negative (DN) for both. DN thymocytes can be further subdivided by CD44 and CD25 expression, and during progression from immature to mature, into DN1 – DN4. DN thymocytes initiate T cell receptor (TCR) rearrangement by recombination of the TCR β-chain. This ends with β-selection in the DN3 state. Afterwards, thymocytes undergo a final clonal expansion and the recombination of the TCR α-chain. At this stage, the cells temporarily express either CD4 or CD8, as immature single positive (ISP) thymocytes. During their further differentiation, thymocytes express both co-receptors in parallel, as double positive (DP) cells. The expansion is completed by

positive selection of the TCR in the DP state, followed by negative selection of the TCR and commitment to either the CD4+ or the CD8+ single positive (SP) lineage. Finally, SP thymocytes leave the thymus via corticomedullary blood vessels. Errors in this process may have dramatic consequences. Probably for this reason, the thymus reacts to septic stress with involution, decreasing the numbers of thymocytes.

Because it is still unclear which thymocyte subpopulation contributes to thymus involution and whether thymocyte emigration is altered during sepsis, we were interested to clarify this question.

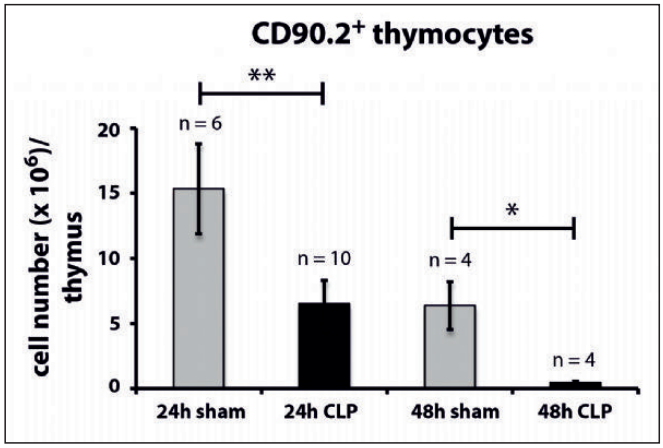
Using the polymicrobial sepsis mouse model of cecal ligation and puncture (CLP) model, thymocyte development was analyzed 24 h and 48 h after sepsis induction by flow cytometry. Analyses of Bim, Delta-like1, Delta-like4, Notch1 and T cell receptor excision circles (TREC) mRNA expression were performed using reverse transcription quantitative polymerase chain reaction (RT-qPCR).

We found that in CLP-dependent sepsis, predominantly immature thymocytes are reduced. The number of ISP thymocytes was markedly diminished, and was associated with the highest apoptosis rate, the reduction in DP thymocytes being associated with a smaller apoptotic response. Analysis of TREC revealed that the emigration of mature thymocytes was not inhibited. RT-qPCR analysis revealed upregulation of pro-apoptotic Bim expression and suggested interference between Notch receptor expression on thymocytes and the respective ligands on thymic stromal cells during CLP-dependent sepsis, which might be responsible for the altered thymocyte viability in CLP-dependent sepsis.

Thymocyte regress during CLP-dependent sepsis is characterized by an early decline in the thymocyte states between DN3 and the less mature DP thymocytes. Our analysis of mature SP thymocytes and TREC analysis rules out active inhibition of mature thymocyte emigration. Further clarification of the apoptotic mechanism leading to acute thymus involution during sepsis might form the basis of a new therapy approach.

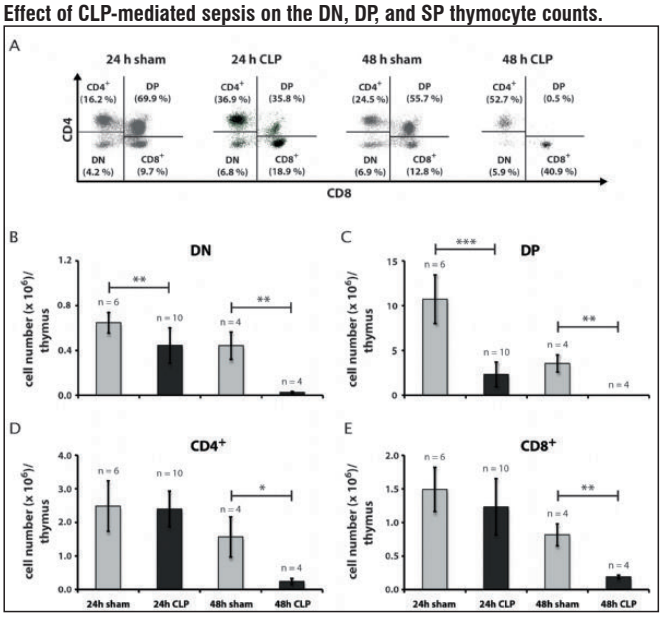
Keywords: CLP, DP, ISP, sepsis, thymocyte depletion

Effect of CLP-mediated sepsis on the CD90.2+ thymocyte count.



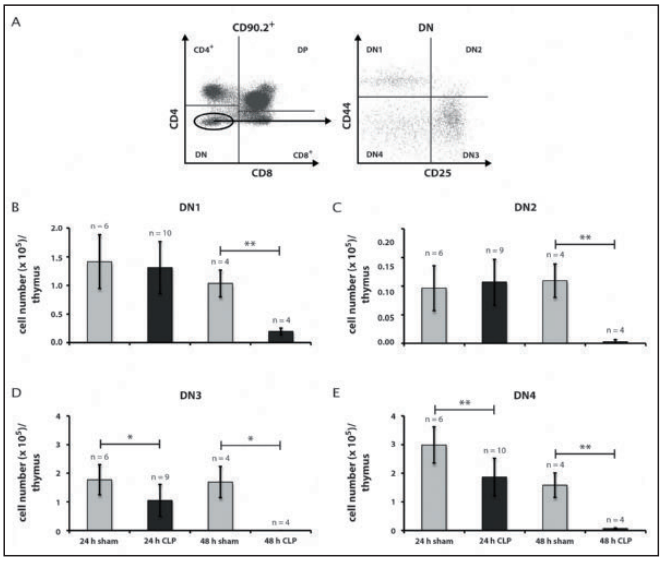
Twenty-four and 48 h following CLP operation, thymuses were isolated and single-cell suspensions were generated. Cells were stained for CD90.2 and positive cells were determined by flow cytomertry. Data are expressed as mean ± SD. n = number of animals. *, ** = *P value versus the sham group (*<0.05, **<0.01). CLP indicates cecal ligation and puncture; SD, standard deviation.*

Effect of CLP-mediated sepsis on the DN, DP, and SP thymocyte counts.



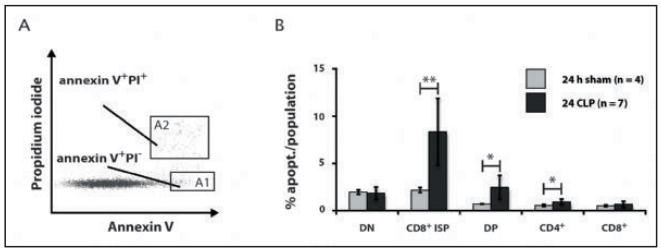
A, Twenty-four and 48 h following CLP operation, thymuses were isolated and single-cell suspensions were generated. Cells were stained for CD90.2, CD4, and CD8. Based on CD90.2-positive thymocytes as described in Figure 1, numbers of cells expressing only CD4 or CD8 (SP), expressing CD4 as well as CD8 (DP) or expressing neither CD4 nor CD8 (DN) were determined by flow cytometry analyses. A representative result is shown. **B–E,** Quantification of flow cytometry analyses as shown in **(A)**. Data are expressed as mean_SD. n = number of animals. *, **, *** = *P value versus the sham group (*<0.05, **<0.01, ***<0.001). CLP indicates cecal ligation and puncture; DN, double negative; DP, double positive; SD, standard deviation; SP, single positive.*

Effect of CLP-mediated sepsis on the DN thymocyte subpopulation counts.



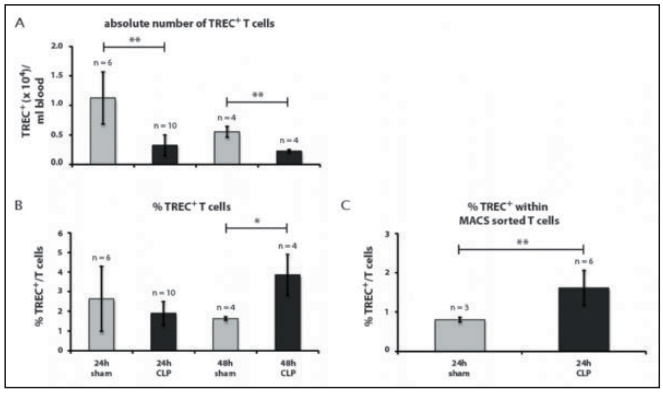
A, Further differentiation of DN thymocytes by CD25- and CD44-staining, progressing from immature to mature, into DN1 (CD25-CD44+), DN2 (CD25+CD44+), DN3 (CD25+CD44-), DN4 (CD25-CD44-). Twenty-four and 48 h following CLP operation, thymuses were isolated and single-cell suspensions were generated. Gating of thymocytes DN for CD4 and CD8 expression was done as described in Figures 1 and 2A. A representative picture is shown. B, Quantification of DN1 to DN4 thymocyte counts 24 and 48 h after CLP- and sham-operation. Data are expressed as mean ± SD. n = number of animals. *, ** = P value versus the sham group (*<0.05, **<0.01). CLP indicates cecal ligation and puncture; DN, double negative; SD, standard deviation.

Effect of CLP-mediated sepsis on thymocyte apoptosis.



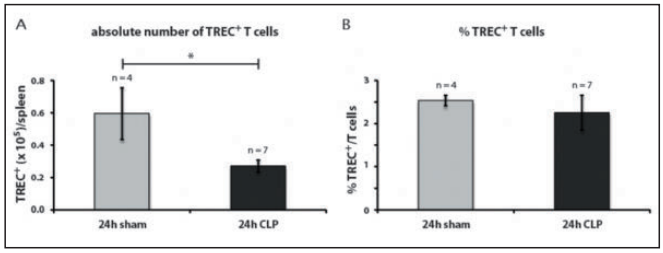
A, Gating of apoptotic thymoctes by PI and annexin V staining. For analysis only, PI+ events (gate A2) were excluded, because these cells also include necrotic cells and cell fragments. **B,** Quantification of flow cytometry analyses of apoptotic thymocytes (annexin V+ PI-) 24 and 48 h after CLP- and sham-operation. DN, DP, and CD4+ thymocyte subpopulations were differentiated as described in Figure 2A and then CD8+ ISP and CD8+ SP by CD3 and CD8. Data are expressed as mean ± SD. n = number of animals. *, ** = *P value versus the sham group (*<0.05, **<0.01). CLP indicates cecal ligation and puncture; DN, double negative; DP, double positive; ISP, immature single positive; PI, propidium iodide; SD, standard deviation; SP, single positive.*

Effect of CLP-mediated sepsis on TREC+ blood T cells.



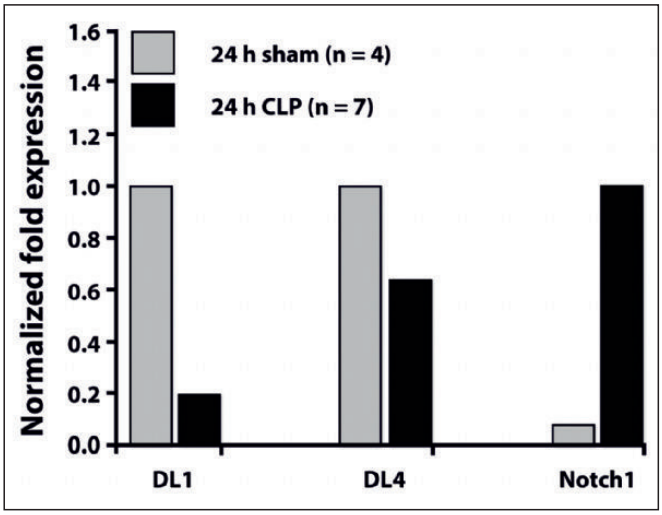
A, Cell count of absolute TREC+ blood T cell number 24 and 48 h after CLP- and sham operation. **B,** TREC+ blood T cell rate per CD3+ T cells 24 and 48 h after CLP- and sham-operation. C, TREC+ blood T cell rate within MACS enriched blood T cells 24 h after CLP- and sham-operation. Calculation of TREC+ T cells was performed by RT-qPCR out of whole blood or out of MACS purified T cells. Data are expressed as mean ± SD. n = number of animals. *, ** = *P value versus the sham group (*<0.05, **<0.01). CLP indicates cecal ligation and puncture; SD, standard deviation; TREC, T cell receptor excision circles.*

Effect of CLP-mediated sepsis on TREC+ spleen T cells.



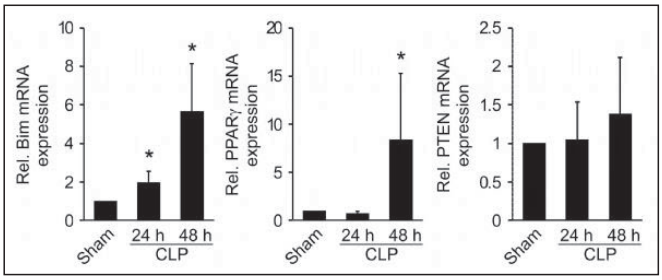
A, Cell count of absolute TREC+ spleen T cell number 24 h after CLP- and sham-operation. **B,** TREC+ spleen T cell rate 24 h after CLP- and sham-operation. Calculation of TREC+ T cells was performed by RT-qPCR. Data are expressed as mean ± SD. n = number of animals. * = *P value versus the sham group (*<0.05). CLP indicates cecal ligation and puncture; SD, standard deviation; TREC, T cell receptor excision circles.*

Effect of CLP-mediated sepsis on Notch signaling.



Delta-like 1 (DL1) and 4 (DL4): mRNA expression of Notch1 ligands DL1 and DL4 on thymic epithelial cells (TEC) 24 h after CLP treatment. Notch1: expression of Notch1 on thymocytes 24 h after CLP treatment. Bars represent the normalized fold expression (ΔCT value) to 18s rRNA. As sample size was too low for PCR of each single-mouse sample, cDNA of all mice was pooled for quantification. Therefore, data are expressed without mean and SD. Twenty-four hours sham and CLP: mice 24 h after sham resp. CLP operation. n = number of animals. CLP indicates cecal ligation and puncture; SD, standard deviation.

Sepsis-dependent upregulation of mRNA expression of pro-apoptotic marker proteins in thymocytes.



Sepsis was induced by CLP. Mice were sacrificed after 24°h or 48°h and thymuses were isolated. Following RNA isolation, **(A)** Bim, **(B)** PPARγ and (C) PTEN mRNA was analyzed in comparison to sham mice. mRNA expression was normalized to 18s rRNA. Sham mice were set as 1. Data represent the means ± SD of five individual mice *(*, p <= 0.05 vs. sham)*.

PP-056

THE AIM2 INFLAMMASOME PREFERENTIALLY ENGAGES ACTIVE BUT UNPROCESSED CASPASE-1 TO INDUCE NONCANONICAL ACTIVATION OF THE NLRP3 INFLAMMASOME

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Inflammasome activation is a hallmark of many infectious or inflammatory diseases. Inflammasomes are thought to activate and cleave caspase-1, stimulate inflammatory cytokines, and induce inflammation. The NLRP3 inflammasome is activated in response to cell damage and K+ efflux, whereas the AIM2 inflammasome is activated in response to cytosolic DNA. We used Legionella pneumophila, an intracellular bacterial pathogen known to activate multiple inflammasomes to elucidate the molecular mechanisms involved in regulation of inflammasome activation. In the absence of NLRC4 and caspase-11, we detected a significant activation of AIM2 by Legionella pneumophila, which leads to caspase-1 activation but not cleavage. Upon infection, AIM2 engaged caspase-1 to induce pore formation, which in turn caused K+ efflux–mediated, noncanonical activation of NLRP3. Thus, AIM2 inflammasome amplifies the signals of infection and triggers noncanonical activation of the NLRP3 inflammasome. During infection, AIM2, caspase-11 and NLRC4 operate independently to trigger membrane damage in infected cells and that the activity of least one inflammasome is sufficient and essential to triggering the cell damage- and K+ efflux-mediated activation of the NLRP3 inflammasome. Our data defines that different inflammasomes cooperate and regulate each other's activity to ensure effective immune response to infection.

Keywords: Inflammasome, AIM2, NLRP3, NLRC4, Macrophages, Legionella

PP-057

ARE 3σ OR MORE LEVELS OF POWER ACHIEVABLE IN INTEGRATIVE MODELS OF INFLAMMATION?

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The 3Rs are in EU legislation (Directive 2010/63/EU) and reducing animal numbers has become a priority. For statistically adequate data predictons of power should be used. However 'n=3' remains a feature of many publications. The reproducibility of academic data is difficult Pharma (Princz et al., 2011; Begley et al., 2012) with poor reproducibility and power. The biomedical field uses $p<0.05$ as the standard level of significance and is the default power calculations to determine animal numbers. In particle physics this level (2σ ($p<0.046$, >2SD) is only considered an 'event' at high risk of 'blips'. 3σ ($p<0.003$, >3SD) is 'Evidence', 5σ ($p<6x10-7$, >5SD) 'Discovery'. We have reported (Blashford-Snell & Seed, 2016) effect size (ES) and 'n' for the mouse carrageenan paw oedema model of inflammation, at $p<0.05$. Here we investigate 3 - 5σ experiments, and discuss achievability.

Archive data was used from a pilot drug trial mouse (balb/c, n=5 per group) carageenan paw oedema. Carageenan (0.1mL 1%) was injected sub-plantar under gaseous anaesthesia, control paws 0.1ml saline. Inflammation was measured blind (plethysmometery, Ugo Basil) and mice dosed p.o. vehicle, 3 drugs and dexamethasone (0.01mg/kg) 2 hrs prior to induction. Outcome was ml increase on paw swelling over control, and expressed as mean±sem. Effect size (ES), power, and sample sizes were calculated (G*Power, Faul et al 2007) at 2, 3, 4, and 5σ. Group SD = (control mean–lowest mean)/√(mean square error). Multiple comparison power analysis, constrained to a single control (Dunnett's Test) used PASS 2015, NCC (USA). PPL and PIL (ASPA, 1986), Queen Mary University of London.

Results were as previously reported, control: 0.084±0.029mL (range 6-13) and dexamethasone 0.023±0.017mL (range 0:4), ES=0.0246, power=0.825 including 4 drug groups ($p<0.01$), 1-way ANOVA post hoc Selected Bonferroni). Figure shows 'n' vs ES for two group comparison. Using Pass 2015 constraining for Dunnett's test the minimum detectable difference (MDD)=0.122, ES=0.0388, power=0.7997. A reasonable MDD of 50% inhibition gives 0.042. For xσ (power:n): 2σ (0.8386:28), 3σ (0.8614:49), 4σ (0.811:78), 5σ (0.8298:111). ANOVA showed dexamethasone significance, despite the >2x range and MDD greater than control.

Levels of 3σ significance can reasonably be planned for this model for an ES of 50%. Repeating studies three times to confirm veracity of the outcome is commonly accepted, and at n=17 per group per study, to give a final blocked dataset (Festing, 2002) of 51, in excess of the numbers (49) required for 3σ. Particle physics uses algorithms to together different studies to achieve 5σ. High σ is achievable especially with refinement.

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Keywords: Carrageena Paw, Inflammation, Power analysis, 3-sigma, 3Rs

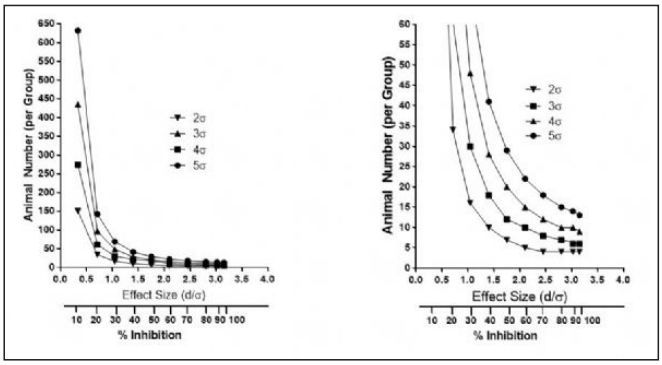

Figure 1. 'Effect Size'/% inhibition vs. sample size (a:-n=0-860; b:-n=0-60) at xσ for t-test.

PP-058

CLINICAL FEATURES OF DRUG REACTION WITH EOSINOPHILIA AND SYSTEMIC SYMPTOMS (DRESS) SYNDROME IN KOREA

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Background: Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome is a life-threatening adverse drug reaction referred to as drug-induced hypersensitivity syndrome. This study aimed to evaluate the incidence of culprit drugs, clinical manifestations, disease course, and outcomes of DRESS in Korea.

Methods: Using the criteria of European Registry of Severe Cutaneous Adverse Reactions (RegiSCAR), a retrospective review of medical records of 25 patients with DRESS was performed between January 2006 and December 2015. Demographic data, culprit drugs, latency periods, clinical and laboratory findings, and outcomes were investigated.

Results: The patients' ages varied from 13–93 years (mean, 58 ± 19.86 years), and the study group consisted of 11 men (44%) and 14 women (56%). The most common culprit drugs were carbamazepine (28%), allopurinol (16%), and anti-tuberculosis drugs (12%). The overall latency period ranged from 4–40 days (mean, 17.6 ± 9.95 days), and the latency periods for anticonvulsants were significantly longer than those for other drugs ($p < 0.05$). However, no statistical differences were found in the RegiSCAR scores for anticonvulsants and other drugs. Disease severity according to the RegiSCAR score was correlated with blood count abnormalities ($p < 0.05$). The mortality rate in the present study was 12%. Three of our 25 patients were managed in the intensive care unit but finally died of septic shock.

Conclusions: The results of our study revealed that anticonvulsants were the leading culprit drugs for DRESS, and carbamazepine was the most common individual drug associated with DRESS in Korea. For the prompt diagnosis and effective management, further studies of drug mechanisms, which can affect the disease prognosis and clinical outcome, are needed.

Keywords: DRESS syndrome, Drug eruption, anticonvulsants

Immunometabolism

PP-061

PARAPANCREATIC ADIPOSE TISSUE IMMUNOMETABOLISM IN EXPERIMENTAL RATS WITH STREPTOZOTOCIN-INDUCED DIABETES AND AFTER INTRODUCTION OF METFORMIN

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Introduction: mTOR is not only a central regulator of lipid metabolism, controlling the processes of adipogenesis and lipolysis, but also a regulator of immunometabolism of immune cells infiltrating the adipose tissue. In its turn, the level of progression of diabetes is largely limited by Treg subpopulation, the complexity and heterogeneity of which is confirmed by the detection of numerous tissue-specific Tregs, including the so-called VAT Tregs (visceral adipose tissue CD4+Foxp3+ regulatory T cells). Therefore, the purpose of the work was to find out the level of expression of mRNA genes of mTOR, Foxp3, IL1β and IL17A in parapancreatic adipose tissue of rats with experimental streptozotocin-induced diabetes after introduction of metformin.

Methods: We use RT-PCR method for investigating of mRNA expression levels of genes mTOR, Foxp3, IL1β and IL17A. To determine the level of target genes was performed RT-PCR in real-time by thermocycler CFX96™ Real-Time PCR Detection

Systems: The relative level of gene expression were studied with rat reference genes GAPDH by the method ΔΔCt. Statistical analysis were conducted using available software «Bio-Rad CFX Manager 3.1» (Bio-Rad, USA).

Results: the development of diabetes causes the transcriptional activation of the gene of the protein kinase mTOR, does not affect the expression of mRNA of Foxp3, increases the level of expression of mRNA of proinflammatory cytokines IL1β and IL17A. At the same the introduction of metformin in diabetic rats inhibits the expression of mRNA of mTOR and increases the level of transcriptional activity of the gene Foxp3 in parapancreatic adipose tissue.

Keywords: diabetes mellitus, adipose tissue, mTOR, Foxp3

PP-062

MOLECULAR CHARACTERIZATION OF METABOLIC HEALTH IN YOUNG ADULTS BY EXPRESSION OF SIRT1, CIRCULATING MIRNAS, CYTOKINES AND ADIPOKINES

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Obesity is a worldwide public health problem and associated with chronic diseases such as type 2 diabetes mellitus, hypertension and cardiovascular disease. Body mass index (BMI) is now considered as inaccurate for evaluating body fat distribution and as indicator of metabolic state. In addition, the metabolic alterations that can be found in individuals who are overweight or obese may also occur in subjects with normal BMI. On the other hand, normal biochemical parameters, anti-inflammatory markers, as well as insulin sensibility can be detected in obese subjects whom are referred to as "metabolically healthy obese". Therefore, it is relevant to stablish the metabolic health and non-metabolic health condition evaluating circulating miRNAs that regulate metabolic pathways, inflammatory markers as cytokines and adipokines and other metabolic sensors such as sirtuin 1 (sirt1), in order to better understand the mechanisms involved in the parameters for metabolic health status.

The aim of this study was to determinate the expression levels of sirt1, circulating miRNAs, cytokines and adipokines from healthy volunteers and their association with metabolic health status and clinical parameters.

We included 40 healthy subjects who were classified according to the following criteria: triglycerides > 150 mg/dL and HDL-col < 40 mg/dL. The Peptide-C, insulin, leptin, resistin, ghrelin, visfatin, glucagon, GIP, GLP-1 and PAI-1 were simultaneously quantified in serum by the Bio-Plex MAGPIX ™. From plasma the total RNA was extracted by the Trizol® method, subsequent specific RT-qPCR was performed for hsa-miR-34a, cel-miR-39 (spike-in normalizer) and hsa-miR-221. Expression levels of miRNAs were detected with a multiplex method using the 2- (ΔΔCq) method. Levels of Sirt1 were determinated in the blood globular fraction by flow cytometry using a primary antibody for sirt1 and the data acquisition was through FACSCanto II (BD).

We detected a higher serum concentration of Peptide-C, GIP, insulin and leptin as well as lower levels of ghrelin in subjects classified as metabolically non healthy compared with subjects metabolically healthy. Regarding to miRNA expression levels, an increase in expression levels of miR-34a and miR-221 was found in metabolically healthy obese subjects. For sirt1 expression, we detected a higher percentage of sirt1 positive cells in subjects metabolically healthy than in the metabolically non healthy.

Based on lipid profile of the volunteers, the altered profile of Peptide-C, ghrelin, GIP, leptin, HOMA-IR and sirt1 expression allowed us to determinate which metabolic parameters are tightly associated with metabolic health. Likewise, a close association was found between the expression of miR-34a and miR-221 with BMI.

Keywords: Obesity, Metabolic Health, miRNAs, inflammation

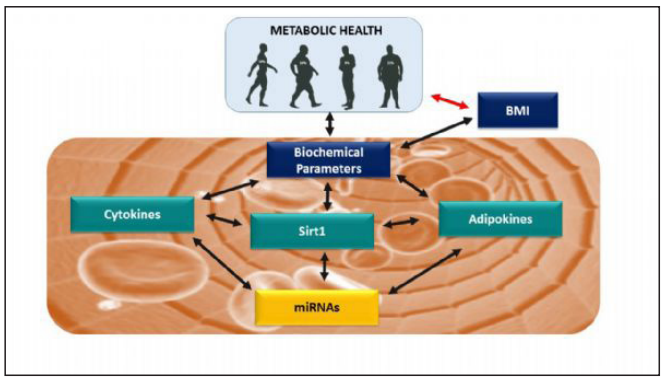

Figure 1. Molecular Interplay determinating Metabolic Health

The use of biochemical parameters and associated with BMI allow better establishing aspects related to metabolic health; Thus, associating them with molecular markers such as cytokines, adipokines and de-acetylases (sirt1), has a great impact in order to better understand the mechanisms between metabolic health status and body weight, which determine the presence of obesity-related comorbidities. Therefore, studying the differences between protein expression profiles, their function and miRNAs expression profiles in young populations considered to be non-metabolically healthy and metabolically healthy, will be important in order to help educidate the mechanisms involved in establishing these conditions

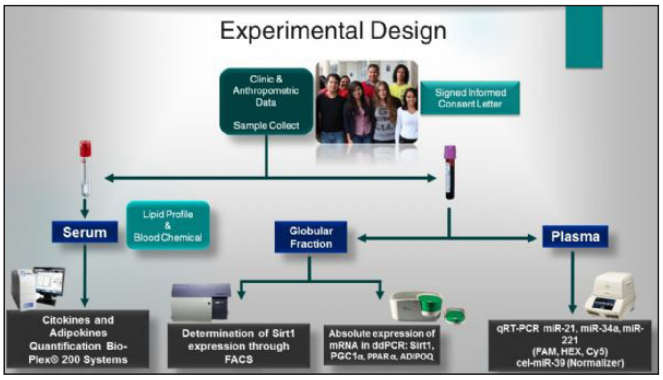

Figure 2. Experimental Design

PP-063

ZINC SUPPLEMENTATION PREVENTS PNEUMOCOCCAL SEPSIS, PNEUMONIA WITH ALCOHOLIC LUNG DISORDER

Seungyeop Lee, Gyu Lee Kim, Sion Lee, Sejin Kim, Bogyeong Kim, Seung Han Seon, Dong Kwon Rhee

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Metal ions like Zn2+ is an essential trace element in biological context for the growth of microorganisms and is strongly associated with their competitive ability to invade and colonise their hosts against pathogenic bacteria. Zinc serves as acatalytic cofactor for a wide and functionally diverse range of hydrolytic enzymes that regulates housekeeping functions in cellular metabolism and gene expression. Zinc also acts as an immunoregulatory, anti-apoptotic and anti-inflammatory agent and upholds innate immune defence mechanism while its deficiency induces a wide range of human diseases including impaired immunity, airway inflammation and cancer. Streptococcus pneumoniae (the pneumococcus) is a formidable human pathogen, responsible to cause sinusitis, conjunctivitis and otitis media, to potentially fatal diseases such as pneumonia, bacteraemia and meningitis. These complications are exceedingly exacerbated by alcohol abuse that impairs host immune defences, rendering the host more vulnerable to severe infections especially pneumonia and sepsis formation. It is well reported that chronic alcohol consumption causes hypoxia and lung dysfunction leading to inflammation that ultimately triggers intracellular zinc release in respiratory tract resulting in activation and recruitment of various immune cells to specific host sites of infection. It is also well known that elevated Zn levels inhibit metabolic processes and virulence pathways in Streptococcus pneumoniae that uses metabolic enzyme alcohol dehydrogenase (adh2), that is involved in ethanol oxidation from acetaldehyde. Thus we investigated that whether Zinc can elicit prevention against pnemonia and sepsis caused by wild type D39 pneumococcal strain with or without alcohol induction in C57BL/6 mice model. We found significant higher death rate and bacterial load in blood and lung samples in C57BL/6 alcohol induced model as compared to zinc treated alcoholic mice.

Our in vivo data show that adh2 deletion mutant (Δadh2) mice were more susceptible with higher growth rate and increased level of virulence factors (pnemolysin and LytA). Similarly, colonisation and adherence was found remarkably higher as compared to wild type strain. Consistent with the

previous observations with the zinc ability to reduce bacterial load and to inhibit bacterial virulence and colonisation in vivo. Taken together, we found that zinc nc supplementation improves bacterial clearance, and hence survival, in chronic alcohol mice with pneumococcal sepsis.

Keywords: Zinc, chronic alcohol consumption, pneumococcal sepsis, pneumoni

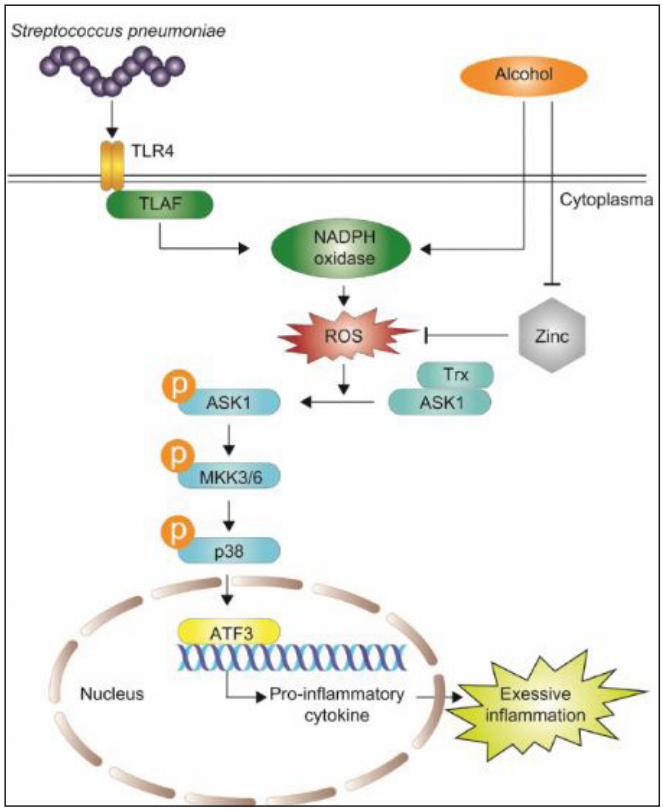

Figure 1. Zinc-Related Signaling pathway in macrophage aginst pneumococcal infection

PP-064

DECREASED EXPRESSION OF CDKN2A/2B ASSOCIATE WITH T CELL IMBALANCE IN HUMAN DIABETES AND CORONARY ARTERY DISEASE

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Type 2 diabetes mellitus (T2DM) and coronary artery atherosclerotic disease (CAD) have been associated with single nucleotide polymorphisms located in the vicinity of the *CDKN2A/2B/2BAS* genes. Decreased levels of regulatory T (Treg)-cells have also been linked with acute coronary syndrome. In this study, the connection between *CDKN2A/2B/2BAS* gene expression and leukocyte phenotype and potential intracellular pathways were investigated in human subjects exhibiting T2DM and CAD. The study included volunteer controls, T2DM patients and T2DM subjects that experienced at least one episode of cardiovascular event. All subjects underwent routine blood biochemical parameter analysis in the hospital clinic. In addition, peripheral blood mononuclear cells (PBMC) were isolated and used for gene expression by qPCR and western blot analysis. Whole blood was analysed by flow cytometry for leukocyte phenotyping. Gene expression analysis showed reduced mRNA levels of the *CDKN2A splice variant 1* ($p<0.02$ and $p<0.008$), *CDKN2B* ($p<0.01$ and $p<0.005$) and *CDKN2BAS* ($p<0.03$ and $p<0.04$) in PBMC from T2DM and T2DM-CAD subjects compared with expression levels in human controls. Consistent with these, protein analysis also revealed diminished levels of the protein products $p16^{Ink4a}$and $p15^{Ink4b}$ in T2DM ($p<0.03$ and $p<0.05$) and T2DM-CAD ($p<0.02$ and $p<0.01$) patients compared with those observed in control subjects. T2DM and T2DM-CAD patients also displayed augmented circulating levels of activated (CD3+CD69+)T-cells ($p<0.03$ and $p<0.02$) and proinflammatory CD14++CD16+-monocyte subpopulation ($p<0.0007$ and $p<0.04$). By contrary, T2DM and T2DM-CAD subjects had diminished (CD4+CD25+CD127-) Treg-cells ($p<0.04$ and $p<0.003$) compared with controls. Correlation analysis showed an inverse correlation between CDKN2B mRNA expression with HOMA-IR index ($p<0.04$) and with common carotid intima-media thickness ($p<0.003$). In addition, Treg levels inversely correlated with HOMA-IR ($p<0.0006$). Plasma cytokine analysis revealed reduced IL4 ($p=0.05$ and $p<0.005$) and IL2 levels in T2DM and T2DM-CAD ($p<0.002$ and $p<0.02$) compared with controls. Interestingly, TGF-β levels were significantly increased in T2DM compared with both controls ($p<0.03$) and T2DM-CAD subjects ($p<0.005$). Activation of MAPK stress pathway analysis showed augmented protein levels of phosphop38 ($p<0.004$) and phosphoERK ($p<0.002$) in PBMCs from T2DM-CAD subjects and increased phosphop38 ($p<0.004$) protein levels in PBMCs of T2DM subjects. Thus, decreased expression of *CDKN2A/2B/2BAS* in T2DM and CAD associate with decreased Treg cells which is accompanied by MAPK activation and decreased anti-inflammatory cytokines.

Funding: This study was supported by grants from the Carlos III Health Institute (CP16/00013, PI16/00091 awarded to H.G.-N.), the European Regional Development Fund (FEDER) and by "Proyecto Paula" a Fundraising initiative for the study of Diabetes.

Keywords: type 2 diabetes, cardiovascular disease, T cells, human subjects

Funding

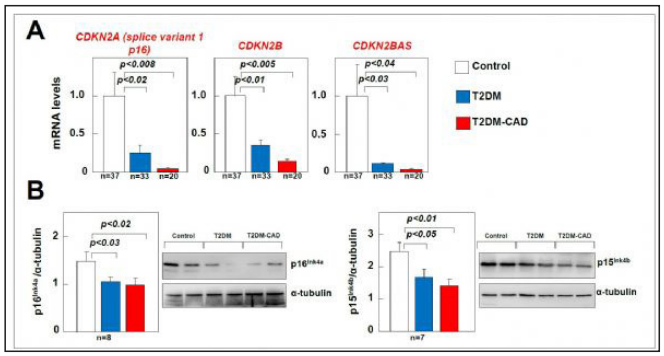

Figure 1. Cdkn2a, Cdkn2b and Cdkn2bas gene expression is decreased in PBMC from T2DM and T2DM-CAD human subjects. **(A)** mRNA expression analysis of peripheral blood mononuclear cells (PBMCs) from controls, T2DM and T2DM-CAD subjects demonstrated significantly lower mRNA levels of Cdkn2a splice variant 1 (p16Ink4a), Cdkn2b and Cdkn2bas in T2DM and T2DM-CAD individuals compared with those in control subjects. **(B)** Protein expression analysis of p16Ink4a and p15Ink4b showed significantly reduced protein levels in both T2DM and T2DM-CAD patients compared with the levels observed in control subjects. mRNA levels were normalised with the endogenous gapdh mRNA levels and relativised to control mRNA levels. Protein levels were normalised with endogenous α-tubulin protein levels. Representative blots are shown. Statistical analysis was performed using the One-way ANOVA.

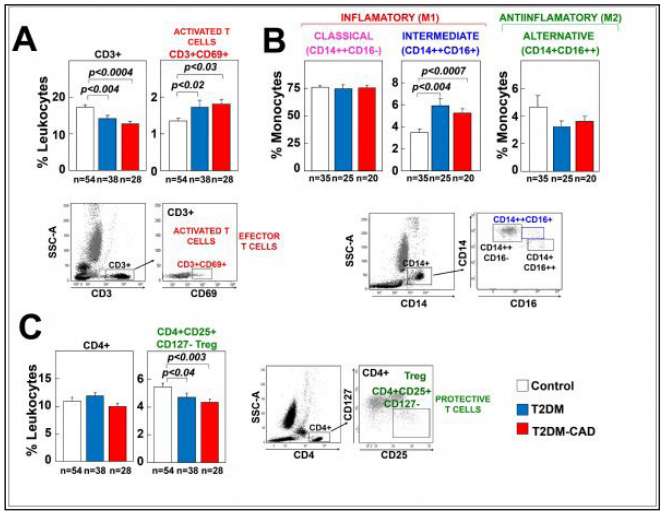

Figure 2. Characterisation of inflammatory cells and mediators in controls, subjects with T2DM and with T2DM-CAD. **(A)** Circulating T(CD3+)-lymphocytes were diminished in T2DM and T2DM-CAD compared with controls while activated CD3+CD69+ T-were significantly enhanced in T2DM and T2DM-CAD patients compared with controls. **(B)** Analysis of circulating monocytes using CD14 and CD16 markers, which differentiate between classical, intermediate and alternative-activated monocytes, demonstrated a significant increase in the percentage of the intermediate CD14++CD16+-monocyte subpopulation in T2DM and T2DM-CAD patients compared with those in control subjects. **(C)** Circulating levels of Treg(CD4+CD25+CD127-)-cells were significantly reduced in both T2DM and T2DM-CAD patients compared with control levels. Representative plots of the gating of the flow cytometry are shown. Statistical analysis was performed using the One-way ANOVA.

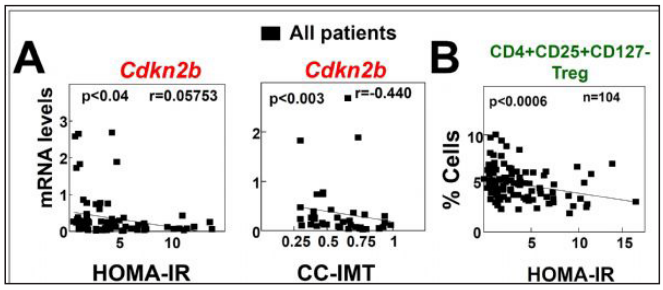

Figure 3. Correlation studies in control, T2DM and T2DM-CAD subjects **(A)** Cdkn2b mRNA levels inversely correlated with CC-IMT and HOMA-IR index. Statistical analysis was performed using. **(B)** Treg (CD4+CD25+CD127-) levels inversely correlated with HOMA-IR index. Statistical analysis was performed using. Statistical analysis was done using the Spearman correlation coefficient.

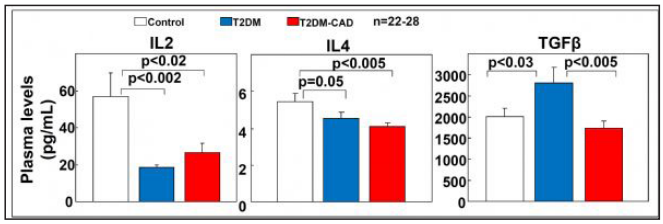

Figure 4. Circulating plasmatic cytokine levels. Plasma levels of IL2, IL4 and TGFβ in Control, T2DM and T2DM-CAD subjects. Analysis was performed in plasma samples by ELISA. Statistical analysis was performed using One-way ANOVA.

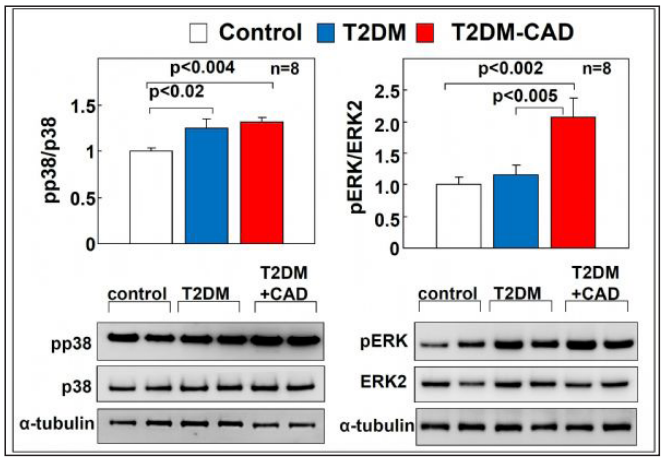

Figure 5. Analysis of the activation of MAPK in controls, subjects with T2DM and with T2DM-CAD. Quantification of the activated (phosphorylated) forms of p38 and ERK and in PBMCs from control, T2DM and T2DM-CAD subjects. Phosphorylated forms were relativised to total unphosphorylated protein. Protein levels of α-tubulin are shown as loading control. Representative blots are shown. Statistical analysis was performed using the One-way ANOVA.

PP-065

GENETIC INACTIVATION OF THE CYTOKINE LIGHT(TNFSF14) DECREASES INSULIN RESISTANCE IN MICE FED A HIGH-FAT HIGH-CHOLESTEROL DIET

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Obesity is a major risk factor for developing insulin resistance (IR) and non-alcoholic fatty liver disease (NAFLD). A major molecular mechanism that connects and promotes the development of these conditions is the activation of inflammatory pathways in the classical insulin target tissues such as adipose tissue and liver. The soluble cytokine LIGHT(TNFSF14) is a proinflammatory cytokine involved in monocyte and T cell activation and maturation. Recent studies suggest a role for LIGHT in both NAFLD and IR since the levels of the cytokine are increased in subjects suffering from these diseases. In the present study, we investigated the role of LIGHT in IR and NAFLD in mice. To this end, Light-deficient (*Light*-/-) mice and *WT* control mice were fed a high-fat high-cholesterol diet for 4 months, which has been shown to produce hepatic steatosis. After the diet, carbohydrate metabolism and inflammatory cells and mediators were characterised. *Light*-/- mice displayed decreased fasting insulin levels ($p<0.03$) compared with *WT* controls without changes in fasting glucose or lipid levels. Carbohydrate metabolism characterisation demonstrated improved glucose intolerance ($p<0.05$) as shown by decreased area under the curve parameter (AUCglucose) of the glucose tolerance test in *Light*-/- mice compared with *WT* mice. *Light*-/- mice also displayed improved insulin sensitivity demonstrated by decreased AUCinsulin ($p<0.02$) of the insulin tolerance test in *Light*-/- mice compared with *WT* controls. A discrete decrease in the triglyceride content of liver, which evaluates hepatic steatosis, from *Light*-/- mice was observed but this was not significant. Flow cytometry analysis of the stromal vascular fractions from epididimal fat indicated increased levels of the alternatively activated F4/80+CD206+ M2 macrophages in *Light*-/- mice compared with *WT* controls ($p<0.03$). No changes in the proinflammatory F4/80+CD11c+ M1 macrophages were observed. Plasma levels of the IL6 and TNFα proinflammatory cytokines were also significantly decreased in *Light*-/- mice compared with *WT* controls. Circulating leukocyte analysis showed increased numbers of total CD3+ T cells ($p<0.0003$) and of CD4+ ($p<0.0003$) and CD8+ ($p<0.004$) T cell subtypes in *Light*-/- mice without changes in T cell activation. These results indicate a potential role of LIGHT in the development of IR that might involve modulation of macrophage phenotype in the adipose tissue.

FUNDING: This study was supported by grants from the Carlos III Health Institute (CP16/00013, PI16/00091 awarded to HG-N), the European Regional Development Fund (FEDER), European Foundation for the Study of Diabetes (EFSD/Novo Nordisk awarded to HG-N) and "Proyecto Paula" (a Fundraising initiative for the study of Diabetes).

Keywords: insulin resistance, metabolism, adipose tissue, LIGHT(TNFSF14)

PP-066

INVOLVEMENT OF GLYCOLYSIS AND PENTOSE PHOSPHATE PATHWAY IN FORMATION OF NEUTROPHIL EXTRACELLULAR TRAPS BY SEPTIC NEUTROPHILS

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Sepsis is one of the most lethal inflammatory conditions and this is mostly due to its systemic nature, independently of the causing agent. Among other leukocytes, neutrophils have a key regulatory role in vascular inflammation (Phillipson & Kubes, Nat Med. 2011;17:1381). The cells had been thought of as simple cells of the innate immune system with a limited array of pro-inflammatory functions, but recently they have been recognized as complex cells capable of an extensive range of specialized functions (Kolaczkowska & Kubes, Nat Rev Immunol. 2013;13:159). One of their characteristics is capacity to form and release neutrophil extracellular traps (NETs). NETs are composed of DNA decorated with histones and granular proteins, including proteases and antimicrobials. They play both beneficial and disadvantageous role as they facilitate catching (and killing) of bacteria (Brinkmann et al. Science. 2004;303:1532) but also bystander injury to host tissues (Kolaczkowska et al. Nat Commun. 2015;6:6673). The latter is of importance especially during sepsis in which NETs are formed and present in blood vessels. Not much is known about metabolic aspects of NET formation, however our studies show that unstimulated neutrophils of (otherwise healthy) obese mice release more NETs upon LPS stimulation. As the mice have elevated glucose levels, and glucose levels are also increased during sepsis, we studied glucose and diet effects on NET formation, and furthermore, involvement of glycolysis and pentose phosphate pathway (PPP) in this process. NETs were induced by liposaccharide (LPS) in BM neutrophils collected from mice with diet-induced obesity (DIO, 60% kcal from fat) and age-matched lean mice. Neutrophils were collected from either septic or healthy animals. The cells were cultured in presence of either "normal" glucose (5.5. mM; NG) or high glucose (22 mM; HG), and additionally a set of glycolysis and PPP inhibitors was used. Interestingly, *ex vivo* HG increased NET formation by neutrophils of lean mice and consequently inhibition of glycolysis and/or PPP down-regulated it, but this effect was absent in the case of neutrophils collected from obese mice chronically exposed to HG *in vivo*. Since neutrophil relay on glycolysis as their main energy source (Kramer et al. Redox Biology. 2014;2:206) we further asked how important is ATP for NET release. Application of various ATPase inhibitors directed against different subunits of the enzyme did not reveal significant differences in NET formation, except for one. Again, this effect was observed only in the case of neutrophils collected from lean animals. We conclude that glycolysis/PPP pathways are involved in NET formation but pre-exposure of neutrophils to elevated glucose affects immunometabolic regulation of the process.

Keywords: NET, neutrophil extracellular traps, sepsis, glucose, obesity, glycolysis

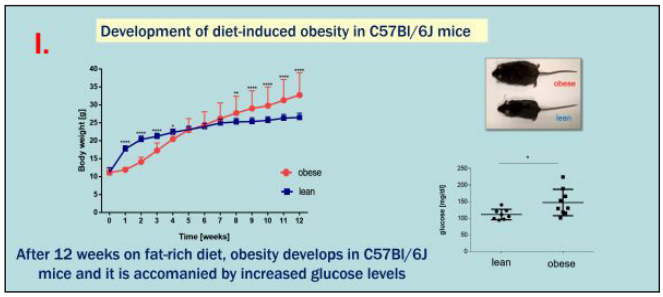

Figure 1.

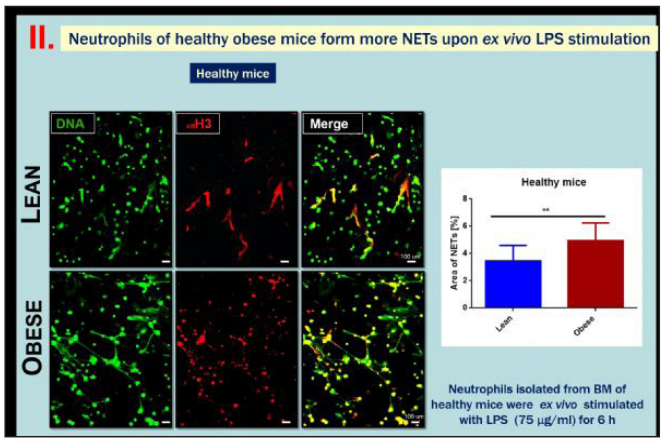

Figure 2.

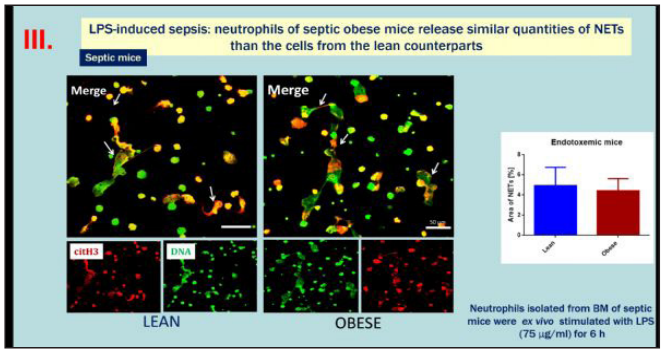

Figure 3.

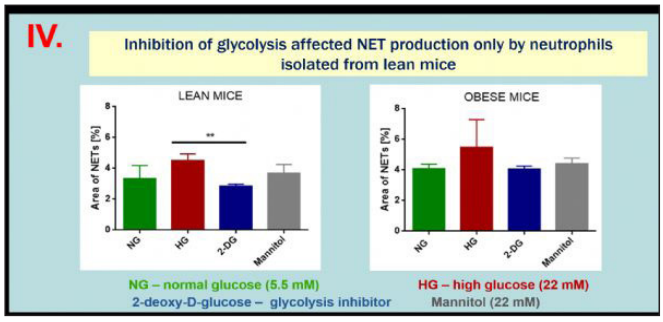

Figure 4.

Infection

PP-067

ADENYLATE KINASE FROM STREPTOCOCCUS PNEUMONIAE IS ESSENTIAL FOR GROWTH THROUGH ITS CATALYTIC ACTIVITY

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Streptococcus pneumoniae (pneumococcus) infection is responsible for more than 1.6 million deaths worldwide. Pneumococcal growth is a prerequisite for its virulence and requires appropriate supply of cellular energy. Adenylate kinases (AdKs) constitute a major family of enzymes to regulate cellular ATP level. Some bacterial AdKs are known to be critical in growth, but the physiological effects of AdKs in pneumococci have been poorly understood at molecular level. Here, by crystallographic and functional studies, we report that adenylate kinase from Streptococcus pneumoniae (SpAdK) is essential for growth through its catalytic activity. We determined the crystal structure of SpAdK in two conformations: ligand-free open form and closed in complex with a two-substrate mimic inhibitor adenosine pentaphosphate (Ap5A). Crystallographic analysis of SpAdK reveals Arg-89 as a key active site residue. We generated a conditional expression mutant of pneumococcus, in which the expression of the adk gene is tightly regulated by fucose. The expression level of adk is consistent with growth rate. Expression of the wild-type adk gene in fucose-inducible strains rescued growth defect, but expression of the Arg-89 mutation did not. SpAdK increased total cellular ATP level. Lack of functional SpAdK attenuated pneumococcal virulence in vivo. Taken together, our results demonstrate that SpAdK is essential for pneumococcal growth and suggest that SpAdK is likely to be linked to pneumococcal virulence.

Keywords: Streptococcus pneumonia, Adenylate kinase, catalytic activity

PP-068

ANTI-INFLAMMATORY EFFECTS OF TRADITIONAL HERBAL EXTRACTS FROM STELLARIA DICHOTOMA VAR. LANCEOLATA ON MYCOBACTERIUM ABSCESSUS-INFECTED MURINE MACROPHAGES

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Stellaria dichotoma var. lanceolata is belong to the family of Caryophyllaceae and acts as the traditional herbal medicine for syndrome of liver and kidney deficiency manifested as bone-steaming, strain fever, tidal fever and night sweat, but its roles during Mycobacterium abscessus (M.abs) infection remains still unknown. Here, we investigated whether the extracts from Stellaria dichotoma var. lanceolata exhibits antimicrobial effects against M.abs. M.abs infection triggered the mRNA and protein expression of tumor necrosis factor α (TNF-α) and interleukin (IL)-6 in murine bone marrow-derived macrophages (BMDMs). However, the extracts from Stellaria dichotoma var. lanceolata significantly attenuated the activation of M.abs-induced production of these cytokines in dose-dependent manner. Additionally,

M.abs infection rapidly activated nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) signaling, which is effectively inhibited by pretreatment of the extracts, as well as the generation of proinflammatory cytokines. Moreover, intraoral administration of the extract improved the survival of mice intravenously infected with M.abs. Collectively, these results strongly suggested the possibility as novel protective agents against M.abs infection.

Keywords: s: Stellaria dichotoma var. lanceolata, Mycobacterium abscessus, Macrophages, Inflammatory response

PP-069

ORPHAN NUCLEAR RECEPTOR SMALL HETERODIMER PARTNER MEDIATES HOST RESISTANCE TO INFECTION WITH TOXOPLASMA GONDII

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Department of Infection Biology, Department of Medical Science, Chungnam National University School of Medicine, Daejeon 301-747, S. Korea

SHP (NR0B2; Mouse Genome Informatics accession code, 1346344), is an atypical orphan nuclear receptor superfamily and mainly contributes to transcriptional regulation of divers metabolic pathways through interactions with various nuclear receptors and transcription factors. We previously reported that SHP is an important negative regulator in endotoxin-induced systemic inflammation, however, the roles of SHP in host protective immune responses against Toxoplasma gondii (T.gondii) have not been fully identified. We first examined whether SHP deficiency modulates in vivo parasitic burden in brain tissues. SHP deficiency resulted in the decreased tissue burden of T.gondii ME49 strain. We next determined serum levels of tumor necrosis factor α (TNFα) and interleukin (IL)-12p40 in T.gondii-infected wild type (WT) and SHP deficient mice. SHP deficient mice infected with T.gondii ME49 strain showed enhanced generation of TNFα and IL-12p40 than WT mice with those. Moreover, intracellular survival and proliferation of T.gondii in SHP deficient primary macrophages were significantly diminished. In consistent of in vivo finding, TNFα and IL-12p40 mRNA expression were highly increased in the SHP deficient macrophages. Taken together, our finding suggested that SHP is a strong suppressor of host innate immune responses upon T.gondii infection.

Keywords: Toxoplasma gondii, SHP, Inflammation, Macrophages

PP-070

INDUCTION OF REACTIVE OXYGEN SPECIES IS IMPORTANT TO SUPPRESS THE INTRACELLULAR SURVIVAL OF *MYCOBACTERIUM SMEGMATIS* VIA ER STRESS RESPONSES

Seonhwa Kim, Yunji Lim, Jiae Choi, Junghwan Lee, Soona Cho, Dam Go, Changhwa Song

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Mycobacterium smegmatis, a rapid growing non-tuberculosis mycobacterium (NTM), is a good model to study the pathogenesis of tuberculosis because *M. smegmatis* is very similar to *Mycobacerium tuberculosis* (Mtb) genetically. Macrophage plays a critical role to remove the bacilli during mycobacterial infection. Macrophage apoptosis is broadly accepted as a host defense mechanism against intracellular bacteria. Previous our results showed that ER stress is important as a host defense mechanism against Mtb infection. In this study, we examined the role of ER stress in *M. smegmatis*-infected macrophages. *M. smegmatis*-induced ER stress was stronger than *M. tuberculosis*-mediated ER stress. We showed that *M. smegmatis*-induced ROS play a critical role in induction of ER stress-mediated apoptosis. Pretreatment of ROS scavenger was effective to suppress *M. smegmatis*-induced ER stress. Elimination of ROS decreased ER stress responses and significantly increased the intracellular survival of *M. smegmatis*. These data suggest that *M. smegmatis*-induced ER stress plays an important role in growth suppression of *M. smegmatis*. Taken together, our results suggest that enhanced ROS generation decreases intracellular survival of *M. smegmatis* via ROS–mediated ER stress. Better understanding the role of ROS could provide new insights into the pathogenesis of tuberculosis.

Keywords: Mycobacterium smegmatis, ER stress, Apoptosis, ROS

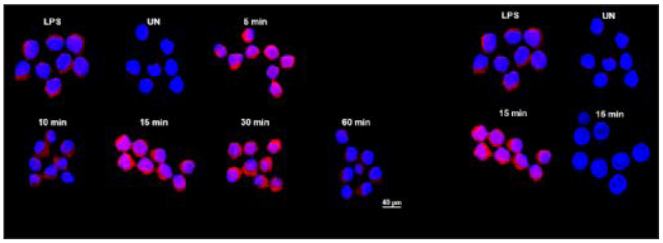

Figure 1. M. smegmatis infection induces intracellular ROS production in RAW 264.7 cells.

M. smegmatis infection increases generation of intracellular ROS in macrophages. **(a)** RAW 264.7 cells were infected with M. smegmatis for 0-1 h. DHE (5 uM) assay was used to assess intracellular ROS levels. LPS (500 nM, 3 h) was used as the positive control for superoxide. **(b)** RAW 264.7 cells were pretreatment of general ROS scavenger (NAC, 30 mM) during M. smegmatis infection for 15 min by Confocal analysis. Representative Data shown are mean ± S.E.M of three independent experiments.

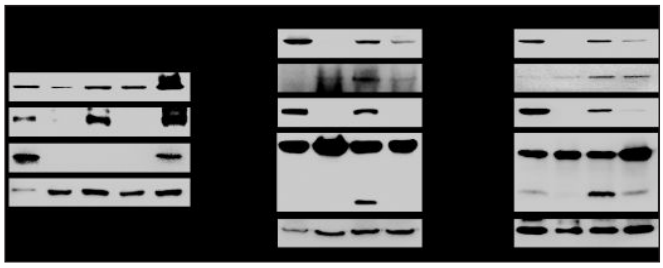

Figure 2. Apoptotic cell death by M. smegmatis infection is associated with ER stress response.

M. smegmatis-induced apoptosis is mediated by ER stress activation in macrophages. **(a)** Immunoblot analysis of ER stress molecules in RAW 264.7 cells infected with M. smegmatis in time-dependent manner (0 - 24 h). **(b)** Immunoblot analysis of ER stress molecules in RAW 264.7 cells infected with M. smegmatis or H37Rv in time-dependent manner (0 - 24 h). **(c)** RAW 264.7 cells were pretreated with ER stress inhibitor (4-PBA, 10 mM) for 1 h prior to M. smegmatis infection for 24 h. Data shown are mean ± S.E.M of three independent experiments. As a positive control, RAW 264.7 cells were treated with 1 ug/ml TM for 6 h. TM; Tunicamycin. 4-PBA; 4-phenylbutyrate.

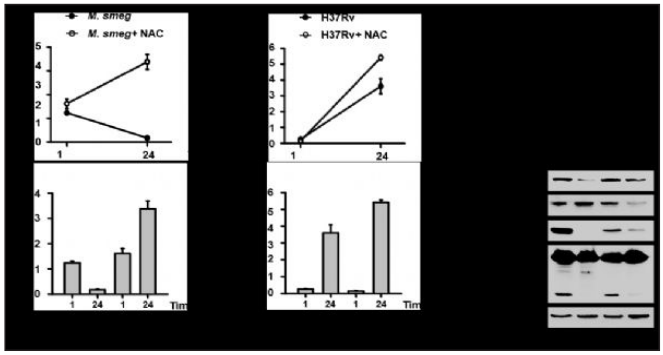

Figure 3. ROS generation by M. smegmatis infection decreases intracellular survival of M. smegmatis via ROS–mediated ER stress.

ROS generation by M. smegmatis infection decreases intracellular survival of M. smegmatis in infected macrophages. RAW 264.7 cells were pretreatment of general ROS scavenger (NAC, 30 mM). **(a)** Intracellular survival of M. smegmatis was measured in M. smegmatis or H37Rv infected Raw264.7 cells for 1h, 24h. **(b)** Immunoblot analysis of ER stress molecules in RAW 264.7 cells infected with M. smegmatis for 24 h. Representative Data shown are mean ± S.E.M of three independent experiments.

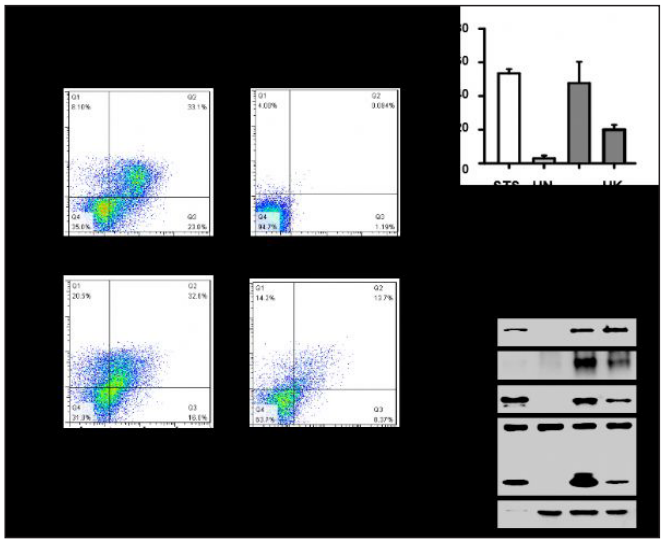

Figure 4. Apoptosis is induced by M. smegmatis infection in RAW 264.7 cells.

RAW 264.7 cells were infected with M. smegmatis or Heat Killed M. smegmatis for 24 h. **(a)** Annexin V and PI staining was used to detect the apoptosis of RAW 264.7 cells during M. smegmatis infection. **(b)** Quantification of apoptotic cells were depicted the percentage of apoptotic cells (sum of early and late apoptotic cells). Data shown are mean ± S.E.M of three independent experiments. As a positive control, RAW 264.7 cells were treated with STS (500 nM) for 6 h. STS; Staurosporine.

PP-072

INHIBITION OF AUTOLYSIS BY LIPASE LIPA IN *STREPTOCOCCUS PNEUMONIAE* SEPSIS

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More than 50% of sepsis cases are associated with pneumonia. Sepsis is caused by infiltration of bacteria into the blood via inflammation, which is triggered by the release of cell wall components following lysis. However, the regulatory mechanism of lysis during infection is not well defined. Mice were infected with Streptococcus pneumoniae D39 wild-type (WT) and lipase mutant (ΔlipA) intranasally (i.n.) (pneumonia model) or intraperitoneally (i.p.) (sepsis model), and survival rate and pneumococcal colonization were determined. LipA and autolysin (LytA) levels were determined by qPCR and western blotting. S. pneumoniae Spd_1447 in the D39 (type 2) strain was identified as a lipase (LipA). In the sepsis model, but not in the pneumonia model, mice infected with the ΔlipA mutant displayed higher mortality rates than did the D39 WT-infected mice. Treatment of pneumococci with serum induced LipA expression at both the mRNA and protein levels. In the presence of serum, the ΔlipA mutant displayed faster lysis rates and higher LytA expression than the WT, both in vitro and in vivo. These results indicate that a pneumococcal lipase (LipA) represses autolysis via inhibition of LytA in a sepsis model.

Keywords: S. pneumoniae, LipA, LytA, sepsis infection

PP-073

VISCERAL LEISHMANIASIS PATIENTS: THE ROLE OF DIFFERENT IMMUNE CELL POPULATIONS

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Visceral leishmaniasis (VL) is an inflammatory chronic disease transmitted by sand flies bites. In Brazil the disease is caused by the protozoan *Leishmania infantum*, and although most patients present asymptomatic forms of the disease, some develop symptoms that may eventually result in death. The aim of our study is to identify differentially expressed genes (DEGs) between groups of VL patients and correlate their expression with the disease outcome. We performed RNA-sequencing of samples from symptomatic (active disease) and asymptomatic patients, as well as symptomatic patients after 180 days of treatment and healthy controls. The comparison of male asymptomatic and active disease patients resulted in greatest number of DEGs. The functional analysis of expression profiles demonstrated that symptomatic patients have enrichment of genes related to neutrophils and citotoxic T cells, possibly contributing to tissue lesions on those patients, while asymptomatic ones revealed an expression profile related to eosinophils responses. Murine validation of those observations employing eosinophils deficient mice infected with *L. infantum* demonstrated that these animals present greater responses of neutrophils and T $CD8^+$ cells in spleen, confirming the patients observations. Our data showed that eosinophils might play a regulatory role on inflammation during *L. infantum* infection. Further studies to comprehend this mechanism are being performed.

Keywords: Leishmania infantum, visceral leishmaniasis, chronic inflammation

PP-074

CAPSULAR POLYSACCHARIDE SYNTHESIS BY GLUCOSYLTRANSFERASE (GTFA) IN *STREPTOCOCCUS PNEUMONIAE*

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Infection of *Streptococcus pneumoniae*, a causative agent of pneumonia, meningitis, and septicemia, into host cells induces removal of capsular polysaccharide (CPS) in the pathogen regardless of serotype. In contrast, invasion into blood induces synthesis of CPS. However, how capsule removal (decapsulation) and encapsulation are controlled by the pathogen during infection is not completely understood. Since exopolysaccharides are synthesized by sucrose phosphorylase (GtfA), which has glucosyltransferase activity for the synthesis of glycopeptide antibiotics, such as vancomycin, we postulated that GtfA could be involved in encapsulation. Here, we show that the *gtfA* mutant showed significantly reduced level of CPS, as well as reduced cytotoxicity to A549 cells *in vitro*. Moreover, adherence of the *gtfA* mutant to HEp-2 and RAW 264.7 cells was increased. Interestingly, the *gtfA* mutant showed resistance to deoxycholate (DOC) -induced autolysis. In addition, ELISA results of pro-inflammatory cytokines (IL-1β and TNF-α) also proved that mutant strain has less inflammatory effect in A549 cells than its wild type counterpart. These results indicate that GtfA is required for *S. pneumoniae* cytotoxicity to lung cells and might have an important role in the pathogenicity of *S. pneumoniae* by regulating CPS.

Keywords: Streptococcus pneumoniae, Glucosyltransferase (GtfA), Capsular Polysaccharide

PP-075

MANNOSE BINDING LECTIN (MBL) DEFICIENCY AND RISK TO TUBERCULOSIS INFECTION IN ANKYLOSING SPONDYLITIS PATIENTS

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Introdution: MBL (mannose binding lectin) deficiency may influence susceptibility to infections. In this study, we verify if MBL deficiency in patients with Ankylosing Spondylitis (AS) predisposes to infections.

Methods: 60 patients with AS diagnosed had their MBL serum levels determinated. Twenty five individuals were identified as MBL deficient (serum values <100 ng/mL) and were paired with 35 "sufficient" MBL producers (median serum level = 700 ng/mL) for gender, age, use of medications and tobacco exposure. Medical records of all patients were retrospectively investigated for the period of five years and the rate of infections occurrence was compared in the two groups.

Results: AS patients with MBL deficiency had higher number of urinary tract infections [p=0.03; IRR =2.33; 95%IC=0.95-6.04] and tuberculosis (p=0.008; IRR =9.8 95% CI=1.2-441.6) than sufficient MBL group.

Conclusion: We found a significant association between MBL deficiency and higher risk of tuberculosis and urinary tract infection in AS patients.

Keywords: MBL, Tuberculosis, Ankylosing Spondylitis, Complement System

PP-076

ZIKA VIRUS INDUCES INFLAMMASOME ACTIVATION IN BMDMS OF C57BL/6 MICE

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Inflammasomes are multimeric complexes formed in response to a variety of physiological and pathogenic stimuli. Inflammasome activation is an essential component of the innate immune response and is critical for the clearance of pathogens or damaged cells. Stress responses triggered by some viral infections

are well stablished for inflammasome activation. However, little information is available about the inflammatory process caused by ZIKV and if inflammasome activation is an important event in this viral infection. Therefore, we investigated if ZIKV is able to infect BMDMs of C57BL/6 mice and how these cells respond to this infection in terms of inflammation (IL-1β, NLRP-3 and CASP-1) and cell death. Our results demonstrated that ZIKV could effectively infect C57BL/6 mice BMDMs and induced IL-1ß release in 24 and 48hours with higher concentrations of this cytokine in ZIKV MOI 5, suggesting that ZIKV could induce inflammasome activation. BMDMs of Nlrp3-/-, Pycard-/-, Casp1/11-/- and Casp11-/- mice were infected with ZIKV in same conditions, and we could observe that in the absence of any NLRP3 inflammasome component as Nlrp3-/-, Pycard-/-, Casp1/11-/- there was not IL-1ß release, indicating that inflammasome activation in ZIKV infection is through NLRP3 platform and is dependent of caspase-1, as it was observed IL-1ß release in BMDMs of Casp11-/- mice. These results may contribute to the increasing knowledge about ZIKV infection features, and may help to understand the inflammatory processes involded in the pathogenesis of this viral infetion.

Keywords: ZIKV infection, inflammasome activation, NLRP3, Caspase-1, IL-1ß release

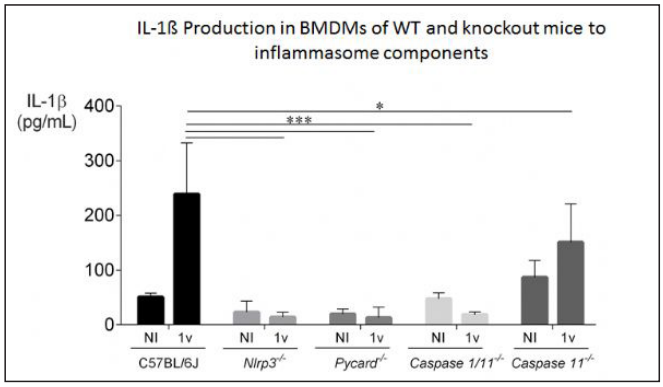

Figure 1. IL-1ß Production in BMDMs of WT and knockout mice to inflammasome components

IL-1ß Production in BMDMs of C56BL/6, Nlrp3-/-, Pycard-/-, Casp1/11-/- mice to investigate inflammasome activation. BMDMs of the different mice were infected with ZIKV with MOI 5. After 24 hours IL-1b production was accessed by ELISA assay.

PP-077

TRANSLATIONAL FEATURES OF A MODEL OF HRSV INFECTION IN BALB/C MICE: EFFICACY OF RIBAVIRIN, RUPINTRIVIR AND OSELTAMIVIR

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Infection with a human Respiratory syncytial virus (RSV) is a leading cause of respiratory illness primarily associated with pneumonia, airway obstruction and airway hyperresponsiveness (AHR) in infants, elderly and immunocompromised patients. Despite the important role of animal models of hRSV in preclinical testing of novel drug candidates, seldom have the selected candidates shown limited success or progressed to clinical trials. This is, at least in part, due to lack of animal models that fully emulate the pathogenesis of hRSV infection in humans. The susceptibility of mouse strains to hRSV infection vary. The BALB/c mouse, with moderate susceptibility to hRSV, has been widely used to study the pathogenesis of hRSV. Here we present a comprehensive evaluation of translational features of hRSV-A2 infection in a BALB/c mouse model. The prophylactic effects of Ribavirin (20 mg/kg/PO/QD), Rupintrivir (1 mg/kg/IP/BID) and Oseltamivir (10 mg/kg/PO/BID) were compared.

Ten-week-old female BALB/c mice were inoculated with RSV (10^7 PFU/animal) and monitored for 4 days for the appearance of bodyweight changes and clinical signs. Treatments started 1hr (Ribavirin, Rupintrivir) or 24hrs (Oseltamivir) prior to infection and were continued for three days. Four days post infection, airway resistance and compliance were measured in anesthetized and ventilated animals by Resistance and Compliance System (Buxco Ltd.-DSI) followed by analysis of viral titers in lungs, differential cell count and cytokine measurements in bronchoalveolar lavage (BAL) samples and lung tissue. Bronchopneumonia severity was assessed using a standard histopathologic score.

Analysis of viral titers four days post-infection revealed that hRSV-A2 has successfully infected and replicated in lungs of infected animals. Infected mice showed significant airway hyperresponsiveness in response to methacholine on day 4 post-infection as well as moderate to severe histopathologic signs of bronchopneumonia in lungs. By day 4 post infection, prophylactic treatment of infected animals with Ribavirin resulted in significantly reduced airway resistance and compliance in comparison to vehicle control. A significant reduction of lung viral titers, neutrophils and mononuclear leukocytes in BALF was also observed in comparison to vehicle control. No protective effects were observed in animals prophylactically treated with Rupintrivir and Oseltamivir.

In this study, a successful infection and propagation of hRSV-A2 virus in female BALB/c mice was confirmed. The observed clinical features recapitulate to a great extent the clinical features of the disease observed in human patients. The responsiveness of the model to Ribavirin, the only anti-viral medication approved for treatment of hRSV infection in humans, was also confirmed. The mouse model of hRSV-A2 infection presented in this study is a reliable research platform for preclinical testing of drug candidates and vaccine development strategies.

Keywords: Respiratory syncytial virus, airway hyperresponsiveness, infection, animal models

PP-078

CLINICAL UTILITY OF IL28B GENOTYPES IN CHRONIC HEPATITIS B WITH ENTECAVIR MONOTHERAPY

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Background: Although genetic polymorphisms at the interleukin 28B gene (IL28B) are associated with therapeutic response to peg-interferon in chronic hepatitis B (CHB), there were no data whether IL28B gene is associated with treatment response of oral nucleoside analogues in CHB patients.

Aims: The aim of this study was therefore to evaluate the clinical utility of IL28B genotyping for predicting virological response (VR) during entecavir (ETV) therapy in CHB patients.

Methods: A total of 124 treatment naïve CHB patients, who subsequently ETV 0.5mg daily for at least 12 months, were consecutively enrolled between 2011 and 2014. Four SNPs of IL28B including rs8105790, rs12979860, rs8099917 and rs10853728 were determined. All patients were monitored at least every 3–4 months by clinical examination, biochemical and virologic assessments during ETV therapy. VR was defined as hepatitis B virus (HBV) DNA negativity at 48 week.

Results: The study population was male dominant (63.7%, 79/124) with 48.1±10.2 years of mean age. Forty six percent had cirrhosis and 51.6% were positive for hepatitis B e antigen (HBeAg) at baseline. HBV DNA and alanine aminotransferase (ALT) levels were 6.65±1.21 log10IU/mLand188.7±221.7IU/L,respectively, in mean. Overall VR was achieved in 80.6% (100/124). In univariate analysis, ALT levels, HBeAg positivity and HBV DNA levels were candidate variables for multivariate analysis ($p < 0.1$). Of the clinical features, high ALT levels, HBeAg negative state, low HBV DNA levels were considered favorable factors for VR. In multivariate analysis, serum ALT and IHBV DNA levels remained as independent predictor for VR (RR 1.004; 95% CI 1.000-1.008; $p = 0.049$ and RR 0.160; 95% CI 0.066–0.384; $p < 0.001$). Genotypes of IL28B gene, however, were not associated with VR ($p > 0.2$).

Conclusion: IL28B may not exert a significant role for predicting therapeutic response to ETV in Korean CHB patients.

Keywords: Chronic hepatitis B, Entecavir, IL28B genotype

Microbiome

[PP-080]

MELATONIN TREATMENT MODULATES INTESTINAL MICROBIOTA ON DSS COLITIS

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Background: Melatonin has strong anti-inflammatory potentials in the GI tract. On our previous study, melatonin reduces various inflammatory cytokines on colon tissues and accelerates recovery from injured mucosa. We aimed to know the effect of melatonin on intestinal microbiota.

Methods: We used 3 groups of C57BL/6 mice. Group I: control, Group II: chronic colitis group: 2% DSS for 7days and followed 2weeks for recovery and then readministered 2% DSS for 7days, Group III: chronic colitis with melatonin group: add daily melatonin treatment. Melatonin(10mg/kg) or saline was injected daily by intraperitoneal route. The mice were sacrificed on 28th day. Stool were collected during last 2days. Genomic DNA from feces was extracted. After amplification of genomic DNA using barcoded primers targeting the V1 to V3 regions of bacterial 16S rRNA genes, pyrosequencing was performed.

Results: Fecal microbial analysis demonstrated that Firmicutes to Bacteroidetes ratio(F/B ratio) is 0.19in GroupI, 0.38 in Group II and 0.21 in Group III. Melatonin treatment significantly decreased F/B ratio comparing with DSS colitis group.($p=0.015$). The increase in bacteroidetes is mainly due to increased bacteroidaceae and prevotellaceae. The decrease in frmicutes is due to decreased lactobacillaceae and erysipelotrichaceae. On principal coodinates analysis, Three groups showed clearly separated each other. A phylogenic tree analysis using MOTHUR showed melatonin treatment group was more close to control Group.

Conclusion: This study showed that DSS induced colitis altered structure of intestinal microbiota. Melatonin treatments on DSS colitis modulate intestinal microbiota and change them close to control. This study suggest that melatonin may modulate microbiota in patients with chronic ulcerative colitis and have protective effects on recurrent flare up.

Keywords: melatonin, microbiota, DSS colitis

Neuroinflammation

[PP-081]

WOMEN'S PREGNANCY HISTORY MAY INFLUENCE ALZHEIMER'S RISK THROUGH ALTERATIONS IN IMMUNE FUNCTION

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Background and Aim: Pregnancy is associated with improvement in immunoregulation that, potentially, persists into the geriatric phase of life. Women with higher gravidity may experience increased regulatory T-cell frequencies in the long-term. Such an increase in immunosuppressive function might protect against Alzheimer's Disease (AD) pathogenesis, because depleted immunoregulatory mechanisms have been implicated in AD aetiology. We hypothesize that women who spend more cumulative months pregnant may experience reduced risk of AD later in life via improved regulation of inflammation. We aim to investigate the relationship between pregnancy history and AD risk, and determine whether the relationship could be attributed to an immunologic mechanism.

Methods: In a case-control, cross-sectional study of elderly British women (N=133), we collected reproductive history information and measured degree of Alzheimer's-type dementia. Cox's proportional-hazards modelling was used to assess the putative effect of cumulative months pregnant on women's AD risk, and the mutually adjusted effects of the counts of first and third trimesters on AD risk.

Results: Cumulative months pregnant is a significant predictor of AD risk after adjusting for age at first birth, reproductive span, breastfeeding, marriages, and occupation. For each

additional month pregnant, women exhibited a 5.5% decrease in AD risk (p=0.02), similarly after adjusting for parity (4.7% decrease, p=0.03). Cumulative number of first trimesters was associated with a lower risk of AD after adjusting for the number of third trimesters (p<0.01), while the latter predictor had no significant effect on AD after adjusting for the former (p=0.31).

Conclusions: This is the first study to suggest pregnancy affects Alzheimer's risk through alterations in the immune system. Our observation that more first-trimesters (but not third-trimesters) conferred protection against AD is more consistent with pregnancy's persisting immunological effects, which are driven by early gestational physiology, than the oestrogenic exposures associated with pregnancy, which are greatest in late gestation.

Keywords: Pregnancy, parity, Alzheimer's Disease, immunoregulation, adaptive immunity, autoimmunity

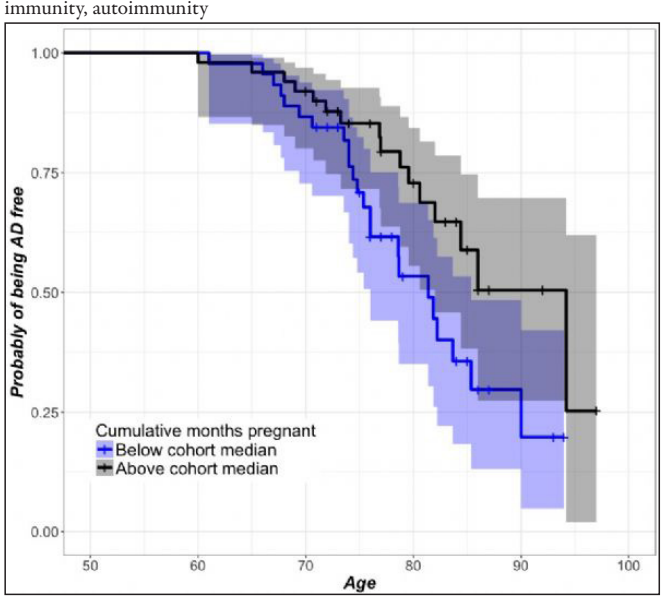

Figure 1. Women with more cumulative months pregnant had lower Alzheimer's risk

For each age, the plot reports the covariate-adjusted probability of being AD-free for women with total lifetime number of months pregnant below the cohort median (lower curve) and above the cohort median (upper curve). Pointwise 95% confidence bands are also shown. The purpose of this plot is to give a visual sense of the magnitude of the effect by dichotomizing the number of cumulative months pregnant variable. Cox regression of the reliance of AD risk on median-split dichotomous characterization of cumulative months pregnant demonstrates that women above the cohort median exhibit 37.01% lower AD risk compared with women below the cohort median (β=-0.99, exp(β)=0.37, se(β)=0.40, p=0.01, 95% CI=[0.17,0.81]). The Cox model reported in the abstract represents a more meaningful analysis by utilizing the continuous cumulative months pregnant variable.

PP-082

THE SYNTHETIC FORM OF MALARIA PIGMENT HEMOZOIN INDUCES NF-KAPPA B-MEDIATED NEUROINFLAMMATION IN BV2 MICROGLIA: IMPLICATIONS FOR CEREBRAL MALARIA

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University of Huddersfield

The most severe neurological complication of acute *Plasmodium falciparum* malaria is cerebral malaria (CM). Several studies have linked elevated levels of pro-inflammatory cytokines and chemokines with the disease severity. Studies have also suggested that the neurocognitive deficit in CM is partly due to activation of microglial inflammatory responses resulting in damage to adjacent neurons. Hemozoin (HZ) is a brown crystalline metabolite of haemoglobin formed by the malarial parasite and released into the systemic circulation. Several lines of evidence suggest that HZ could play a role in CM pathophysiology related to cytokine overproduction in the brain. Previous studies on macrophages showed that malarial HZ induced the production of IL-1β and IL-18 via NLRP3 inflammasome complex. In this study we report induction of NF-kappa B-mediated neuroinflammation in cultured BV2 microglia by a synthetic form of HZ. Cultured BV2 microglia were stimulated with HZ (200 and 400 μg/ml) for 24 h. Levels of pro-inflammatory cytokines (TNFα, IL-6 and IL-1β), PGE_2 and nitrite were measured in culture supernatants. Western blotting was used to evaluate effects of HZ on phosphorylation of I kappa B protein and p65 sub-unit. Results show that exposure of cells to HZ induced a significant (p<0.05) elevation in the production of TNFα, IL-6 and IL-1β, nitrite and PGE_2 from BV2 microglia. Further experiments showed that HZ treatment resulted in phosphorylation of I kappa B and p65 subunit. Experiments to further elucidate the role of NF-kappa B in the induction of neuroinflammation by HZ showed that in the presence of the NF-kappa B inhibitor, BAY11-7085 (10 μM), there was significant reduction in the production of TNFα, IL-6 and IL-1β, nitrite and PGE_2 following exposure to HZ (400 μg/ml). Furthermore, phosphorylation of both I kappa B and p65 sub-unit were attenuated following pre-treatment with BAY11-7085 (10 μM) prior to stimulation with HZ (400 μg/ml). These results show that hemozoin produces neuroinflammation through mechanisms involving activation of NF-kappa B signalling in the microglia. The outcome of these studies is significant in our understanding of the mechanisms involved in neuroinflammatory events during CM and provides opportunities for developing new pharmacological adjuncts for treating the condition.

Keywords: Cerebral Malaria, Hemozoin, Neuroinflammation, Microglia, NF-kappa B

PP-083

TRPV1 IN ALLERGIC CONTACT DERMATITIS

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Objective: Allergic contact dermatitis (ACD) is a hypersensitive reaction to allergens that causes rash or skin lesion at the site of exposure. Transient Receptor Potential Vanilloid Type 1 (TRPV1) is a member of TRP family of ion channels. It plays a role in the transmission and modulation of pain, and it may contribute to neurogenic inflammation via the release of neuropeptides. Recently, tacrolimus was promoted as a good substitute for glucocorticoids in the treatment of ACD. In addition to its immunosuppressant actions, tacrolimus was reported to produce desensitization of TRPV1, and such action was suggested to be beneficial in attenuating skin inflammation. This study aims to investigate the importance of TRPV1 in ACD.

Methods: Dinitrofluorobenzene (DNFB) was used as the inducer of ACD in male 6-week-old ICR mice. On days 1, 2, and 3 (sensitization), the mice received single topical administration of DNFB onto their shaved dorsal skin. On days 7, 8, and 9 (challenge), DNFB was topically applied onto the dorsum of their test ears. Their contralateral ears received parallel vehicle application to serve as internal controls. Symptoms of ACD were assessed one day after the last challenge. Involvement of TRPV1 was examined by desensitization or antagonism of TRPV1 using tacrolimus and capsazepine respectively, or by depletion of sensory neuropeptides using resiniferatoxin.

Results: DNFB treatment increased the mice ear weight and thickness. Microscopic examination confirmed spongiosis and increased numbers of immune cells in the affected ears. Tacrolimus (100 nmol) inhibited all these ACD symptoms, whereas, capsazepine (1 μg/kg) suppressed the increases in ear thickness and weight, but not the increase in infiltration of immune cells. Resiniferatoxin (10 nmol) had no effect on ear weight, but slightly reduced ear thickness, and suppressed the immune cell numbers. The effects of tacrolimus and resiniferatoxin were similar in control and capsazepine-treated animals.

Conclusions: Desensitization of TRPV1 by Tacrolimus and antagonism of TRPV1 by capsazepine suppressed the increases in weight and thickness of DNFB-treated mice ears. However, only tacrolimus reduced the number of infiltrated immune cells in the affected ears, and co-administration of capsazepine with tacrolimus did not enhance the inhibitory effects of tacrolimus. This indicates tacrolimus is more efficacious than capsazepine, and tacrolimus alone can attain maximum inhibition on its own. Depletion of neuropeptides by resiniferatoxin decreased the levels of immune cells, and slightly attenuated ear thickness without affecting ear weight. Curtailment of TRPV1 activities might therefore provide additional benefits to depletion of neuropeptides. These findings support the notion that inhibition of TRPV1 activities is a plausible approach for treatment of ACD. Further studies on TRPV1 knockout mice would be useful to consolidate the present findings.

Keywords: TRPV1, allergic contact dermatitis, tacrolimus

PP-084

ANTI-INFLAMMATORY AND NEUROPROTECTIVE EFFECTS OF CO-ULTRAPEALUT IN A MOUSE MODEL OF VASCULAR DEMENTIA

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Vascular dementia (VaD), the second most common cause of cognitive impairment in the population, is a disease that results from reduction in regional cerebral blood flow and involves oxidative stress and inflammation. Co-ultramicronized PEALut (co-ultra PEALut) is a new compound with beneficial effects, which include anti-inflammatory and anti-oxidant properties. Recently, co-ultraPEALut has been shown to exhibit neuroprotective effects in models of Parkinson's disease, cerebral ischemia and Alzheimer's disease. However, its effects on VaD remain unknown. Therefore, the purpose of the present study was to highlight the potential neuroprotective actions of co-ultraPEALut containing N-Palmitoylethanolamine (PEA) and the anti-oxidant flavonoid luteolin (Lut) (10:1 by mass) in a mouse model of VaD induced by bilateral carotid arteries occlusion. At 24 hours after VaD induction, mice were orally treated with 1 mg/kg co-ultraPEALut daily for 15 days. On the 15th day, brain tissues were processed for histological, immunohistochemical, western blot and immunofluorescent analysis. Our results clearly demonstrate that co-ultraPEALut improved learning, memory ability, locomotor activity and the reciprocal social interaction. In addition, the mice subjected to VaD and treated with the co-ultraPEALut showed a reorganization of CA1 and CA3 regions of the hippocampus and restored the number of hippocampal neurons as evidenced by NeuN expression, a specific marker of neurons. Furthermore following carotid arteries ligation, mice treated with co-ultraPEALut, showed a modification of pro-inflammatory, pro-apoptotic proteins and of oxidative stress as evidenced by the expression of IκB-α, NF-κB p65, Bax, Bcl-2, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). In order, co-ultraPEALut treatment restored VaD-induced loss of brain-derived neurotrophic factor (BDNF) and neurotrophins 3 (NT-3) expression in mice. These results confirmed that the neuroprotective effects of co-ultraPEALut were associated with its anti-inflammatory and anti-oxidant properties.

Keywords: Neuroprotection, Palmitoylethanolamide, Luteolin, Oxidative Stress, Inflammation

PP-085

PSEUDOBULBAR AFFECT ASSOCIATED WITH AUTOIMMUNE ENCEPHALITIS

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Pseudobulbar affect (PBA), which features involuntary emotional displays incongruent with an individual's mood, has been demonstrated to occur secondary to neurological insults such as traumatic brain injury. Described here is a case of PBA secondary to autoimmune encephalitis, which has not previously been reported. A.B. was a 49-year-old man with bilateral lower extremity spasms at night, which progressively worsened in intensity and frequency over three months, without significant response to cyclobenzaprine or tizanidine. A neurological exam revealed inappropriate bursts of crying without feeling sad, indicating the presence of PBA, as well as gaze paresis of both eyes with leftward gaze, spontaneous myoclonic jerks of both lower extremities, clumsy foot tapping bilaterally, reduced light touch and vibration sensation at both feet, and diffuse hyperreflexia. A MRI of the brain and entire spinal cord with and without gadolinium was only remarkable for a left frontal cavernoma and cervical and lumbar degenerative changes, which did not sufficiently explain the patient's symptoms. A routine electroencephalogram revealed no epileptiform abnormalities. An analysis of the cerebrospinal fluid revealed elevated leukocyte and protein levels, with normal glucose level and no acute viral or bacterial infection. A paraneoplastic panel was negative, but an autoimmune panel revealed the presence of anti-glycine receptor antibodies. The patient was treated with intravenous methylprednisolone for autoimmune encephalitis, dextromethorphan/quinidine for pseudobulbar affect, and diazepam as needed for spasms. With spasms better controlled, he could ambulate with a walker and

was discharged to home with an outpatient physical therapy course. Six months later, he had resolution of PBA and spasms, and could walk independently, without the need to continue any medications for symptomatic treatment. This case represents an example of PBA secondary to autoimmune encephalitis, which resolved with effective treatment of the underlying disease. A differential diagnosis for patients presenting with PBA should include the autoimmune encephalitis as a possible etiology.

Keywords: Pseudobulbar affect, Autoimmune encephalitis, Anti-glycine receptor antibody, Dextromethorphan/quinidine, Myoclonic jerk

MRI Brain

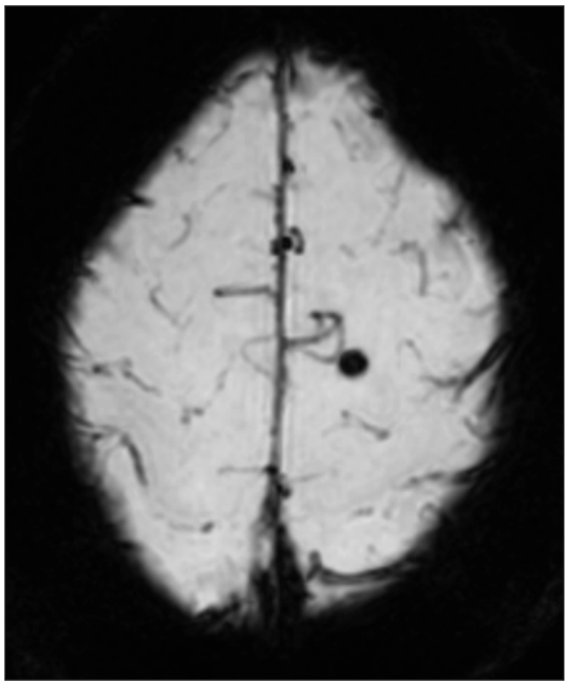

Figure 1. Incidental left frontal cavernoma, without other gross abnormalities, as seen on MRI Brain with and without gadolinium.

PP-086

HARMANE AMELIORATES DEPRESSIVE-LIKE BEHAVIORS IN CHRONIC MILD STRESS-TREATED RATS: THE POSSIBLE INVOLVEMENT OF THE NEUROINFLAMMATORY PATHWAY

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Objectives: Major depressive disorder is a condition, which is commonly associated with chronic stress. Chronic inflammation is a contributing factor in stress-induced depression, which in turn can increase the levels of proinflammatory cytokines. Alterations in structure and functions of certain brain areas due to increased inflammation may be associated with the pathophysiology of depression. In a previous study, we have shown that harmane, which is an endogenous ligand for imidazoline binding sites, has a potent antidepressant effect in forced swim test (FST) when administered acutely to rats. Therefore, current study was designed to investigate the effect of chronic harmane treatment on behavioral dysfunction and neuroinflammation induced by chronic unpredictable mild stress (CUMS) model of depression in rats.

Methods: Male adult Wistar Albino rats weighing 220-360 g were used in our study. CUMS treatment was performed for a total duration of 6 weeks. Harmane was administered intraperitoneally once a day at two different doses (5 and 10 mg/kg) for the last 3 weeks of the experiment along with CUMS procedure. At the end of CUMS, depressive-like behaviors were evaluated by sucrose preference (SP) and FST. Proinflammatory cytokines such as interleukin (IL)-1β, IL-6 and IL-18 in addition to nuclear factor kappa-B (NF-κB), apoptosis-associated speck-like protein (ASC) and caspase-1 were determined in prefrontal cortex by real time-PCR (RT-PCR).

Results: It was demonstrated that the harmane ameliorated depression-like behaviors in CUMS-treated rats, as indicated by body weight changes (BW), %SP, and the immobility time in FST. mRNA levels of proinflammatory cytokines (IL-1β, IL-6,and NF-κB but not IL-18) as well as ASC and caspase-1 were profoundly enhanced in the prefrontal cortex of the stress-treated rats. Activation of caspase-1 was considered as a trigger for the induction of major proinflammatory cytokines associated with depression whereas ASC expression as a marker linked to the altered innate immune responses in depression. Harmane (10 mg/kg) significantly reduced the elevated mRNA expressions of IL-1β, IL-6 and caspase-1 in rats exposed to CUMS but not IL-18, NF-κB and ASC in prefrontal cortex.

Conclusions: The naturally occurring beta-carboline, harmane, has been implicated in several functions in the central nervous system. Previous literature indicates that harmane may modulate monoamine levels through monoamine oxidase inhibition, which may at least partially play a role in its antidepressant effect. The current findings showed that harmane may also modulate neuroinflammation by inhibiting proinflammatory cytokines and caspase-1 induced by chronic stress thereby suggesting the immunomodulatory action of harmane in depression.

This study was funded by Scientific Research Project Foundation of Marmara University (SAG-C-YLP-110915-0415).

Keywords: Harmane, beta-carboline, depression, cytokine, neuroinflammation

PP-087

ANNEXIN A1 CONTROLS PPARΓ AND CD36 EXPRESSIONS BY BV2 CELLS

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Inflammation is a key process in maintaining homeostasis and is essential for the physiological response to injury in the central nervous system (CNS). Protein annexin A1 (AnxA1) and peroxisome proliferated-activated receptors (PPAR) are

important factors responsible for inflammation control in the CNS, as both inhibit development of inflammation; however, all of the underlying mechanisms responsible for modulation of both of these factors are still unknown. In this study, we aimed to investigate whether AnxA1 could modulate the expression of PPARγ in BV2 microglia cells. Our findings demonstrate that: 1) depletion of endogenous AnxA1 reduced gene and protein expression of PPARγ, and both were rescued by recombinant AnxA1(rAnxA1); 2) depletion of endogenous AnxA1 reduced expression of transcription factors STAT-6 and CREB, and both seems to be not involved on control of AnxA1 on PPARγ expressions and 3) depletion of endogenous AnxA1 impaired the expression of surface protein CD36, which was not rescued by rAnxA1. Nevertheless, rAnxA1 rescued gene transcription of CD36, via FPR2 as both pan FPR and FPR2 antagonists blocked the rAnxA1 effect. Our data demonstrate the molecular control of AnxA1 on PPARγ and CB36, evidencing different roles for endogenous and exogenous AnxA1 on the control of mechanistic pathways. A better elucidation of such effects will contribute to the comprehension of the pathophysiology of neuroinflammation and possible novel therapeutic approaches.

Keywords: resolution of inflammation, formyl peptide receptor, Boc-2, WRW-4, STAT-6, CREB

PP-088

EHD1 MODULATES IL-6/JAK2/STAT3 SIGNALING IN A LIPID RAFT DEPENDENT MANNER

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Lipid rafts are specialized membrane microdomain serving as signaling platform for signal transduction during immune and inflammatory responses. However, a precise molecular mechanism involved in lipid rafts is not clear. IL-6 stimulation increases lipid raft clustering and the lipid raft-association of IL-6Rα, gp130 and JAK2 in microglia. Lipid rafts disruption using methyl-β-cyclodextrin and nystatin results in a significant reduction of IL-6-induced lipid raft-clustering and lipid raft-association of gp130 and JAK2 followed by the inhibition of STAT3 phosphorylation but not ERK phosphorylation. Here, we investigated lipid raft-associated proteins that is altered by IL-6 stimulation. Using lipid raft proteome analysis, we found Eps 15 homology domain-1 (EHD1) is increasingly localized to lipid rafts by IL-6 stimulation. Lipid raft disruption also causes a reduction in lipid raft-association of EHD1 and its co-localization with gp130 and JAK2. The siRNA-based-silencing of EHD1 markedly inhibits IL-6-induced association of gp130 and JAK2 with lipid rafts. It also blocks IL-6-induced STAT3 phosphorylation but does not ERK phosphorylation. Point mutation of EHD1 W485 that interacts with proteins containing asparagine-proline-phenylalanine (NPF) motifs leads to inhibit IL-6-induced STAT3 phosphorylation. These results indicate that IL-6 induces a modification of lipid raft composition and lipid raft association of EHD1 has a role in IL-6/JAK2/STAT3 signaling.

Keywords: IL-6, STAT3, EHD1, lipid raft, microglia

PP-089

DIMETHYL FUMARATE ATTENUATES NEUROINFLAMMATION AND NEUROBEHAVIORAL DEFICITS INDUCED BY EXPERIMENTAL TRAUMATIC BRAIN INJURY

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TBI is a serious neuropathology that causes secondary injury mechanisms, including dynamic interplay between ischemic, inflammatory and cytotoxic processes. Moreover, the damage induces massive cell death and outcomes in extensive dendrite degeneration leading to persistent cognitive, sensory and motor dysfunction and resulting in a permanent neurobiological alteration. Fumaric acid esters (FAEs) showed beneficial effects in preclinical models of neuroinflammation, neurodegeneration and toxic oxidative stress, so the aim of the present work was to evaluate the potential beneficial effects of dimethyl fumarate (DMF), the most pharmacologically effective molecules among the FAEs, in a murine model of TBI induced by controlled cortical impact (CCI). Mice were orally administered with DMF at the doses of 1, 10 and 30 mg/Kg, 1h and 4h after CCI. DMF treatment notably reduced histological damage and improved behavioral function, observed by Rotarod and Elevated Plus Maze (EPM) tests. Moreover, DMF treatment was able to reduce edema and brain infarctions as evidenced by decreased 2,3,5-triphenyltetrazolium chloride staining (TTC) and a blocked apoptosis process increasing B-cell lymphoma 2 (Bcl-2) expression in the injured cortex. Furthermore, DMF treatment up-regulated Nrf-2 pathway, inducing activation of manganese superoxide dismutase (Mn-SOD) and heme-oxygenase-1 (HO-1). Also, regulating NF-κB pathway, DMF treatment decreased the severity of inflammation through a modulation of neuronal nitrite oxide synthase (nNOS), interleukin 1 (Il-1β), tumor necrosis factor (TNF-α) and ionized calcium-binding adapter molecule 1 (Iba-1) expression, and cyclooxygenase 2 (COX-2) and myeloperoxidase (MPO) activity. Our results showed important protective effects of DMF in an animal model of TBI, sustaining the thesis that DMF could provide a valuable support to the therapies for brain trauma available today.

Keywords: traumatic brain injury, dimethyl fumarate, Nrf-2, Nf-kb

PP-090

INHIBITION OF MAMMALIAN TARGET OF RAPAMYCIN (MTOR) IMPROVES NEUROBEHAVIORAL DEFICIT AND MODULATES INFLAMMATORY RESPONSE AFTER TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) induces primary and secondary damage on endothelium and brain parenchyma, leading neurons die rapidly by necrosis. The mammalian target of rapamycin signalling pathway (mTOR) mediates many aspects

of cell growth and regeneration and is up-regulated after moderate to severe traumatic brain injury (TBI). The significance of this increased signalling event for recovery of brain function is presently unclear, here we used two different selective inhibitors of mTOR activity to explore the functional role of autophagy in an validated model of TBI, the controlled cortical impact injury (CCI).

We treated animals with KU0063794, a dual mTORC1 and mTORC2 inhibitor, and with Rapamycin a well-known inhibitor of mTOR, 1 and 4 hours after TBI.

Our results demonstrated that mTOR inhibitors, especially KU0063794, significantly improve motor and cognitive recovery after controlled cortical impact, as well as reduce lesion volumes. Moreover we observed that mTOR inhibitors treatment ameliorate the neuroinflammation associated to TBI and showed that this acute treatment significantly diminished the extent of neuronal death, astrogliosis and apoptotic process after trauma.

Our findings suggest that the neuronal mTORC1/2 activity afterTBI is deleterious to brain function, and that acute intervention with mTORC1/2 inhibitor after trauma may represent an effective therapeutic strategy to improve recovery after brain trauma.

Keywords: Traumatic brain injury, mTOR, neuroinflammation

PP-091

MULTIPLE MECHANISMS OF DIMETHYL FUMARATE IN AMYLOID B-INDUCED NEUROTOXICITY IN HUMAN NEURONAL CELLS

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Alzheimer disease (AD) is characterized by a complex heterogeneity of pathological changes and any therapeutic approach categorically requires a multi-targeted way. It has been demonstrated that together with the hallmarks of the disease such as neurofibrillary tangles and senile plaques, oxidative and inflammatory stress covered an important role. Dimethyl fumarate (DMF) is an orally bioavailable methyl ester of fumaric acid and activator of Nrf2 with potential neuroprotective and immunomodulating activities. Therefore, the aim of the present work was to evaluate the potential beneficial effects of DMF in an in vitro Alzheimer's model by using SH-SY5Y human neuroblastoma cell lines stimulated with amyloid-beta (Aβ). DMF pretreatment (30 μM) preserved cellular viability from Aβ 1μM stimulation, reducing tau hyper-phosphorylation. Moreover, DMF was able to induce an activation of manganese superoxide dismutase (Mn-SOD) and heme-oxygenase-1 (HO-1), decreasing the severity of oxidative stress. Our results showed important multi-protective effects of DMF pretreatment from Aβ stimulation in SH-SY5Y cells, highlighting a Nrf2/ NF-κB dependent mechanism, that could provide a valuable support to the therapies for neurodegenerative diseases today.

Keywords: Alzheimer's disease, dimethyl fumarate, Tau hyper-phosphorylation, NfkB, oxidative-stress, Nrf2

PP-092

THE EFFECT OF ANTIDEPRESSANT-ACTING NOS INHIBITORS ON NOD-LIKE RECEPTOR PROTEIN 1 (NLRP1) INFLAMMASOME-MEDIATED STERILE NEUROINFLAMMATION IN A RAT MODEL OF DEPRESSION

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Objectives: Innate immunity-mediated sterile inflammation is involved in the progression of depression. The activation of inflammasome forming Nod-like receptor protein (NLRP), particularly NLRP3, a member of pattern recognition receptors, has been recently addressed in depression. Based on our previous work, agmatine (AGM), decarboxylated L-arginine and an endogenous modulator of nitric oxide synthase (NOS), might exert antidepressant-like effects via inhibiting neuroinflammation. Here, we investigated the possible involvement of neuronal NLRP1 inflammasome cascade in the antidepressant-like effect of AGM compared to specific NOS inhibitors (NOSi) in chronic unpredictable mild stress (CUMS) model of depression.

Methods: Adult male Sprague Dawley rats (290-320 g) were divided into groups; Control (vehicle), CUMS (vehicle), CUMS+IMI (Imipramine; 10 mg/kg i.p.), CUMS+AGM (40 mg/kg i.p.), CUMS+inducible (i)NOSi (Aminoguanidine; 50 mg/kg i.p.) and CUMS+neuronal (n)NOSi (3-Bromo-7-Nitroindazole; 20 mg/kg i.p.) (n=10-12/group). In CUMS model, various stressors were applied for 6 week. After 3rd week, chronic treatments were started (for 21 days). Anhedonia-like behavior was assessed by sucrose preference test (SPT) in every two weeks. Finally, forced swim test (FST) was performed. Brain prefrontal cortex (PFC) was used for real-time PCR analysis of NLRP inflammasome cascade and NOS isoforms. Microglial activation was assessed by Iba1 immunostaining. One/two-way ANOVA were used for statistical analysis.

Results: CUMS resulted in depressive-like behaviors that were ameliorated by chronic treatments. AGM, iNOSi and nNOSi treatments showed reduced iNOS levels while nNOSi downregulated nNOS. AGM stimulated eNOS and nNOS. AGM, iNOSi, nNOSi but not IMI reduced caspase-1, ASC, IL-1β and IL-6 mRNA levels induced by CUMS. Although AGM and iNOSi treatments decreased CUMS-induced NLRP1 levels, the reduction was significant in nNOSi-treated group. AGM reduced CUMS-induced Iba1(+) cells in hippocampus.

Conclusions: While microglial NLRP3 inflammasome activation has lately been the focus of depression, the possible involvement of neuronal NLRP1 inflammasome was not addressed before. Here we show for the first time that NLRP1 inflammasome activation might also play role in psychological stress. We found that AGM produced antidepressant-like effects comparable to specific iNOS and nNOS inhibitors possibly by inhibiting neuronal NLRP1 inflammasome activation and subsequent proinflammatory responses. Nevertheless, the post-transcriptional regulation of NLRP1 and (also) NLRP3

cannot be ruled out and protein analysis in specific brain regions are needed. Overall, we suggest that NO might interact neuronal inflammasome complex and interfering this communication may help inhibiting innate immunity responses in depression.

This study was funded by Scientific Research Project Foundation of Marmara University (SAG-C-DRP-110915-0417).

Keywords: Nitric oxide synthase, agmatine, depression, NLRP1, sterile neuroinflammation

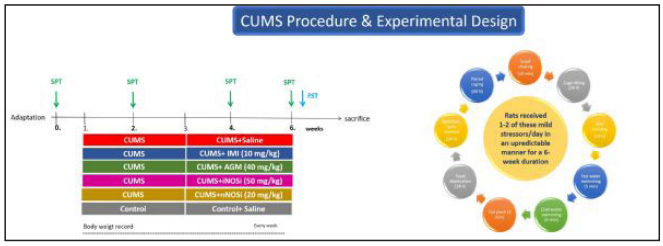

Figure 1. CUMS procedure and experimental design

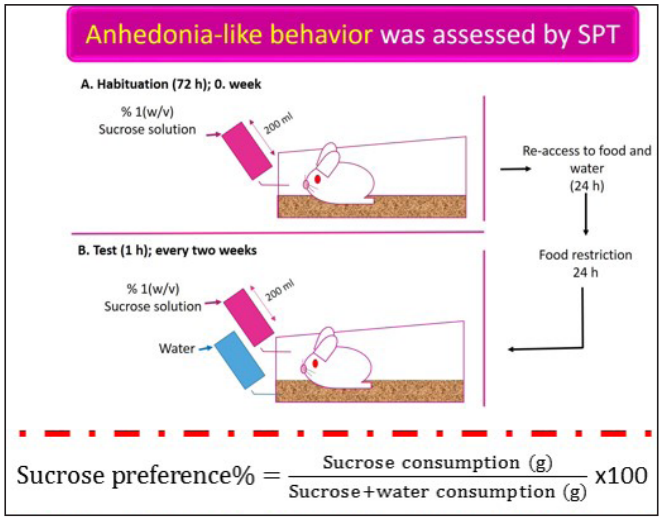

Figure 2. Sucrose Preference Test

Figure 3. Forced Swim Test

Forced swim test was conducted at 6th week after CUMS and treatment schedule. All data are expressed as mean ± S.E.M. **p<0.001 versus control, #p<0.05, ##p<0.01 versus CUMS. One-way ANOVA followed by Tukey's post-hoc test was used for statistical analysis (n=10-12/group). CUMS; chronic unpredictable mild stres, IMI; imipramine, AGM; agmatine, iNOSi; iNOS inhibitor (aminoguanidine), nNOSi; nNOS inhibitor (3-Bromo-7-Nitroindazole)

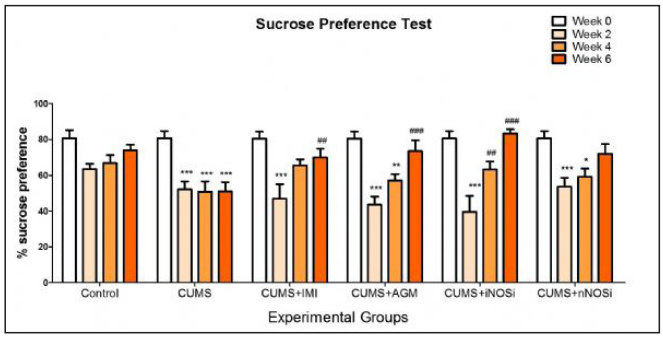

Figure 4. Sucrose Preference Test

Sucrose preference test was conducted in every two weeks throughout CUMS procedure. Treatments were started at the end of 3rd week.. All data are expressed as mean ± S.E.M. *p<0.05, **p<0.01, ***p<0.001 versus Week 0, #p<0.05, ##p<0.01, ###p<0.001 versus Week 2. Two-way ANOVA was used for statistical analysis (n=7-8/group). CUMS; chronic unpredictable mild stres, IMI; imipramine, AGM; agmatine, iNOSi; iNOS inhibitor (aminoguanidine), nNOSi; nNOS inhibitor (3-Bromo-7-Nitroindazole)

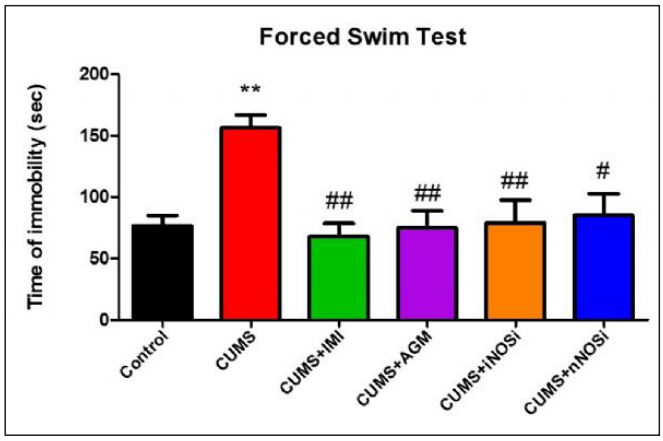

Figure 5. Forced Swim Test

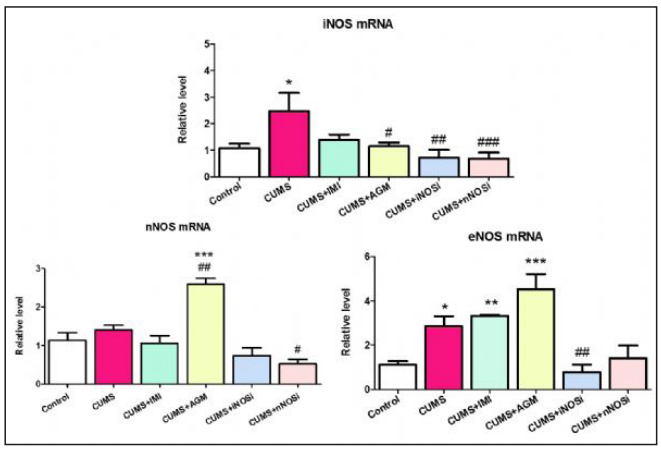

Figure 6. The effect of CUMS and treatments of relative mRNA levels of different NOS isoforms in PFC of rats

*p<0.05, ***p<0.001 versus control, #p<0.05, ##p<0.01, ###p<0.001 versus CUMS group. All data are expressed as mean ± S.E.M. and represented as fold change relative to control. One-way ANOVA followed by Tukey's post-hoc test was used for statistical analysis (n=7/group). CUMS; chronic unpredictable mild stres, IMI; imipramine, AGM; agmatine, iNOSi; iNOS inhibitor (aminoguanidine), nNOSi; nNOS inhibitor (3-Bromo-7-Nitroindazole)

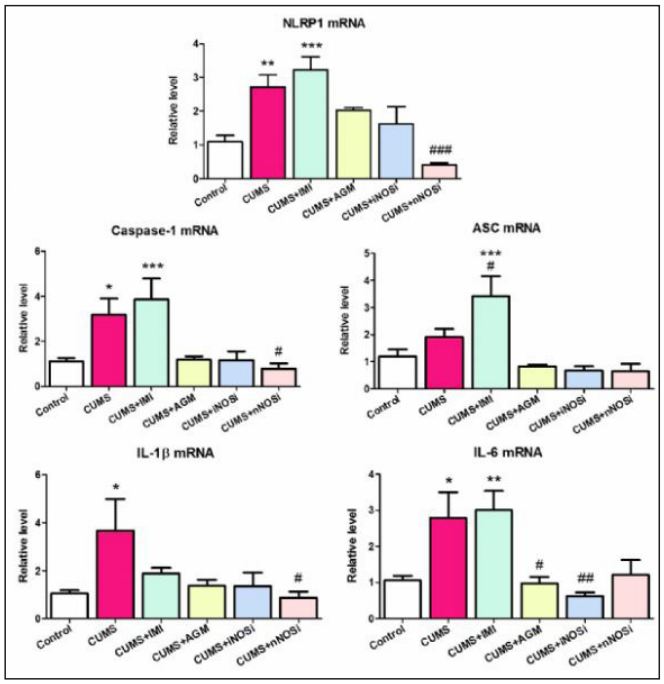

Figure 7. The effect of CUMS and treatments of relative mRNA levels of NLRP1 inflammasome components and inflammatory mediators in PFC of rats.

*p<0.05, **p<0.01, ***p<0.001 versus control, #p<0.05, ##p<0.01, ###p<0.001 versus CUMS group. All data are expressed as mean ± S.E.M. and represented as fold change relative to control. One-way ANOVA followed by Tukey's post-hoc test was used for statistical analysis (n=7/group). CUMS; chronic unpredictable mild stres, IMI; imipramine, AGM; agmatine, iNOSi; iNOS inhibitor (aminoguanidine), nNOSi; nNOS inhibitor (3-Bromo-7-Nitroindazole)

PP-093

A-CHLOROFATTY ACID INDUCES ENDOPLASMIC RETICULUM STRESS AND INFLAMMATION IN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

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Neurodegenerative disorders are chronic diseases with a significant inflammatory component, accompanied by alterations in brain lipid composition. In these diseases persistent inflammation coupled with oxidative stress contributes to blood-brain barrier (BBB) dysfunction. Under inflammatory conditions peripheral leukocytes can exacerbate neurovascular dysfunction by releasing cytotoxic mediators that induce BBB breakdown. One of these mediators is hypochlorous acid (HOCl), which is formed via the myeloperoxidase (MPO)/H2O2/chloride system of activated leukocytes. HOCL targets the vinyl ether bond of the cellular ether-phospholipid (plasmalogen) pool, resulting in generation of chlorinated fatty aldehydes (e.g. 2-chlorohexadecanal; 2-ClHDA) and the corresponding remnant lysophospholipid. 2-ClHDA can be metabolized to 2-Chlorohexadecanoic acid (2-ClHA) and 2-Chlorohexadecanol (2-ClHOH) by neutrophils and endothelial cells. 2-ClHDA has deleterious effects on brain microvascular endothelial cell (BMVEC) barrier function and induces mitochondrial dysfunction, ATP depletion, and apoptosis.

The present study aimed to elucidate the role of 2-ClHA unfolded protein response (UPR) activation and concomitant cell death in BMVEC. UPR is a homeostatic mechanism to re-establish endoplasmic reticulum (ER) function. It is stimulated by a variety of pathophysiological conditions, such as Alzheimer's and Parkinson`s.

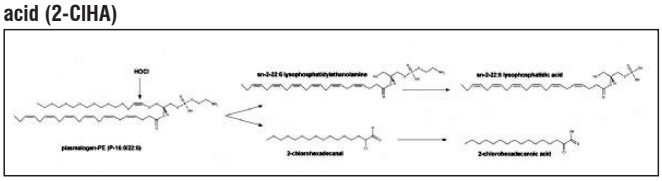
The cytotoxic effect of 2-ClHA was examined by Annexin V-PI double staining and flow cytometry. Results showed that 2-ClHA incubation resulted in a significant increase of early and late apoptotic cells. To clarify if 2-ClHA is a potent activator of UPR-mediated signalling, the expression of proteins involved in ER stress mediated signalling cascades were analysed. Stimulation of hCMEC/D3 cells with 2-ClHA resulted in induction of eIF2α phosphorylation and raised expression of ATF4 and CHOP, two transcription factors involved in PERK mediated signalling. In parallel a modulator and major chaperon GRP78/BiP was upregulated. Taken together, ER stress related protein expression is significantly upregulated in response to 2-ClHA stimulation.

Moreover, 2-ClHA mediated UPR activation is accompanied by an increase in the expression and release of inflammatory cytokines (Il-6 and IL-8). The ER stress related receptor PERK mediates the expression of transcription factors that are capable of transactivating anti-oxidative stress response genes, including heme oxygenase-1. qRT-PCR analyses showed that 2-ClHA elevated the expression of Nrf-2, EGR-1 and HO-1 significantly, suggesting a crucial role of oxidative stress in protein-misfolding-related cellular dysfunction.

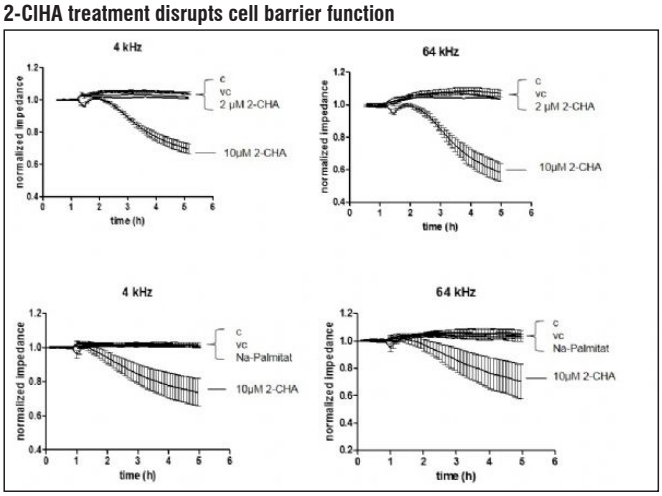
In conclusion, the current data provide evidence that α-chloro fatty acids which are generated under inflammatory conditions in vivo are able to induce BBB dysfunction via induction of ER stress by the PERK-mediated signaling cascade and trigger the realease of inflammtory cytokines.

Keywords: Blood brain barrier, inflammation, ER stress, oxidative stress

2-Chlorohexadecanal (2-ClHDA) can be metabolised to 2-Chlorohexadecoanoic acid (2-ClHA)

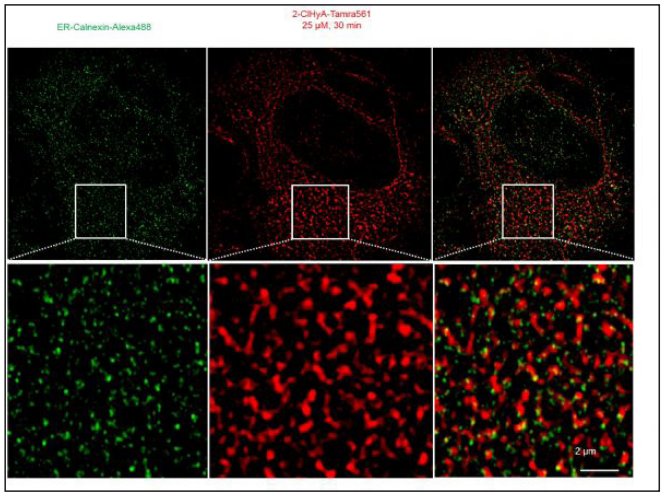


2-ClHA treatment disrupts cell barrier function



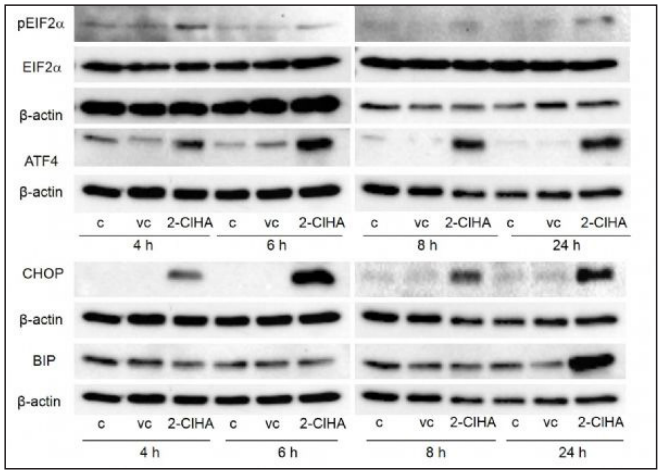
To study the impact of 2-ClHA on cell barrier function, hCMEC/D3 cells were stimulated with various concentrations of 2-ClHA or Na-palmitate as indicated and impedance was monitored over time using the ECIS system. The live time measurements revealed that 2-ClHA induced barrier dysfunction (4 kHz) and breakdown of the cell monolayer integrity (64 kHz). Importantly, the structural analogue of 2-ClHA, Na-palmitate showed no effect on cell monolayer function. Na-Palmitat is the sodium salt of Palmitic acid, a saturated long-chain fatty acid with a 16-carbon backbone. In contrast 2-ClHA is a long-chain fatty acid but posses a chlorine group (Figure 1).

2-ClHyA localizes to the ER in hCMEC/D3 cells



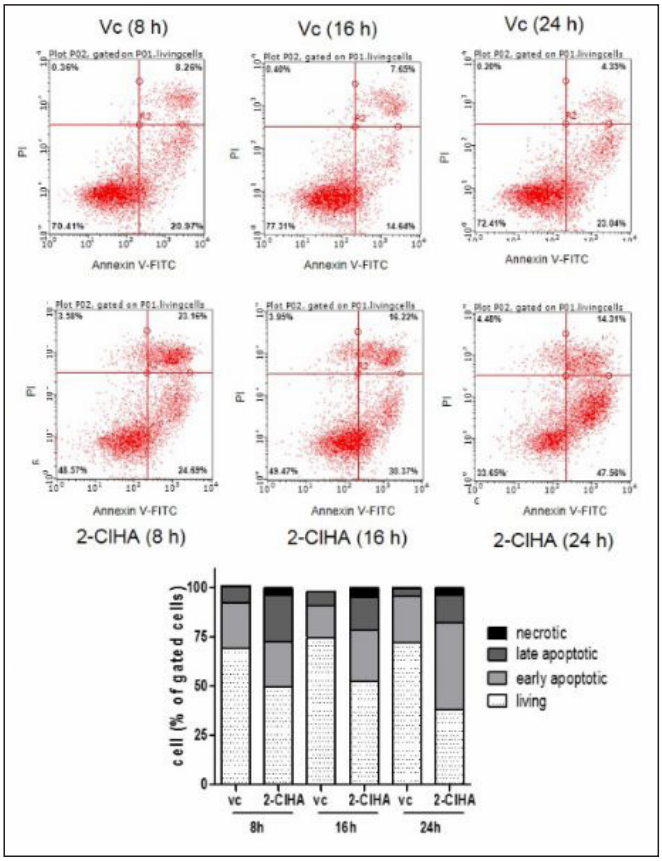
Cells were seeded onto 4-well plastic chamber slides and cultured until confluence. After serum starvation, cells were stimulated with 25 μM 2-ClHyA for 30 min followed by subsequent incubation with NaCNBH3 for reducing Schiff base formation. Cells were fixed in methanol, followed by permebilization with Trtion-X100 and subsequently subjected to click chemistry with N3-Tamra (red) and incubated with an Alexa488 conjugated antibody against the ER marker calnexin.

2-ClHA induced the expression of proteins involved in UPR mediated signalling cascade



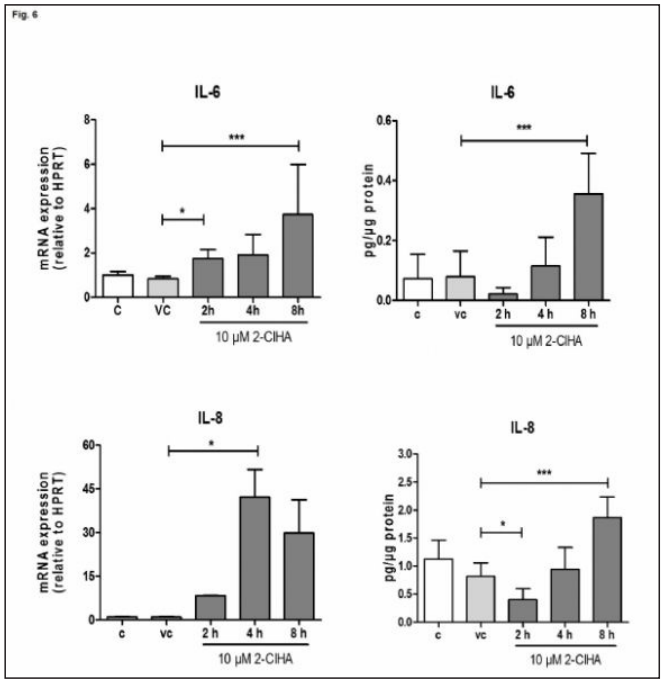
HCMEC/D3 cells were plated onto collagen type I coated 6-well culture plastic plates and treated with 10 μM 2-ClHA for indicated time periods. DMSO (0.2 % (v/v) per well) served as a vehicle control (vc). Cellular Western blot analysis was performed. Pan- or phospho-specific monoclonal and polyclonal antibodies against pEIF2-α, ATF4, CHOP and BiP were used as primary antibodies. β-Actin expression was used as a loading control.

2-ClHA mediated induction of apoptosis in hCMEC/D3 cells.



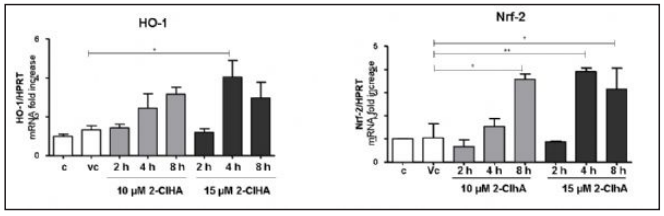
HCMEC/D3 cells were stimulated with DMSO (0.2 %(v/v)) or 10 μM2-ClHA for the indicated time periods. Cells were trypsinized followed by Annexin V FITC and propidium iodide (PI) double staining and subsequent flow cytometry analysis using a Guava easyCyte 8 Millipore flow cytometer.

2-CIHA induces expression of pro-inflammatory cytokines

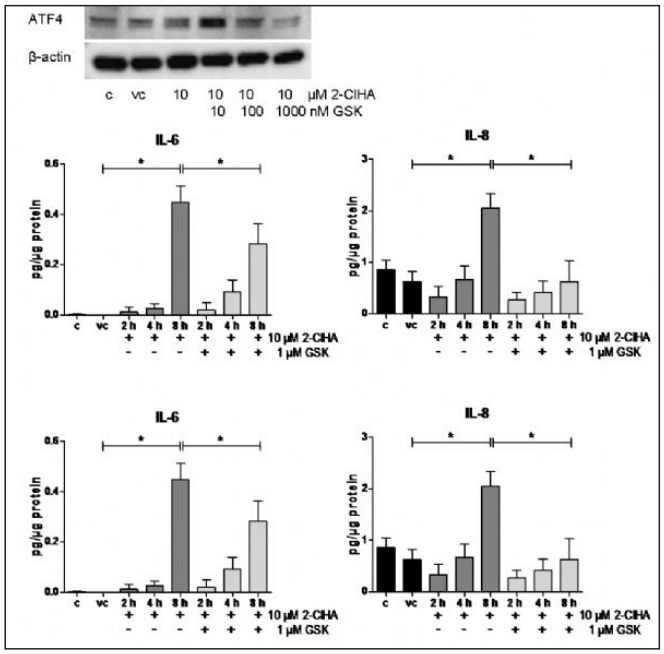


hCMEC/D3 cells (starved of serum overnight) were stimulated with 10 μM 2-CIHA for indicated time periods. Gene expression profiles were quantified by qPCR analysis and normalized to HPRT housekeeping gene. Data are shown as mean + SD from 3 independent experiments, each performed in triplicate. Expression profiles were calculated using the 2-ddCt method. hCMEC/D3 cells were seeded onto 24-well plates and serum starved overnight prior to the experiment. After incubation with vehicle (0.2 % (v/v) DMSO) or 10 μM 2-CIHA for indicated time periods, cell supernatants were collected. IL-6 and IL-8 concentrations were quantified using ELISA. Results shown represent mean + SD from three independent experiments performed in triplicate. (*p<0.05; **p<0.01; ***p<0.001; one-way ANOVA with Bonferroni correction).

2-CIHA treatment elevates the mRNA expression of anti oxidative response proteins

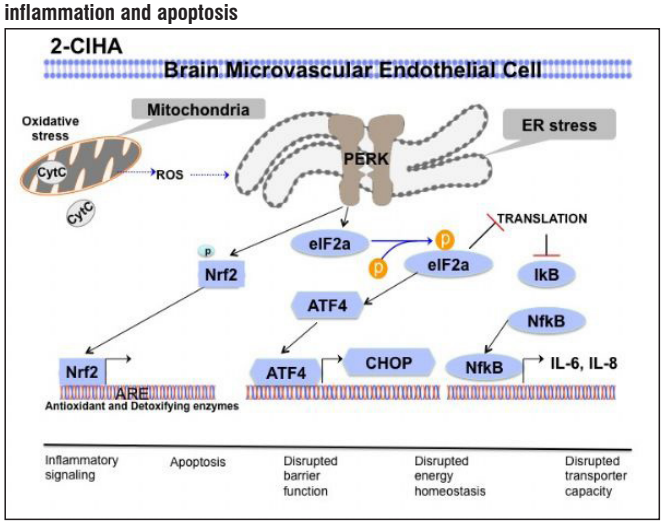


PERK inhibition resulted in reduced inflammatory cytokine release



Serum starved hCMEC/D3 cells were stimulated in presence of vehicle (DMSO, (0.2 % (v/v)), 10 μM 2-CIHA or 2-CIHA (10 μm) plus GSK2606414 for the indicated time periods and concentrations. Expression levels of ER stress marker ATF4 was examined by Western Blot analysis. Overnight serum starved hMCEC/D3 cells were cultivated with 2-CIHA with or without 1 μM GSK2606414 for indicated time periods. DMSO (0.2 % (v/v)) served as vehicle control. IL-6 and IL-8 mRNA levels were determined by qPCR and mRNA levels were normalized to HPRT housekeeping gene. Values are expressed as mean + SD from three independent experiments performed in triplicate. Expression profiles were calculated using 2-ddCt method. (D) HCMEC/D3 cells were seeded onto rat collagen type-1 coated 24-well cell culture plates. Cells were stimulated with 10 μM 2-CIHA in presence and absence of 1 μM GSK2606414 for indicated time periods. Supernatants were collected and IL-6 and IL-8 concentrations were determined using ELISA. Results shown are presented as mean+SD from three independent experiments performed in triplicate. (*p<0.01; one-way ANOVA with Bonferroni correction).

2-CIHA initiates UPR activation via the ER stress receptor PERK and mediates inflammation and apoptosis



PP-094

MULTI-TRACER BRAIN IMAGING DETECTS REGIONAL MICROGLIAL OVER-ACTIVATION IN A MOUSE MODEL OF SYSTEMIC INFLAMMATION

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Background: Peripheral systemic inflammation could exert diverse changes in the brain, some of which can be detected with in vivo imaging. However, the early effects of systemic inflammation on brain perfusion, metabolism and neuroinflammation are currently unclear.

Methods: Systemic inflammation was induced by intravenous lipopolysaccharide. 99mTc- hexamethylpropyleneamine-oxime was used for cerebral perfusion imaging. Brain tissue tracer uptake data were further corrected with the thus obtained brain perfusion data. Neuroinflammation dynamics were imaged with 125I-iomazenil. Changes in glucose metabolism were described by PET/ MRI imaging using 18F-fluorodeoxyglucose. Results of the in vivo studies were correlated to ex vivo brain glutathione levels and immunohistochemistry (CD45 and P2Y12) studies.

Results: In the lipopolysaccharide-treated group significantly ($p<0.05$) reduced perfusion values (in all examined brain areas) and significantly ($p<0.001$) enhanced fluorodeoxyglucose uptake values were registered. When compensated for perfusion, a significantly ($p<0.05$) enhanced iomazenil uptake was registered in the cerebellum and hippocampus while relevant but not significant difference could be seen in iomazenil uptake in the whole hemispheres and the neocortex. The correlation studies showed highly positive correlation between the uptakes of FDG and iomazenil in each brain region while highly negative correlation was found between FDG and HMPAO-derived perfusion and between the uptake levels of iomazenil and HMPAO within the examined brain areas in the LPS-treated group.

No significant differences were detected in the measured glutathione and glutathione disulfide levels by the lipopolysaccharide treated and control groups. The CD45 and P2Y12 double labeling immunohistochemistry validated the results of in vivo imaging. Both the percentage of activated/all microglia and the number of activated microglia/area were significantly ($p<0.01$) higher in the lipopolysaccharide-treated group compared to the control group in all brain regions.

Conclusion: Benzodiazepine receptor over-expression and increased glucose intracellular transport signalled early neuroinflammatory changes in the hippocampus and the cerebellum. The activation of microglia decoupled from the blood perfusion of these regions in the early phase of systemic inflammation.

Keywords: systemic infection, neuroinflammation, LPS, SPECT, PET/MRI

PP-095

ACTIVATING TRANSCRIPTION FACTOR-3 REGULATES INFLAMMATORY CYTOKINES AGAINST LUNG INFECTION BY ACTIVATING THE MAPK-JNK PATHWAY

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School of Pharmacy Sungkyunkwan University Suwon Republic of Korea

Signal-dependent transcription factors bind to primed active enhancers. In pneumonia infection models, activating transcription factor-3(ATF3) was expressed highly in various cell lines in vitro and many organs in vivo. ATF3 also plays an important role in regulation of innate immunity and hypoxia condition. Gram positive bacteria including Streptococcus pneumoniae, Listeria monocytogenes induced pro-inflammatory cytokine using ATF3 via Toll-like receptor 4 (TLR4) and jnk, p38 and ERK signaling. ATF3-mediated cytokine induction protected the host from gram positive bacteria. In the pneumonia infection model, the wild-type mice were more resistant than the ATF3 knock-out (KO) mice. Taken together, we can conclude that ATF3 is involved in regulating cytokine expression to prevent gram positive bacterial infection.

Keywords: ATF3, Streptococcus pneumoniae, Listeria monocytogenes MAPK-jnk pathway

PP-096

EVIDENCE FOR GENDER-DEPENDENT IN EXPERIMENTAL PNEUMOCOCCAL MENINGITIS

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Estrogen, via estrogen receptor beta (ERβ), promotes beneficial effects on metabolism and immune system by enzymatic activity and hormonal anti-inflammatory in the central nervous system. Neurodegenerative processes in several CNS disorders including stroke, Alzheimer's and Parkinson's Disease are associated with activation of microglia. Here, we have shown that ERβ can function as a neuroprotective agent and it revealed increased survival of bacterial meningitis. Furthermore, our studies were designed to test the ERβ agonist promotes anti-inflammatory effects in microglia, specifically via up-regulation Levels of brain-derived neurotrophic factor (BDNF). Subsequently, ERβ agonist decreased exaggerated expression of pro inflammatory mediators in response to streptococcus pneumoniae infection including TNFα and IL-1β. Our results indicate that ERβ agonist decrease bacterial meningitis has not previously been reported for gender dependent in mice model. These findings may explain the hormonal effect of fetal estradiol on the bacterial meningitis to promote neuronal recovery.

Keywords: pneumococcal meningitis, Estrogen, gender-dependent

PP-097

EFFECTS OF GYY-4137 ON K/BXN SERUM TRANSFER INDUCED ARTHRITIC TRPA1 WT AND KO MICE

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Decades of intensive research into the biological aspects of hydrogen sulfide (H2S) have led to the revelation that – beyond its toxic properties – H2S is a gasotransmitter influencing a wide range of physiological functions and pathological conditions. The mitochondrial effects of H2S were among the first to be discovered, followed by it's influence on the nervous, cardiovascular, and immune systems. Prior experiments have demonstrated the anti-inflammatory and antinociceptive attributes of capsaicin-sensitive sensory neurons and also revealed their "triple function" – sensory afferent, local efferent and systemic efferent". A subpopulation of these neurons also expresses a non-selective cation channel: the transient receptor potential ankyrin 1 (TRPA1). It is known that H2S activates the TRPA1 channel. Therefore, we aimed to examine the effects of slow-releasing H2S donor GYY4137 (GYY) on the K/BxN serum transfer arthritis in TRPA1 wild type (WT) and knockout (KO) mice.

Experiments were carried out on four-month-old male TRPA1 WT and KO mice. Immune arthritis was induced by a single i.p. administration of 300 μL K/BxN serum. Animals received a daily i.p. dose of 50 mg/kg GYY and were treated for the entire duration of the experiment. Body weight, semi quantitative clinical score and results of grip test were registered daily. Mechanical threshold of nociception and swelling of the hind paws were measured by dynamic plantar aesthesiometer and plethysmometer on days 0, 2, 4, 6, 8, 10, 12 and 14. Myeloperoxidase (MPO) activity and extravasation was determined on days 0, 2 and 6 by in vivo fluorescent and luminescent imaging. The control group received non-immunogenic BxN serum and vehicle of GYY.

Mice genetically lacking TRPA1 receptors developed more severe mechanical hyperalgesia, had deteriorated grip strength, larger clinical arthritis scores, more expressed plasma extravasation and MPO activity in the inflamed limb in response to K/BxN arthritis. Alternatively, GYY ameliorated hyperalgesia and arthritis score in animals expressing TRPA1. GYY treatment had no significant effect on the BxN mice.

Conflicting data on the effect of H2S in inflammation and nociception have been published since the advent of sulfide research. According to our novel results protective actions of the gasotransmitter are associated with TRPA1 receptor activation whilst detrimental effects are mediated by other mechanisms in murine arthritis. This investigation provides hints on the importance of TRPA1 channels for the development and application of sulfide therapeutics.

Keywords: GYY-4137, sulfide, TRPA1, KBxN, inflammation

PP-182

PRO-RESOLVING AGONISTS OF FPR2 CAN RESTRAIN MICROGLIAL ACTIVITY, CONTROLLING INFLAMMATION AND OXIDATIVE STRESS

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Introduction: Inflammation and oxidative stress are both central contributors to a variety of neurodegenerative diseases [1,2]. Microglia are the resident immune cells in the brain and spinal cord, providing the first line of immunological defence. However, in disease, these cells become chronically activated, contributing to extensive neuronal damage [3]. Dampening this activation and promoting the restoration of a normal environment may provide neurons with critical protection against chronic inflammatory and oxidative damage. The aim of this study was to determine whether lipopolysaccharide (LPS)-induced inflammation and reactive oxygen species (ROS) release could be inhibited through the activation of the receptor Fpr2, a key promoter of inflammatory resolution in the periphery [4].

Methods: Immortalised murine microglia (BV2 cells) were stimulated with LPS (50ng/ml) for 1h prior to treatment with either of two Fpr2 ligands, Cpd43 or Quin-C1 (both 100nM). Cytokine (TNFα and IL-10) and nitric oxide (NO) production was monitored at 24 and 48h using commercial ELISAs and the Griess reagent, respectively. ROS production was detected over time with carboxy-H2DCFDA. LPS was administered for 30 minutes prior to Cpd43 or Quin-C1, with ROS production recorded every 5 minutes for up to 1h.

Results: Treatment with both Cpd43 and Quin-C1 significantly reduced LPS-induced production of TNFα and NO at 24 and 48h. Aligning with previous data investigating Fpr2 signaling [5], SB203580 (2μM) or FR180204 (10μM), p38 MAP kinase and ERK1/2 inhibitors respectively, was added 10 minutes prior to either Fpr2 agonist and LPS-induced NO release was assayed at 24h post-stimulation. NO suppression of both agonists was blocked through the addition of SB203580, but not FR180204. Further, both agonists enhanced production of IL-10 48h post-LPS exposure. Strikingly, both Cpd43 and Quin-C1 completely abolished LPS-induced ROS, reversing production to baseline levels.

Conclusions: Together, these data suggest that Fpr2 may be a viable therapeutic target for the control of neuroinflammation, increasingly recognized as a major contributor to the pathologies of numerous neurodegenerative disorders.

Keywords: Inflammation, Oxidative Stress, Microglia, Fpr2

PP-186

EFFECT OF ACUPUNCTURE AT NEUROGENIC INFLAMMATORY SPOTS ON CAPSAICIN-INDUCED PNEUMONIA IN RATS

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Visceral disorders frequently produce a referred pain at topographically distinct body surfaces due to the convergence of visceral and somatic afferents on the same neuron in the sensory

pathway. In multiple sites of skin overlying the referred pain, local tissue responses, known as neurogenic inflammation (neurogenic spots), are observed and can be visualized experimentally in the skin by systemic injection of Evans blue dye. Our previous study proposed that neurogenic spots resemble the physiological features of acupoints.

To investigate whether (1) neurogenic spots were found in the anatomical location of traditional acupoints in acute pneumonia, and (2) stimulation of neurogenic spot could alleviate the symptoms of acute pneumonia, in an acute pneumonia rat model induced by an intratracheal injection of capsaicin, we compared anatomical locations of Evans blue dots (EBD) with classical acupoints, and examined the effect of acupuncture at EBD on breathing patterns and capacity. Capsaicin injection significantly increased Penh values expressed as [Penh= (Te/RT-1)*(PEF/PIF)] during first 10 min after capsaicin injection, compared to those of pretreatment or sham group. The blue spots, ranging in diameter from 0.5 to 3 mm, started to appear approximately 5-10 min after intravenous injection of Evans blue dye (50 mg/kg) in acute pneumonia rats, while very few spots were observed in control rats.

The majority of blue dots were observed over skin such as LU11 and LU9. Electroacupuncture (0.5mA, 2Hz, 10min) at the blue dots normalized the increased Penh values by an intra-tracheal capsaicin, compared to control groups. It suggests that the majority of neurogenic spots in acute pneumonia model coincides with the location of acupoints commonly used in acupuncture medicine for treatment of pneumonia and acupuncture-like stimulation of the dots can alleviate the symptoms of pneumonia.

Other

PP-098

PROTECTIVE ROLE OF P-COUMARIC ACID IN DIABETES-INDUCED PERIODONTITIS AND ALVEOLAR BONE LOSS IN MICE

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Institute of Oral Biosciences and School of Dentistry, Chonbuk National University, Cluster for Craniofacial Development and Regeneration Research

Introduction: Hyperglycemia, the main features of diabetes mellitus is related with heterogeneous group of complications. Recently, it was depicted that chronic hyperglycemia heighten the severity of periodontal diseases by an exacerbated inflammatory response, triggered by elevated glycation end products. Therefore, anti-inflammatory agents play a vital role as potential inhibitors of these pathological complications. In particular, p-coumaric acid (4-hydroxycinnamic acid) has been shown to possess anti-inflammatory and anti-oxidants properties mediated by its polyphenol content.

Objectives: This study investigated the mechanisms by which p-coumaric (p-CA) acid weaken the spontaneous onset of diabetes-induced periodontitis and bone loss in mice.

Methods: Diabetes was induced in mice via a single intraperitoneal injection of streptozotocin. Mice were divided into three groups- sham, diabetic mice treated with DMSO and diabetic mice treated with p-coumaric acid (50 mg/kg). All mice were analyzed at 40 days after diabetes induction. The pattern of bone destruction was evaluated by histology and micro-CT analysis. Further, immunohistochemistry (IHC) and tartrate-resistant acid phosphatase (TRAP) staining was performed to evaluate inflammatory molecules and bone resorption activity. Morphometric measurements of the distance from the cemento-enamel junction (CEJ) of the maxillary root of the first and second molar to the alveolar bone crest (ABC) were performed to assess bone loss.

Results: The results from micro-CT revealed that diabetics group led to greater alveolar bone resorption with extensive surface erosion at the interproximal space between the maxillary molars, compared with the sham group. This was inhibited by treatment with p-CA. Histological analysis supported that p-CA treatment protects against periodontitic damages. The diabetics group also showed greater distance between the cemento-enamel junction (CEJ) and the apex of alveolar bone (AB), compared with the sham group. P-CA treatment significantly ($p < 0.05$) inhibited the periodontal destruction showing a CEJ/AB distance smaller to that of the diabetics group. Furthermore, the diabetics group showed greater positive TRAP staining, compared to the others. In addition, IHC staining indicated that the induction of TNF-α, COX-2 and RANKL proteins was higher in the diabetics group than the sham or p-CA group.

Conclusion: Although, additional experiments are required, we suggested that p-CA could be an effective bioactive molecules to treat bone destruction caused by persistent inflammation and excessive osteoclast formation generated by diabetes.

Acknowledgements: A grant from the national research foundation of Korea (NRF-2014R1A1A2008488) supported a part of this study.

Keywords: Diabetes, p-coumaric acid, periodontitis, inflammation, osteoclastogenesis, bone loss

PP-099

BAX INHIBITOR-1 INHIBITS ACETAMINOPHEN-INDUCED HEPATOTOXICITY BY REDUCING OXIDATIVE ER STRESS THROUGH REGULATING THE RIDD ACTIVITY OF IRE1A

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Acetaminophen (APAP) overdose is the most frequent cause of acute liver failure in young adults and is primarily caused by CYP2E1 driven conversion of APAP into NAPQI, a hepatotoxic metabolite. This will lead to the ER stress, activation of UPR and the pro apoptotic events, due to the reduced glutathione level and perturbation in the redox balance. Bax inhibitor-1 (BI-1) is an evolutionarily conserved ER-membrane resident protein that suppresses cell death by regulating ER stress response. In this study we examined the role of BI-1 in APAP induced ER stress and in regulation of IRE1alpha, an endoribonuclease UPR molecule known to degrade the mRNA through the process called RIDD. Our results showed that APAP induced ER stress was reduced in BI-1 over expressing cells. BI-1 knockout mice showed massive hepatic toxicity and large number of cytoplasmic vacuoles as revealed by H&E staining. Further it increased ALT and AST levels, protein oxidation and lipid peroxidation. We observed reduction of CYP2E1, a RIDD substrate, expression in BI-1 overexpressing cells. To examine the possible relation of BI-1 in CYP2E1 lower expression in the ER stress, we hypothesized that BI-1 may regulate the IRE1 response. As, it is known that XBP1s requires oligomer sate of activated IRE1a and this

XBP1s is reduced in BI-1 expressing cells but at the same time phosphorylation of IRE1 was also observed. So, It indicates that in BI-1 overexpressing cells IRE1alpha is activated but held in dimer state for extended time compare to PC cells. In consequence, the dimer state of activated IRE1a has the RIDD activity and helps in initial adaptive stress response by reducing the further load of protein synthesis during ER stress. As a consequence CYP2E1 mRNA degraded and resultantly lesser conversion of APAP to toxic metabolite. So, our results suggest a role for BI-1 in the regulation of RIDD activity in early adaptive responses against APAP induced ER stress.

Keywords: Acetaminophen, Bax inhibitor-1, Oxidative ER stress, RIDD, IRE1α

PP-100

PROTECTIVE EFFECTS OF DUNALIELLA AGAINST TO SUPPRESSION OF CO-ADMINISTRATION OF ETHANOL AND DEHP ON GLUTATHIONE PEROXIDASE ACTIVITY AND TOTAL ANTIOXIDATIVE CAPACITY IN HEPG2 CELLS

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Phthalic acid esters are commonly used as plasticizers to impart flexibility to plastics, particularly poly(vinyl chloride) (PVC) polymers, which have a wide variety of biomedical and other uses. Di(2-ethylhexyl) phthalate (DEHP) is known to leach out from finished PVC products into blood, physiological fluids, commercial solvents and food materials. Acute and chronic administrations of DEHP cause variable effects on liver, depending upon the species of the animal. In general, after administration of phthalates caused enlargement of the liver, which declined to or below normal weight on prolonged or discontinued exposure. An increase in hepatic cytochrome P-450 content, alcohol dehydrogenase activity and persistent inhibition of aniline hydroxylase activity in livers of rats receiving DEHP for prolonged periods indicates its interaction with biotransformation mechanism. However, it is less known whether ethanol influences the effects of DEHP on antioxidative capacity of the liver. Dunaliella has been shown to protect the liver from CCl4-induced damage. Human HepG2 hepatocytes were used to study effects of DEHP on cell viability, glutathione peroxidase (GPx), total antioxidative capacity (TAC) when co-administration with ethanol. The protective effects of Dunaliella extract were also assessed in these studies. Results showed that DEHP alone did not induce acute cytotoxicity, whereas co-administration of ethanol (between 25 and 100 mM) with DEHP caused significant cytotoxicity. Ethanol also enhanced DEHP-suppressed GPx activity and TAC in HepG2 cells. The inhibition of GPx activity and TAC by co-administration of ethanol and DEHP was reversed by extract of Dunaliella in a dose-dependent trend.

Keywords: Di(2-ethylhexyl) phthalate (DEHP), HepG2 cells, cytotoxicity, Dunaliella

PP-101

INHIBITORY EFFECTS OF CORDYCEPS EXTRACT ON UV-INDUCED MMP-1 EXPRESSION AND DEGRADATION OF COLLAGEN

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Solar UV radiation (UVA and UVB) damages human skin, affecting skin tone and resiliency and leading to premature ageing (photoaging), which is thought to be due to increases in matrix metalloproteinases (MMPs) expression and collagen degradation. MMPs are crucial factors involved in the connective tissue remodeling accompanying UV irradiation-induced skin damage. Collagen is one of the major components of extracellular matrix, which is responsible for skin tone. The production and fibrillar organization of collagen in skin was reduced by UV irradiation. The Cordyceps mushroom is one of the best anti-aging materials in Traditional Chinese Medicine. Cordyceps aqueous extract has been reported to exert inhibitory effects on macrophages based anti-inflammatory property. Although preventive effects of Cordyceps aqueous extract on UVB-induced MMP-1 expression has been shown, its effects on UVA-induced MMP-1 and UV-induced degradation of collagen have not been studied before.

Skin fibroblasts Hs68 were exposed to UVB and UVA, MMP1 expressions and activities were measured by Western blotting and ELISA kits 24h later. Pro-collagen mRNA expression (RT-PCR) and Type I collagen Western blotting) were also studied.

Doses between 0.1 and 10 μg/ml of Cordyceps aqueous extract did not produce any cytotoxicity on skin fibroblasts (Hs68). Cordyceps aqueous extract (between 0.25 to 1 μμg/ml) significantly prevented UVB (25 mJ/cm2) and UVA (2.5 J/cm2)-induced MMP-1 expressions and activities. The same treatment of UVB and UVA suppressed collagen mRNA and protein expressions. These reductions were also prevented by the treatment of Cordyceps aqueous extract at dose 0.1 and 1 μg/ml.

Cordyceps aqueous extract is commonly known as an anti-aging material, it also reveals skin anti-aging effects. Thus, this material can be used as an active ingredient in cosmetic products.

Keywords: Skin fibroblasts, collagen, MMP-1

PP-102

APPROACHES TO THE REGULATION OF CHRONIC LOW-INTENSITY INFLAMMATION THAT FOLLOW METABOLIC DISORDERS

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Medical Academy of V.I.Vernadsky Crimea Federal University

Pro-inflammatory factors are regarded as key triggers in the pathogenesis of metabolic syndrome (MS) and diabetes mellitus type 2(DM2). Nowadays their role is connected withantiendotoxin mechanisms following the increase of chronic low-intensity inflammation's markers, proteolytic enzymes' activity,free radical oxidation (FROX) and misbalance in antioxidative oxidative system (AOS-HEOX). In those disturbances' correction, natural compounds with antioxidant activity, particularly polyphenols,

could be widely adopted. That is why the study of grape processing products (GPP) application in correction of oxidative stress andchronic low-intensity inflammation in MS and DM2 is considered a topical problem.

The experimental study was performed on 54 white male rats of the weight of 180-200gr with the fructose-induced model of MS during 8 weeks. Clinical study was carried on 259 patients, suffered from DM2 complicated ischemic heart disease and arterial hypertension. The correction both in the experimental study and in clinic (in addition to the basic therapy) was performed with GPP in the dosage of 10mg of total polyphenols per kilo of body weight. The influences of GPP on the concentration of inflammatory markers (C-reactive protein (CRP)), FROX and proteolytic activity in blood serum were studied. Additionally in clinic antiendotoxin immunity was investigated by the measurement of serum antibodies to enterobacterial lipopolysaccharides (anti-LPS-Ab) - anti-LPS-IgA, anti-LPS-IgM and anti-LPS-IgG.

In the MS modelling the obesity, more than 25% of abdominal fat tissue comparing control was resulted. That followed the reliable rise of nonspecific proteinases' activity and FROX, and drop of antioxidants proving by the increase of tiobarbituric acid active products (TBA-AP) of 50,4 % ($p<0,01$) in animals with MS in comparison with the control group. In the patients with DM2 FROX rise, breakdown of inhibitors abilities, sings of low-intensity inflammation and anti-LPS-Ab misbalance took place.

GPP application led to the restoration of AOS-HEOX proving by the reliable reduction of TBA-AP of 34% and restoration of ceruloplasmin; in clinical study patients with DM2 demonstrated the rise of antioxidant enzymes (superoxide dismutase activity, catalase-like activity) and the decrease of FROX.Besides that, CRP-the main marker of low-intensity inflammation-demonstrated steady decline under the exposure of GPP. The tendency to the stabilization of antiendotoxin immunity, reflected in the drop of anti-LPS-IgA, anti-LPS-IgM and anti-LPS-IgG was registered.

Thereby, application of GPP in MS and DM2 both in the experiment and in the clinical study promoted the reduction of pro-inflammatory changes in an organism, which allow recommendingGPP for the correction of metabolic disturbances, connected with pathogenesis of low-intensity inflammation.

Keywords: Metabolic syndrome, inflammation, antiendotoxin immunity, polyphenols

PP-103

INTESTINAL ANTI-INFLAMMATORY ACTIVITY OF MUSA SPP (AAA) INVOLVES INCRESEAD PRODUCTION OF SHORT-CHAIN FATTY ACIDS

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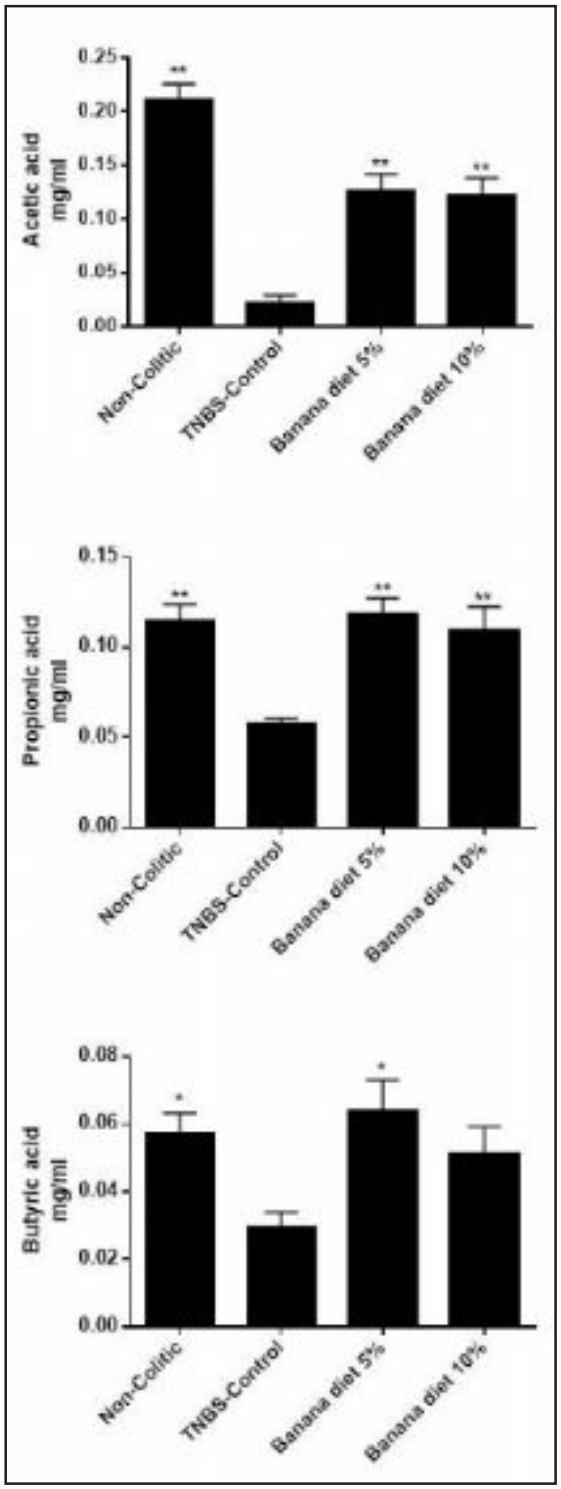
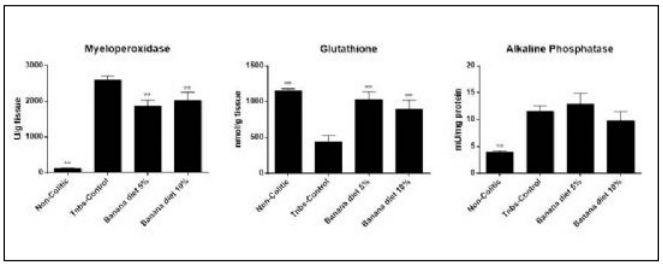
Inflammatory Bowel Disease (IBD) includes ulcerative colitis and Crohn's disease, chronic inflammatory disorders of the gastrointestinal tract. The limitation of the IBD patients due to the low response associated with several side effects has leading to search new sources of compounds. Based on the IBD aetiology, new treatment and prevention approaches have been studied, including intestinal microbiota modulators. Dietary products presenting prebiotic responses have been used to promote protective effects in inflammatory process. In fact, several prebiotics are source of short chain fatty acids (SCFAs), which have been associated with a lot of beneficial effects, including anti-inflammatory and immunomodulatory activities. Approximately 80-90% of the SCFAs produced by prebiotics are absorbed in the colon, while the remainder is excreted in faeces and used as biological markers. Green Dwarf Banana (Musa sp AAA) is a food rich in resistant starch, potentially useful as source of SCFAs by the fermentative process. In previous studies in our research laboratory, a dietary intervention with green dwarf banana flour showed intestinal anti-inflammatory activity in the TNBS model of intestinal inflammation. These results encourage us to study whether anti-inflammatory effects were related to prebiotic effects. For this, green dwarf banana flour was incorporated in a standard ration for rats at the proportion of 5 or 10%. Rats received dietary intervention for 35 days before intestinal inflammation induction by intracolonic instillation of TNBS. 48 hrs after, rats was killed for colon samples collection. Colonic segments were used to myeloperoxidase (MPO) activity, glutathione (GSH) content and morphological studies in haematoxylin-eosin and PAS-Alcian blue techniques. In addition, faeces were collected to SCFAs quantification by gas chromatography technique with mass spectrometry (GC/MS). Treatment of animals with banana flour diet 5 and 10% significantly reduced MPO activity and counteracted GSH depletion. Indeed, banana diet 10% reduced the extent of injury. In the histological analysis the diet with banana flour showed of mucosa with tubular glands with normal crypt, reduced oedema, villus preserved, besides, showed a higher proportion of mucus producing cell in relation to the TNBS control group. Dietary intervention with green dwarf banana flour increased 2-fold butyrate (at 5%), 3-fold propionate and 5-fold acetate when compared with TNBS-control group in both concentrations. Dietary intervention with green dwarf banana flour is an intestinal anti-inflammatory product acting by prebiotic effects, as evidenced by increased production of SCFAs. Antioxidant and mucosal healing effects also were observed. Since banana is an abundant fruit, low cost and easy to access, green dwarf banana flour could be used as a complementary product at the treatment of patients with inflammatory bowel disease.

Keywords: Inflammatory bowel disease, functional foods, banana, Musa, short-chain fatty acids

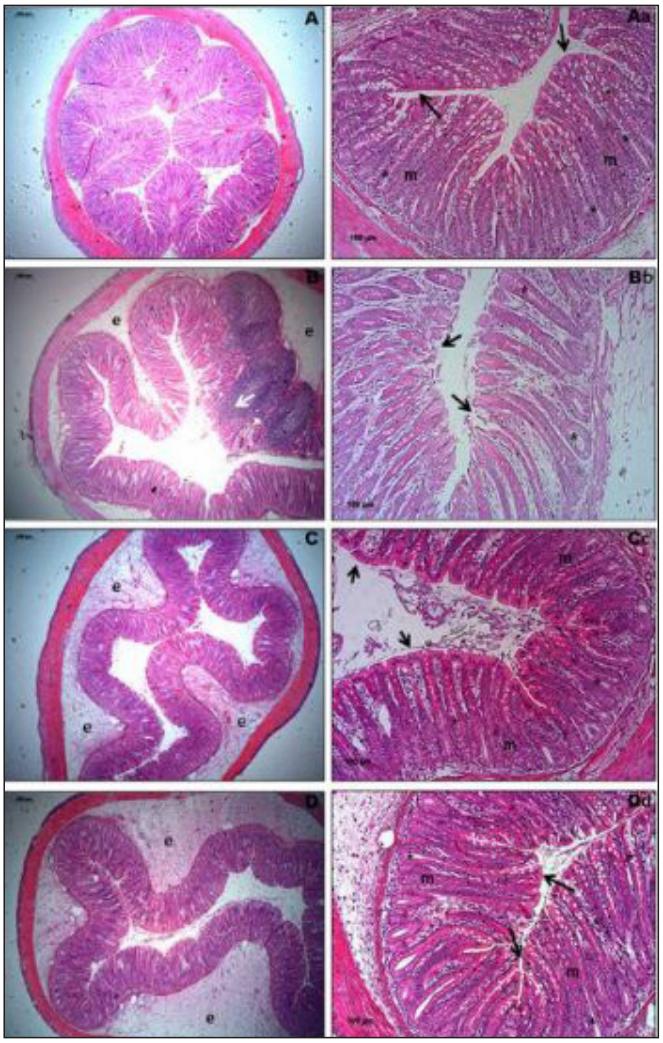


Concentration (mg/ml wet faeces) of the SCFAs in rat faecal samples. Values are expressed as the mean value ± S.E.M. * P<0.05 and **p<0.01.



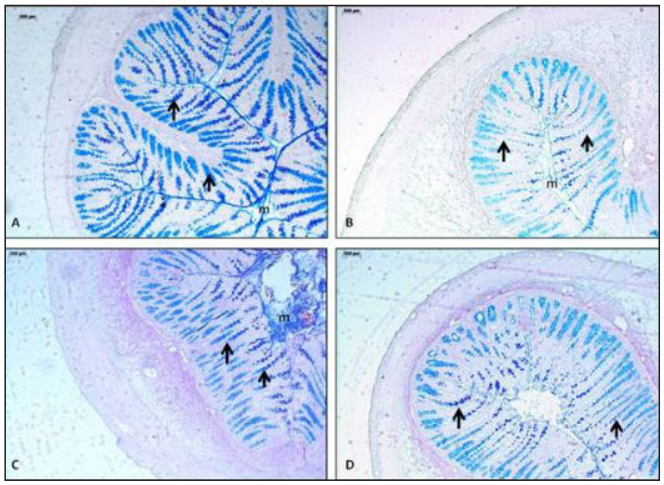
Effects of the Banana diet (5% or 10%) on glutathione (GSH) content, myeloperoxidase (MPO) and alkaline phosphatase (AP) activities in intestinal inflammation induced by trinitrobenzenosulfonic acid.

Photomicrographs of colonic mucosa stained with haematoxylin and eosin in health and colitic animals



(Aa) non-colitic; **(Bb)**TNBS-control; **(Cc)** Banana Diet 5%, **(Dd)** Banana Diet 10%. In A (non-colitic group): mucosa (m) contains numerous straight tubular glands with many lightly stained goblet cells; crypt (*) and luminal epithelium was intact with a typical morphology associated with normal villus *(black arrow)*. In B (TNBS-control group): tubular glands with normal crypt (*) were reduced with loss of membrane integrity *(black arrow)* and the goblet cells were atypical; a disruption *(white arrow)* with extensive oedema **(e)** and a large area of ulceration. In C (Banana 5% diet group): colon cytoarchitecture was recovering and included the restoration of mucosa (m) with straight tubular glands with normal crypt (*), which were similar to healthy animals; oedema **(e)** was reduced and villus was recovered (black arrow). D (Banana 10% diet group): Colon cytoarchitecture was recovering and included the restoration of mucosa (m) and tubular glands, lightly stained goblet cells and crypt (*) preserved.

Photomicrographs of colonic mucosa stained with PAS/Alcian Blue in health and colitic animals



(A) non-colitic; **(B)**TNBS-control; **(C)** Banana Diet 5%, **(D)** Banana Diet 10%. In non-colitic group **(A)** there is several mucous cells containing mixed acid and neutral mucins (stained purple) *(arrow)*, combined with a membrane-bound mucus layer. In the TNBS-control group notes a large reduction of mucus and the number of goblet cells *(arrow)*. In banana diet group both 5% and 10% **(C and D)**, there is a higher proportion of mucus producing cell in relation to the TNBS control group *(arrow)*, and a greater amount of mucus in the intestinal lumen from the banana flour 5% (m).

PP-104

LRRK2 G2019S MUTATION DISRUPT ER CALCIUM HOMEOSTASIS AND INCREASE ER STRESS-INDUCED CELL DEATH BY INHIBITING SERCA ACTIVITY

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Growing evidence from studies in Parkinson's disease (PD) indicates that endoplasmic reticulum (ER) stress is a common feature of the disease and several genes are responsible for ER dysfunction. However, little is known about the potential pathophysiological interplay between these two PD-related phenomena. Here, we defined the mechanism how astrocytes carrying a pathogenic mutation in PD-associated gene, G2019S LRRK2 (GS-Tg), accelerated ER stress and cell death, compared to non-Tg astrocytes. When cells were treated with α-synuclein, the expression of ER stress proteins and resulting cell death were increased in Gs-Tg. Intriguingly, we found that GS-Tg localizes to ER membrane, where it profoundly interacts with sarco-endoplasmic reticulum calcium adenosine triphosphatase (SERCA) and suppress SERCA activity by inhibiting displacement of SERCA inhibitor, phospholamban. Calcium imaging further revealed that elevated cytosolic calcium levels and decreased ER calcium load were observed in Gs-Tg astrocytes. In addition, treatment with inhibitors of LRRK2 kinase suppress the ER localization of Gs-Tg, restored SERCA activity and reduced severe ER stress and cell death. Collectively, Our data demonstrate that G2019S mutation of LRRK2 involve ER calcium homeostasis which determines cell survival, contributing to the development of PD pathogenesis.

Keywords: Parkinson's disease, LRRK2, ER stress, SERCA, Calcium homeostasis, Astrocytes

PP-105

OPTIMIZATION OF A CHRONIC COLITIS MODEL IN MICE INDUCED BY DEXTRAN SULPHATE SODIUM (DSS)

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Background: Ulcerative colitis is an inflammatory bowel disease that affects millions of people worldwide. Dextran Sulfate Sodium (DSS) model of colitis is widely used to explore disease mechanism and to evaluate potential new therapies. In the present study, a chronic version of the DSS-induced colitis model in mice has been optimized.

Methods: Mice received DSS in sterile drinking water and underwent washout with sterile water in six different protocols: 1A, B) three cycles of 7 days of DSS with 7 days of washout in between (1A) and further 7 days washout at the end (1B); 2A, B) three cycles of 5 days of DSS with 7 days of washout in between (2A) and further 7 days washout at the end (2B); 3A, B) three cycles of 4 days of DSS with 3 days of washout in between (3A) and further 3 days washout at the end (3B). Stool samples were evaluated daily for softness and presence of occult blood. Body weight was also taken daily. At necropsy, colons were collected for histopathological evaluation. Colon weight and length were also taken at necropsy. In-life data were summed to obtain a disease activity index (DAI) score. Whole colon sections were stained for semi-quantitative colitis scoring as well as for CD3+ cells. Effects of three different test agents (Prednisolone 1mg/kg, Sulfasalazine 200mg/kg and Cyclosporine A 40mg/kg, once daily oral gavage) were also evaluated using the protocol 2A.

Results: Mice from 7 days of DSS treatment groups (1A, B) showed poor health conditions. In terms of disease induction, groups 1A, B and 2A, B had higher DAI scores than groups 3A, B. Colon weight length ratios in these chronically treated groups were comparatively higher than the acute one cycle DSS treated animals. The mean ratio was highest in groups 1A, B and lowest in groups 3A, B. In microscopic semi-quantitative colitis evaluation, colitis scores were higher with higher DSS treatment days. Moreover, a decrease in colitis scores was observed in groups 1B and 2B that underwent additional washouts compared with groups 1A and 2A where there was no final washout phase. In contrast, colitis scores were higher in the group 3B compared with group 3A. Similar results were observed for CD3+ staining as well. Based on in-life and histology evaluation results, protocol 2A was chosen for examining effects of test agents. While sulfasalazine had no effect throughout the duration of the study, prednisolone further aggravated DAI scores during the last two weeks of the study. On the other hand, cyclosporine A attenuated DAI scores during the first two weeks of the study.

Conclusion: In conclusion, we have identified an optimal DSS duration and washout cycles protocol for induction of chronic colitis in mice. Moreover, we found that cyclosporine A can partially reverse chronic colitis in this model.

Keywords: Inflammatory bowel disease, dextran sulphate sodium, colitis, chronic, mice

[PP-106]

EFFECT OF KOREAN RED GINSENG EXTRACTS ON DRUG-DRUG INTERACTION

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Ginseng and its constituents has been the subject of many experimental and clinical researches to reveal the diverse biological properties. It is regarded as a traditional medicine employed as immunostimulatory, antithrombotic, anti-oxidative, anti-inflammatory and anti-cancer agent. As a common practice, ginseng may interact with concomitant medications, and alter metabolism and/or drug transport. Such an interaction in metabolism or transport can alter the known efficacy and safety of a drug. However, there is no clear documented evidence of its role when taken together with other medications till date. Therefore, we assessed the effect of Korean red ginseng (KRG) on enzymes responsible for drug metabolism (cytochrome p450 [CYP]), transporters (MDR and OAT), and disposition after administration of KRG to mice for 15 days. Results showed that 30 mg/Kg of KRG significantly increased the expression level of CYP3A11 protein in liver. 100 mg/Kg of KRG increased the expression of OAT1, both at mRNA level and protein levels in kidney. Similarly, KRG significantly increased expression of OAT1, OAT3, and MDR1 at both mRNA and protein levels in the liver. Also the substrate of MDR1 drug was significantly inhibited by KRG pretreatment in the MDR1 expressed cell. Taken together, KRG can mediate CYPs and drug transporters thus affecting drug disposition.

Keywords: Korean Red Ginseng (KRG), Drug-Drug interaction (DDI), Cytochrome P450 (CYP), Drug transporter, Pharmacokinetics

PP-107

THE ROLE OF NONSPECIFIC PROTEINASES IN THE DEVELOPMENT OF INFLAMMATION OF THE PARANASAL SINUSES

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Activation of nonspecific proteinases of granulocytes occurs during the development of leukocyte infiltration in inflammatory process. Moreover, the destruction of neutrophils or their exocytosis, leading to the accumulation of nonspecific proteinases, can cause damage to mucous membranes with formation of proteinase inhibitory imbalance. Therefore, measured parameters of nonspecific proteinases and their inhibitors can be effectively used as markers for development of the inflammatory process, including in the nasal cavity and paranasal sinuses.

The study assessed the nature and severity of the inflammatory process in patients with acute rhinosinusitis (RS) based on changes in nonspecific proteinases and their inhibitors in peripheral blood and synonasal secret. 93 patients were divided into 2 groups: the first had 45 patients with acute catarrhal RS, the second 48 patients with purulent RS. The control group included 12 healthy people - volunteers. Elastase-like activity, trypsin-like activity, antitriptic activity and acid-stable inhibitors were determined by enzymatic methods using synthetic substrates in serum and synonasal secret samples. Statistical analysis was performed using variational statistic methods.

Results of the study show that measured parameters for acute RS in the blood serum and the synonasal secret in the dynamics of changes are significantly different. The blood revealed a nonspecific reaction of the studied parameters, by increase in the activity of proteinases and their inhibitors, depending on the severity of the inflammatory process. In the synonasal secretion in catarrhal RS there was a moderate increase in proteolytic activity during increase in the level of inhibitors of proteinases. With purulent RS at the local level, there was a notable imbalance in the system, which is characterized by a marked increase in proteolytic activity against the background of a decrease in proteinase inhibitors. These results suggest that during the progression of the inflammatory process, the protective properties of the local antiproteinase system start to deplete, which can be associated with both excessive consumption of proteinase inhibitors in the inflammatory focus and with a decrease in the synthesis and secretion of local inhibitors due to mucosal damage.

Thus, determination of the activity of nonspecific proteinases and their inhibitors in the synonasal secretion of patients with acute rhinosinusitis reflects the nature of the acute inflammatory process and the risk of destructive changes in the nasal mucosa and paranasal sinuses. The formation of a notable local imbalance in the proteinase inhibitor system, manifests by an increase in the activity of nonspecific proteinases and a decrease in the activity of their inhibitors, creates conditions for the development of destructive disorders in the nasal mucosa and paranasal sinuses and formation of a tendency to chronic inflammation.

Keywords: proteinases, proteinase inhibitors, rhinosinusitis, inflammation

PP-108

ACCELERATED DEVELOPMENT OF AGING-ASSOCIATED AND INSTABILITY-INDUCED OSTEOARTHRITIS IN 12/15-LIPOXYGENASE DEFICIENT MICE

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Objective: 12/15-Lipoxygenase (12/15-LOX) catalyzes the generation of various anti-inflammatory lipid mediators, and has been implicated in several inflammatory and degenerative diseases. However, there is currently no evidence that 12/15-LOX has a role in osteoarthritis (OA). The aim of this study was to investigate the role of 12/15-LOX in the pathogenesis of OA.

Methods: The development of aging-associated and destabilization of the medial meniscus (DMM)-induced OA were compared in 12/15-LOX-deficient (12/15-LOX-/-) and wild-type (WT) mice. The extent of cartilage damage was evaluated by histology. The expression of OA markers was evaluated by immunohistochemistry and RT-PCR. Cartilage explants were stimulated with IL-1α in the absence or presence of the 12/15-LOX metabolites, 15-HETE, 13-HODE or LXA4, and the levels of MMP-13, NO and PGE2 were determined. The effect of LXA4 on the progression of OA was evaluated in WT mice.

Results: The expression of 12/15-LOX in cartilage increased during the progression of DMM-induced OA and with aging in WT mice. Cartilage degeneration was more severe in 12/15-LOX-/- mice compared to WT mice in both models of OA,

and this was associated with increased expression of MMP-13, ADAMTS5, iNOS, and mPGES-1. Treatment of cartilage explants with 12/15-LOX metabolites, suppressed IL-1α-induced production of MMP-13, NO and PGE2, with LXA4 being the most potent. Intra-peritoneal injection of LXA4 reduced the severity of DMM-induced cartilage degradation.

Conclusions: These data suggest an important role of 12/15-LOX in the pathogenesis of OA. They also suggest that activation of this pathway may provide a novel strategy for prevention and treatment of OA.

Keywords: 12/15-LOX, LXA4, Cartilage, Osteoarthritis

PP-109

A NEW INFLAMMATION RESOURCE: INTRODUCING THE IUPHAR GUIDE TO IMMUNOPHARMACOLOGY (GTOIMMUPDB)

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A 2016 global pharmaceutical R&D review lists the top-three general mechanisms of action as immuno-stimulant, anticancer immuno-therapy and immune-suppressant, covering 1706, 399 and 221 therapeutic agents, respectively[1]. The increasing dominance of these categories is reflected in growing academic and commercial research in the pharmacology of immunity, inflammation and infection (I-I-I). Data exchange between these three research communities is therefore critical to the development of new drugs. Our Wellcome Trust-funded project to produce the International Union of Basic and Clinical Pharmacology (IUPHAR) GtoImmuPdb addresses this need by providing a new portal/web-interface designed to provide a unique 'GtoImmuPdb view' of the data held within the existing IUPHAR/BPS Guide to PHARMACOLOGY database (GtoPdb)[2,3], highlighting content of immunological relevance (explicitly encompassing the inflammation domain) and prioritising immunological data in search results and display. New curation input tools have been designed in Java and the Postgres database tables expanded to encompass GtoImmuPdb specific data. Targets and ligands of immunological relevance are now 'flagged' for inclusion in GtoImmuPdb (current contents: >430 targets and >720 ligands). GtoImmuPdb has developed top-level categories for immunological processes and cell types which are underpinned by the recognised ontologies and controlled vocabularies used by the Gene Ontology[4] and the Cell Ontology[5] respectively. This allows for higher resolution annotation and facilitates interoperability between new data types in GtoImmuPdb and external resources that use these same ontologies. Development has ensured that all GtoImmuPdb data is fully downloadable. GtoImmuPdb is designed to be both 'immunologist-friendly' for pharmacological information and 'pharmacologist-friendly' for accessing immunological agents and targets. GtoImmuPdb is now in beta release (www.guidetoimmunopharmacology.org), with current funding supporting development and expansion until autumn 2018.

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Keywords: database, immunopharmacology, immunological processes, website

PP-110

IMMUNOMODULATION OF HUMAN COMPLEMENT SYSTEM BY SUPERCRITICAL EXTRACTS OF *MUSA PARADISIACA L.* INFLORESCENCE

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The complement system is intimately related with the activation of the inflammatory process. It comprises a group of plasma soluble and membrane bound proteins that can mediate important biological functions such as, cell lysis, opsonization, phagocytosis, removal of immune – complexes and apoptotic cells and inflammation. The complement proteins can be activated by tree different pathways: classical pathway, alternative pathway or lectin pathway. Activation of the three pathways converge to a standard proteolytic cascade that promotes immune/inflammatory responses. Although *Musa paradisiaca L.* is known to present anti-inflammatory properties, the mechanism of action is not completely clarified. The purpose of this study was to verify the action of different *M. paradisiaca* supercritical extracts on the activity of alternative pathway and classical pathway of complement. Two extracts where obtained via supercritical fluid extraction with propane at 3Mpa/95°F (M1) and carbon dioxide at 25Mpa/104°F (M2). The activity of both extracts on the lytic effect of alternative and classical pathway was evaluated using complement fixation test. A human fresh serum pool was the used as complement source. The results are expressed in percentages of hemolysis being considered significant when $p<0,05$. The results showed that at concentrations of 333-5,20 μg/ml, the M1 extract inhibited the activity of alternative but had no effect on the classical pathway. On the other hand, the M2

extract showed no significant effect on the lytic activity of both alternative and classical pathways.

Conclusion: Only the supercritical extract M1 showed an inhibitory effect on the alternative pathway of complement. This result suggests that different temperature and pressure parameters used in the extraction process may be responsible for the observed M1 biological activity. In addition, this anti-inflammatory activity of M1 supercritical extract might holds promises for its use in the treatment of diseases affected by excessive activation of complement, such as inflammatory and autoimmune disorders.

Keywords: Musa paradisiaca L., Complement System, Immunomodulation

PP-111

REGULATORY ROLE OF PERIPHERAL PEPTIDERGIC SENSORY NERVES IN THE PROTEOGLYCANE-INDUCED CHRONIC AUTOIMMUNE ARTHRITIS MODEL OF THE MOUSE

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Introduction: The immunological aspects of rheumatoid arthritis are well-known, but there is significantly less information about the role of sensory-immune interactions in this condition. Capsaicin-sensitive sensory nerves densely innervating the synovium and the joint capsule, release pro- and anti-inflammatory neuropeptides which have important regulatory functions in various diseases associated with pain and inflammation. Therefore, we investigated the role of these sensory nerves in a translational mouse model of chronic autoimmune arthritis.

Methods: The capsaicin-sensitive sensory nerves were selectively inactivated by resiniferatoxin (RTX, capsaicin analog) pretreatment in female BALB/c mice (desensitization). Arthritis was induced by i.p. proteoglycan aggrecan obtained from human osteoarthritic cartilage (PGIA). Mechanonociception was measured by esthesiometry, paw volume by plethysmometry, arthritis severity by semiquantitative scoring, joint function by grid test, bone structure by micro-CT, myeloperoxidase activity by luminescence and plasma extravasation by fluorescence imaging during the 4-month experimental period.

Results: From the 7th week 30-35% decrease of mechanonociceptive threshold (hyperalgesia), 40-60% paw swelling, macroscopic arthritic signs and functional damage developed. The increase of myeloperoxidase activity and plasma protein extravasation also reached their maximum by this timepoint. Ankylosis was detected from the 12th week, and increased bone mass on the 17th week. Inactivation of the capsaicin-sensitive peptidergic nerves significantly decreased hyperalgesia, paw edema, arthritis score, functional damage and myeloperoxidase activity.

Conclusion: This is the first complex functional characterization of the proteoglycane-induced chronic autoimmune arthritis mouse model. We provided evidence that the capsaicin-sensitive sensory nerves increase edema, have a role in functional damage and mediate mechanical hyperalgesia. Identification of the released neuropeptides responsible for these actions provide novel insight in the pathophysiology of the disease and help to identify new drug targets.

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Keywords: capsaicin-sensitive sensory nerves, arthritis, pain, inflammation

PP-112

VALIDATION OF AN ELECTROCHEMILUMINESCENCE-BASED ELISA FOR HIGH-SENSITIVITY MEASUREMENT OF THE OXIDATIVE STRESS BIOMARKER 3-NITROTYROSINE

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The generation of 3-nitrotyrosine (3NT), within proteins, is a post-translational modification resulting from oxidative/nitrative stress. It has been suggested that this protein modification could be used as a biomarker in inflammatory diseases. Mass spectrometry-based methods allow precise measurement of 3NT levels. However, in a high-throughput clinical setting, measurement of 3NT by ELISA is likely to be more cost-effective. On the other hand, many of the existing commercially available ELISAs are insufficiently sensitive for detection of 3NT in healthy human plasma/serum.

We have developed a novel electrochemiluminescence-based ELISA for 3NT providing superior sensitivity (e.g. a 50-fold increase in sensitivity compared with one of the tested commercial colorimetric ELISAs). This 3NT ELISA assay had the following characteristics: lower limit of quantitation 0.04 nM nitrated albumin equivalents; intra- and inter-assay CV: 6.5% and 11.3%; mean recovery: 106 ± 3%; mean linearity: 0.998 ± 0.001. The new ELISA was validated using mass spectrometry; analysis of the same set of samples (chemically modified bovine albumin) showed good agreement in the relative levels of nitration in each sample.

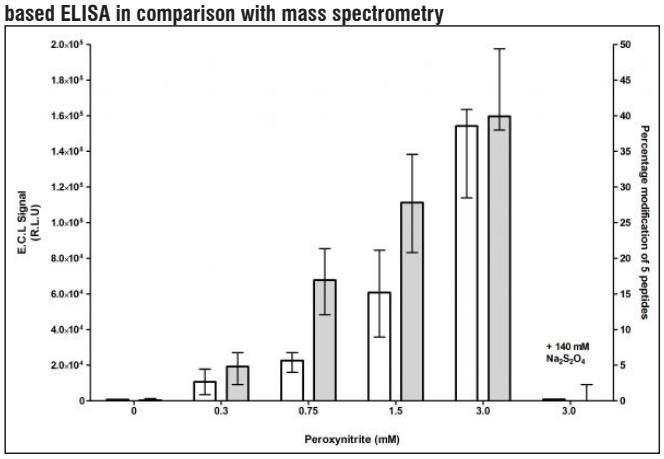
We observed the following median (IQR) levels of 3NT (fmol nitrated albumin equivalents/mg protein) in healthy human samples: plasma 1.03 (0.72-2.06), red blood cells 11.2 (8.20-14.4) and mononuclear cells 743 (494-770). Most quantitative measurements of nitration in published clinical studies have been applied to plasma/serum. However, our results showed that nitration levels in healthy plasma were far lower than in human blood cell populations. Median 3NT levels in a cultured cell line, U937 (3160 (3080-3510) fmol nitrated albumin equivalents/mg protein) were higher than in both plasma and peripheral blood cells. The assay was also applied to serum samples from patients undergoing elective surgery, providing an example of an acute inflammatory response. Matched serum samples were collected before, and one day after,

surgery. An increase in median 3NT levels (fmol nitrated albumin equivalents/mg protein) was detected following surgery: 0.59 (0.00-1.34) before surgery and 0.97 (0.00-1.70) post-surgery.

In conclusion the reported assay is suitable for 3NT determination in human plasma/serum samples, and may also be used as a means to determine oxidative stress in primary and cultured cell populations.

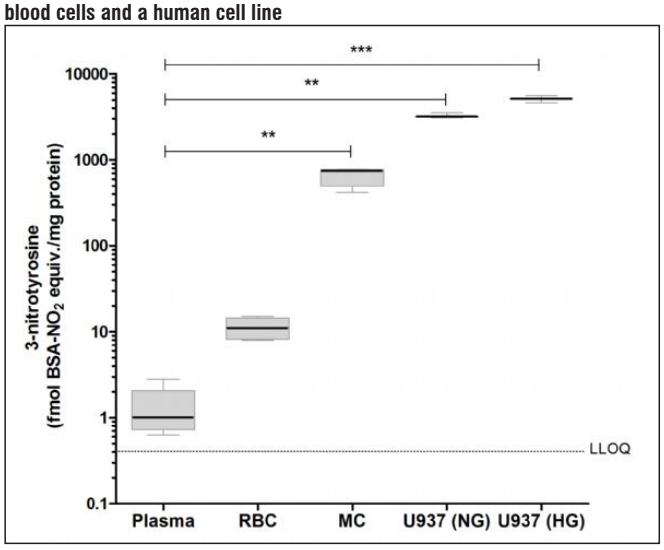
Keywords: 3-Nitrotyrosine, ELISA, oxidative stress

Relative nitration of native bovine serum albumin and peroxynitrite-treated bovine serum albumin, as detected by the new electrochemiluminescence-based ELISA in comparison with mass spectrometry



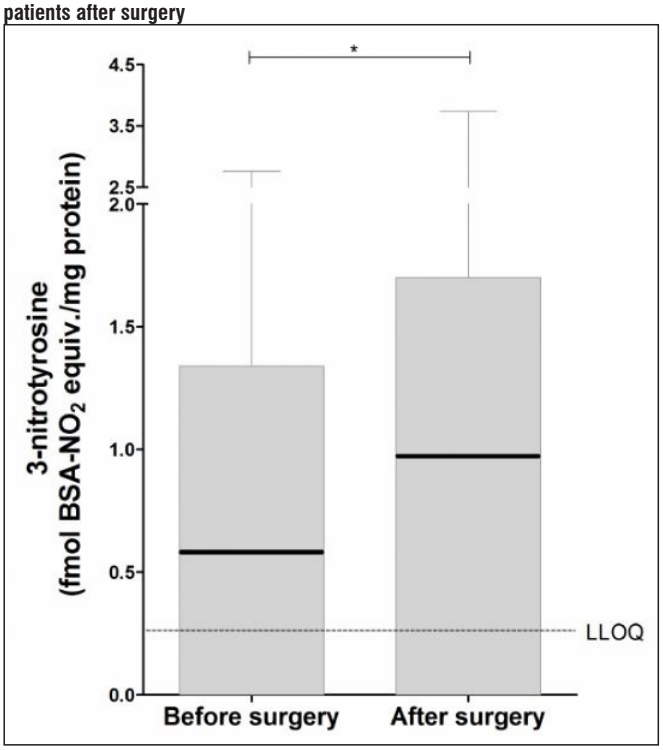
Chemically modified BSA was measured by the ECL-ELISA (white bars) and by LC-MS/MS (grey bars). A sample of BSA, modified using 3.0 mM peroxynitrite, was additionally treated with sodium dithionite (indicated on the graph as "+ 140 mM Na2S2O4"). Median and interquartile ranges are shown (n=4 for each treatment). The LC-MS/MS peptides selected for analysis were the peptides identified as undergoing nitration in the majority of samples (K.YLYEIAR.R, R.RHPEYAVSVLLR.L, K.YICDNQDTISSK.L, K.LGEYGFQNALIVR.Y and K.DAFLGSFLYEYSR.R). Where peptides contained more than one Tyr residue the signals for mono- and di-nitrated peptides were both used for calculation of the percentage modification.

Median 3-nitrotyrosine levels in healthy human plasma, compared with human blood cells and a human cell line



Levels of nitration were far lower in the plasma (n=15) compared to cellular samples. RBC (red blood cells, n=6), MC (mononuclear cells, n=4) and U937 cells (histiocytic lymphoma cell line) at a normal glucose (NG; 5.6 mM, n=3) and high glucose (HG; 24 mM, n=3) concentration. A log scale has been used for the y-axis. The boxes represent the lower and upper quartiles and the central line is the median. The whiskers show the maximum values. **p<0.01, ***p<0.001 (Dunn's Multiple Comparison Test).

Median serum 3-nitrotyrosine levels before surgery, compared with the same patients after surgery



3-Nitrotyrosine was in paired patient serum samples, before and after surgery (n=35 in each group). Serum nitration levels increased following major elective surgery (*p=0.02, Wilcoxon matched pairs). The dashed line represents the lower limit of quantification (LLOQ). The boxes represent the lower and upper quartiles and the central line is the median. The whiskers show the maximum values.

Pain

PP-113

THE DEVELOPMENT OF TRANSLATIONAL BIOMARKERS AS A TOOL FOR IMPROVING THE UNDERSTANDING, DIAGNOSIS AND TREATMENT OF CHRONIC NEUROPATHIC PAIN

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Chronic neuropathic pain (CNP) is one of the most significant unmet clinical needs in modern medicine. Alongside the lack of effective treatments, there is a great deficit in the availability of objective diagnostic methods to reliably facilitate an accurate diagnosis. We therefore aimed to determine the feasibility of a simple diagnostic test by analyzing differentially expressed genes in the blood of patients diagnosed with CNP of the lower back and compared to healthy human controls. Refinement of microarray expression data was performed using correlation analysis with 3,900 human 2-color microarray experiments (Figure 1). Selected genes were analysed in the dorsal horn of Sprague Dawley rats after L5 spinal nerve ligation (SNL), using qRT-PCR and ddPCR, to determine possible associations with

pathophysiological mechanisms underpinning CNP and whether they represent translational biomarkers of CNP. We found that of the 15 potential biomarkers identified (Table 1), tissue inhibitor of matrix metalloproteinase-1 (TIMP1) gene expression was upregulated in chronic neuropathic lower back pain (CNBP) ($p = 0.0049$) which positively correlated ($R = 0.68$, $p = <=0.05$) with increased plasma TIMP1 levels in this group ($p = 0.0433$). Moreover, plasma TIMP1 was also significantly upregulated in CNBP than chronic inflammatory lower back pain ($p = 0.0272$) (Figure 2). In the SNL model, upregulation of the Timp1 gene was also observed ($p = 0.0058$) alongside a strong trend for the upregulation of melanocortin 1 receptor ($p = 0.0847$) (Table 2). Our data therefore highlights several genes that warrant further investigation, and of these, TIMP1 shows the greatest potential as an accessible and translational CNP biomarker.

Keywords: Neuropathic pain, biomarker, Plasma, Dorsal Horn, Back Pain, Inflammatory pain

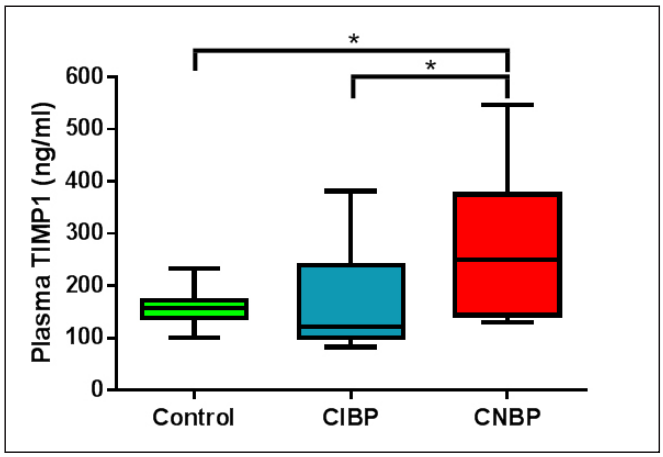

Figure 2. Plasma TIMP1 concentrations in healthy controls and patients with CIBP or CNBP

Analysis of plasma TIMP1 concentrations in healthy controls (n =10), CIBP patients (n = 12) and CNBP patients (n = 10) was carried out using an ELISA. Diluted plasma samples were exposed to human TIMP1 monoclonal antibody coated wells and treated with human TIMP1 antibody conjugated to biotin. After Streptavidin-Peroxidase treatment, addition of substrate allows for colourmetric detection at 450nm. Greater absorbance recordings correlate to higher plasma TIMP1 levels. *p = <= 0.05 (Mann-Whitney).

PP-114

INVOLVEMENT OF TH1CELL-DEPENDENT RESPONSES IN THE TRANSITION OF NERVE INJURY-INDUCED ACUTE PAIN TO A CHRONIC PAIN STATE

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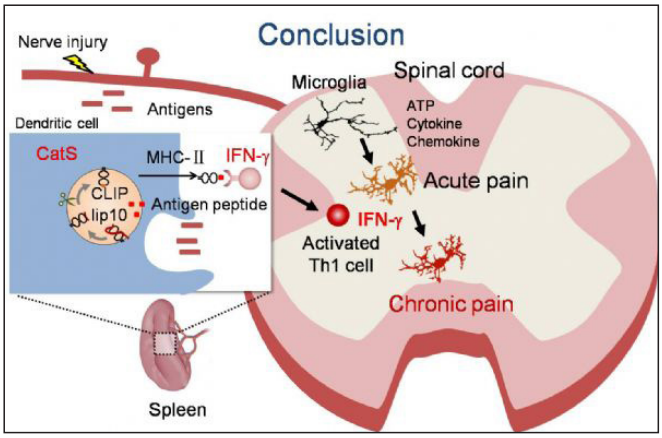
We have previously reported that inhibition of cathepsin S (CatS) activity, either through genetic deletion or via a pharmacological inhibitor,Z-Phe-Leu-COCHO (Z-FL), significantly attenuated the maintenance of neuropathic pain. However, the precise roles of CatS in maintenance of neuropathic pain remain unclear. There is increasing evidence that CD4+ T cell-dependent responses are associated with the maintenance of neuropathic pain. Wehave thus examined a possible involvement of CatS in CD4+ T cell-dependent responsesfollowing spinal nerve injury, because CatS is responsible for the final proteolyticcleavage of the MHC class II-associated invariant chain(Ii) in antigen presenting cells, which is an essential step for the CD4+ T cell-dependent responses.We found that CatS deficiency or Z-FL treatmentsignificantly attenuated the proliferation of splenic CD4+ T cells, the final cleavage of Ii and infiltration of CD4+ T cells that expressed IFN-γ in the dorsal spinal cord following spinal nerve injury. Furthermore, the adoptive transfer ofCD4+ T cells prepared from splenocytes of neuropathic wild-type mice significantly increased the pain level of CatS-/- mice. It was also noted that STAT1 was activated exclusively in microglia. These results show a peripheral pivotal role of CatS in the development of neuropathic pain through the antigen-specific activation of CD4+ T cells. After activation, CD4+ T cellsinfiltrate into the dorsal spinal cord and secrete IFN-γto further activate microglia, which contribute to the transition of acute pain to a chronic pain state. Therefore, peripherally active selective CatS inhibitors could be a safer therapeutic option for the pharmacological treatment of neuropathic pain.

Keywords: cathepsin S, dendritic cells, interferon-ganmma, microglia, neuropathic pain, Th1 cells

Conclusion:



Our present observations strongly suggest that peripherally active selective CatS inhibitors could be a safer therapeutic option by avoiding the induction of off-target side effects for the pharmacological treatment of neuropathic pain.

PP-115

ANTINOCICEPTIVE EFFECTS OF METHANOL EXTRACT OF *CALLOPHYLLUM INOPHYLLUM*: POSSIBLE ROLES OF THE OPIOIDERGIC, ADRENERGIC AND CHOLINERGIC PATHWAYS

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The antinociceptive activityies of the methanol extract of *Callophyllum inophyllum* (CI) leaves were investigated using thermal and chemical tests of nociception: tail flick, the hot plate, acetic acid-induced writhing and the formalin-induced paw licking test. In the thermal tests, the extract delivered per oral (p.o) dose dependently resulted in the prolongation of both the hot plate and the tail flick latencies at all doses. The

extract dose dependently reduced the number of abdominal constrictions in the acetic acid-induced writhing test and also considerably reduced the licking times in both phases of the formalin-induced paw licking test. While naloxone had no effect on the antinociceptive activity of the extract, both atropine and prasozin produced an inhibition of its antinociception as revealed by significant reductions in the thermal latencies compared with the control. It may therefore be concluded from this study that *Callophyllum inophyllum* antinociception is mediated via the activation of the cholinergic and alpha adrenergic pathways.

Keywords: Callophyllum inophyllum, Antinociceptive, Opioid, Adrenergic, Cholinergic

PP-116

GASTROINTESTINAL SAFETY AND EFFICACY OF LONG-TERM GCSB-5 USE IN PATIENTS WITH OSTEOARTHRITIS: A 24-WEEK, MULTICENTER STUDY

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Ethnopharmacology relevance: A previous study indicated non-inferiority of GCSB-5 to celecoxib regarding efficacy and safety in treating OA; however, the gastrointestinal (GI) safety data was limited to 12 weeks. Accordingly, a longer term study with a larger number of patients was necessary to establish the GI safety of GCSB-5.

Aim of study: The primary goal was to determine the safety and efficacy of 24-week use of GCSB-5. The secondary goal was to compare the GI safety data of GCSB-5 with that of the previously reported Celecoxib Long-term Arthritis Safety Study (CLASS).

Method: This was a 24-week, multicenter, single-arm phase IV Study for the safety and efficacy of GCSB-5. A total of 761 patients were enrolled and 756 patients received at least one dose of GCSB-5. Among them, 629 patients (82.7%) completed the 24 week follow up. The primary goal was to determine the safety and efficacy of GCSB-5 for 24 weeks. The secondary goal was to compare the GI safety data of GCSB-5 with that of the previously reported Celecoxib Long-term Arthritis Safety Study (CLASS).

Results: The incidence of GI disorders of GCSB-5 was 23.7%. The annual rate of perforation, ulcer obstruction, or bleeding (PUB) incidence was 0.0%. The drop-out rate due to GI disorders following GCSB-5 use was 4.8%. Compared to celecoxib data from CLASS, the incidence of GI disorders (23.7% vs. 31.4%, $p<0.001$), annual rate of PUB and gastroduodenal ulcers (0.0% vs 2.2%, $p=0.004$), and drop-out rate due to GI disorders following GCSB-5 use were significantly low (4.8% vs 8.7%, $p<0.001$). Efficacy was proven by significant improvements in Western Ontario McMaster Questionnaire (WOMAC) scale, Korean Knee Score (KKS), 100-mm pain visual analogue scale (VAS), and physician's global assessments of patient's response to therapy (PGART).

Conclusions: The safety and efficacy profile of GCSB-5 are comparable to celecoxib. These results indicate GCSB-5 is safe for a long-term treatment of knee OA patients.

Keywords: GCSB-5, GI safety, Herbal medicine, Osteoarthritis, Shinbaro

PP-118

DIMETHYL TRISULFIDE AND SODIUM POLYSULFIDE BOTH AMELIORATE MECHANICAL HYPERALGESIA INDUCED BY CARRAGEENAN IN A TRPA1 RECEPTOR-DEPENDENT MANNER

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Organic trisulfides and inorganic polysulfides have recently been reported to interact with transient receptor potential ankyrin-1 (TRPA1) ion channels. TRPA1 channels are mostly located on primary sensory neurons. Interestingly, both hyperalgesic and antinociceptive effects are attributed to TRPA1. Excitation of nociceptors might mediate the former and release of neuropeptides the latter. Organic trisulfides and inorganic polysulfides interact with proteins by forming di/trisulfide or persulfide metabolites with cysteine residues, respectively. Based on different chemical interactions with TRPA1, different biological effects of the two species are possible. We investigated TRPA1 receptor-mediated effects of dimethyl trisulfide (DMTS) and sodium polysulfide in CHO cells expressing human TRPA1, as well as on nociception of TRPA1 transgenic mice undergoing carrageenan-induced paw inflammation.

Activation of TRPA1 channels by DMTS and inorganic polysulfide was assessed in CHO cells expressing human TRPA1. Cells were loaded with Fluo-4-AM before stimulation and calcium signals were detected by flow cytometry. One hindpaw of TRPA1 wild-type (WT) or knockout (KO) mice was injected with carrageenan (10 μL of 3% solution in saline) intraplantarly. Contralateral paws received saline. Animals were treated with DMTS (250 μmol/kg) or sodium polysulfide (50 μmol/kg) i.p. 30 min before carrageenan challenge and every hour thereafter for 6 hours. Mechanical hyperalgesia was tested by dynamic plantar aesthesiometry before and 2, 4, 6 h after carrageenan injection.

DMTS and sodium polysulfide evoked calcium signals in CHO cells expressing human TRPA1. Cells lacking the channel did respond to neither substances. Carrageenan produced mechanical hyperalgesia in TRPA1 WT and KO animals. DMTS and sodium polysulfide significantly blunted mechanical hyperalgesia in TRPA1 WT mice, but not in TRPA1 KO ones.

Inhibitory action of repeated systemic DMTS and sodium polysulfide administration on mechanical hyperalgesia induced by carrageenan is mediated by TRPA1 channels. Antihyperalgesic effect of the two compounds might be mediated by a release of anti-nociceptive neuropeptides from the TRPA1-expressing primary sensory nerve endings. These peptides are able to inhibit the excitation of nociceptors and might prevent the activation of inflammatory cells, too. Our data might move the development of analgesic therapeutics based on organic trisulfides and inorganic polysulfides forward.

Keywords: organic trisulfide, polysulfide, TRPA1, mechanical hyperalgesia, carrageenan

PP-119

ADIPOKINE CONTRIBUTION TO ENHANCED INFLAMMATORY PAIN IN RODENT MODELS OF OBESITY

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Glasgow Caledonian University

Obesity and chronic pain are positively correlated in humans. This association is not limited to musculoskeletal pain but also includes conditions such as migraine and fibromyalgia. A causal link may be the dysregulated secretion of bioactive peptides or 'adipokines' from expanding adipose tissue. This study set out to characterise the impact of obesity on nociception and the development of inflammatory pain in two rodents model of obesity and analyse the secretion profile of key inflammatory adipokines in these animals.

Responses to thermal and mechanical stimulation of the hindpaw were assessed in adult male Zucker fatty rats and their lean littermates (a model of genetic obesity; n=6-9/group), and in adult male Wistar rats fed a high fat diet (HFD; 22%) or normal diet for 16 weeks (model of dietary-induced obesity; n=6/group) in absence of inflamation, and then in response to intradermal hindpaw injection of carrageenan (3%; 50μl), a model of acute inflammation. Spinal cord, white adipose tissue (WAT) and plasma were collected and adipokine mRNA expression, cholesterol and triglycerides (TAGs) measured using real-time PCR and ELISA techniques.

Obese Zucker rats were significantly heavier than controls (551 ± 35g vs. 377 ± 27g; $P<0.01$), as were HFD rats (502 ± 12g vs. 444 ± 7g; $P<0.01$ vs. lean controls), and both displayed plasma hyperinsulaemia and hypercholesterolaemia (both $P<0.05$ vs. lean controls). Acute nociceptive responses were unchanged in obese Zucker and HFD rats but both ddisplayed potentiated mechanical and thermal hyperalgesia and increased paw oedema (all $P<0.05$ vs. lean controls) in response to carrageenan. Significant changes in levels of adiponectin, resistin and visfatin (but not IL1β, RAGE or lipocalin-2) were detected in obese rat serum. In spinal cord, visfatin, resistin and IL1β were significantly up-regulated, while adiponectin was down-regulated (RAGE and lipocalin-2 remained unchanged) in obese rats compared to lean controls.

The increased pain sensitivity and inflammatory response observed in rats with an obese phenotype fits well with the hypothesis that obesity is a chronic low-grade inflammatory disorder, producing a state where responses to inflammatory challenge are potentiated. The aaltered adipokine secretion profile observed in obese rats suggests that some adipokines may be useful biomarkers for monitoring initiation and progression of pain with obesity, or may even be involved in the development of co-morbid pain in obese individuals.

Keywords: pain, adipokine, spinal cord, obesity

PP-120

PILOT EPIDEMIOLOGICAL SURVEY OF PATIENTS SUFFERING FROM LOIN PAIN HAEMATURIA SYNDROME (LPHS)

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Loin pain haematuria syndrome (LPHS) is considered to be the most painful syndrome suffered by humankind. It occurs in mainly Caucasian females of young age (Spetie et al, 2006). It is charaterised by unilateral or bilateral flank pain, with exacerbations that are catastrophically incapacitating. It is accompanied by macroscopic or microscopic haematuria and mediators from blood might contribute. LPHS can be of primary or secondary origin. Primary LPHS is diagnosed through the absence of causative pathologies such as infection or glomerular nephritis. It is poorly understood and validated diagnostic criteria lacking. Diagnosis is thus through exclusion. Numerous theories have been produced as to the aetiology of LPHS, including glomerular basement membrane defects. A plethora of therapeutic options are attempted including renal denervation (RDN) and autotransplantation (RAT). No global epidemiological surveys have been carried out, this pilot survey will provide a starting point for further research.

This study has UEL SREC Institutional ethics approval. Questions were generated from an appraisal of the literature, and in order to provide a focus for a full study. S.1 contains demographic questions, as well as number of chlidren and contraceptive use. S.2 interrogates length to diagnosis, whether primary or secondary, and if well managed. S.3. details the quality of pain and impact on life. S.4 determines aspects of haematuria. S.5 medical and surgical interventions. S.6 other conditions and S7 treatment effictiveness.

The survey took place between the dates of the July 2016 to May 2017. Data presented is mid term (n=21). Members of 2 closed Facebook groups were invited to complete an online questionnaire to a total of 40 of a possible 272.

95.24% of participants were female and 90.48% participants wereCaucasian. There was an average period of 5.57 years between age of onset of LPHS and age of diagnosis. 71.43% participants suffer from comorbid diseases. 61.90% of participants initially presented with bilateral flank pain and 100% of participants experienced haematuria. 66.67% of participants feel their LPHS is not under control. 95.00% of participants suffered from primary LPHS; 100% of participants undertook medical intervention including opiates and NSAIDs and 66.67% participants underwent surgical intervention. The most common forms of surgical intervention observed were RAT and RDN (19.05%) there was a ratio of 3:1 double RAT to single.

LPHS is severely debilitating. Comparisons of effective anf ineffective therapies will inform good practice. Optimal treatment cannot be achieved until the pathobiology of LPHS is better understood, and the inflammatory role of the haemauturia is currently being investigated (see paired poster).

References

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Spetie D et al (2006) Proposed pathogenesis of idiopathic loin pain-haematuria syndrome. Am J Kidney Dis; 47:419.

Keywords: Loin Pain Haematuria Syndrome, LPHS, epidemiology, treatment, haematurai, renal autotransplantation

Resolution of Inflammation

PP-121

IFNB IS PRODUCED BY RESOLUTION-PHASE MACROPHAGES AND PROMOTES THE RESOLUTION OF INFLAMMATION

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[3]The Forsyth Institute, Boston, MA

The engulfment of apoptotic leukocytes (efferocytosis) by macrophages during the resolution of inflammation is essential for tissue homeostasis and results in macrophage reprogramming/immune-silencing. However, a distinct subset of resolution phase macrophages loss their phagocytic potential following the uptake of large numbers of apoptotic PMN, and hence were termed satiated macrophages. Here, we show using an unbiased RNA-Seq analysis that satiated macrophages upregulate the expression of a distinct IFNβ-related gene signature. Unexpectedly, we found peritoneal IFNβ levels peaked during the onset as well as the resolution phase of peritonitis. Consequently, we used IFNβ$^{-/-}$ mice to determine IFNβ limited the onset of inflammation by enhancing neutrophil apoptosis. Moreover, IFNβ enhanced macrophage efferocytosis and reprogramming to an anti-inflammatory/pro-resolving phenotype. These findings indicate for the first time that IFNβ is a key effector cytokine in resolving inflammation.

Keywords: Resolution of Inflammation, Macrophages, Phagocytosis, IFN

PP-123

MOLECULAR AND STRUCTURAL ALTERATIONS AFTER *BIDENS PILOSA* L. SUPERCRITICAL CO2 EXTRACT TREATMENT IN INTESTINAL INFLAMMATION INDUCED BY TNBS IN RATS

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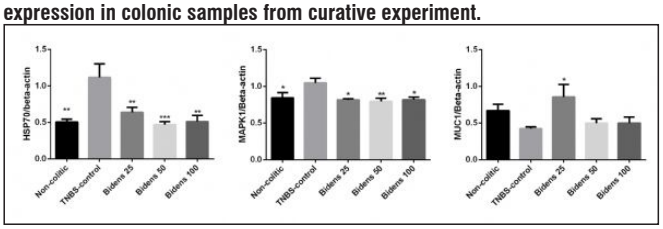
Inflammatory bowel disease (IBD) refers to a group of conditions characterized by a chronic inflammation in the intestinal tract that comprises Crohn's disease (CD) and ulcerative colitis (UC). The aetiology of these diseases has not been fully elucidated but is currently presumed to result from a complex interplay among genetic, environmental, microbial and immune factors. Restriction of pharmacological treatment of IBD is due to low response associated with several side effects has leading to search new sources of compounds, including natural compounds with several mechanism of action. Bidens pilosa L. (Asteraceae), is an herb widely distributed in tropical and subtropical countries, where have been used in traditional medicine for its anti-inflammatory properties. Among other constituents, B.pilosa contains polyunsaturated fatty acids, that show anti-inflammatory and antioxidant properties, with potential effects in intestinal inflammatory processes such as inflammatory bowel disease (IBD). Previous studies from our group found that B.pilosa extract is capable to diminish intestinal inflammation caused by trinitrobenzenesulphonic (TNBS) acid, decreasing myeloperoxidase, IL-1β and TNF-α, inflammatory markers besides maintaining glutathione, endogenous antioxidant, level. Based on this, we proposed to study the molecular and structural effects of B. pilosa supercritical CO2 fluid extract (BPE-CO2) after TNBS instillation in rats. Intestinal inflammation was induced in rat by intracolonic instillation of TNBS. Rats orally received 25mg/Kg, 50mg/kg or 100mg/kg of BPE-CO2 starting 2 hours after colitis induction and continuing 7 days thereafter. Rats were killed in the 8th day after colitis induction; colonic segments were obtained after laparotomy for macroscopic, biochemical and gene expression analysis of Hsp70, Heparanase, NF-kB, Mapk1, 3, 6 and 9 and Muc1, 2, 3 and 4. For structural analysis, colon segments were analysed by scanning electron and transmission electron microscopy and histochemical by PAS/Alcian Blue staining. In this protocol Hsp70 and Mapk1 were decreased by all doses and the dose of 25mg/kg was capable to enhance Muc1 gene expression. The structural analysis shown that occurs an improvement in colonic appearance, indicating minor oedema and membrane disruption and a mucin production enhance in all doses analysed. In conclusion, B. pilosa extract improved structurally the colon tissue as demonstrated by electron microscopy and diminishes genes related to inflammatory process. Altogether indicates that Bidens pilosa L. extract can be used to ameliorate the inflammation caused by TNBS and can be a potential source of compounds useful to treat IBD in humans.

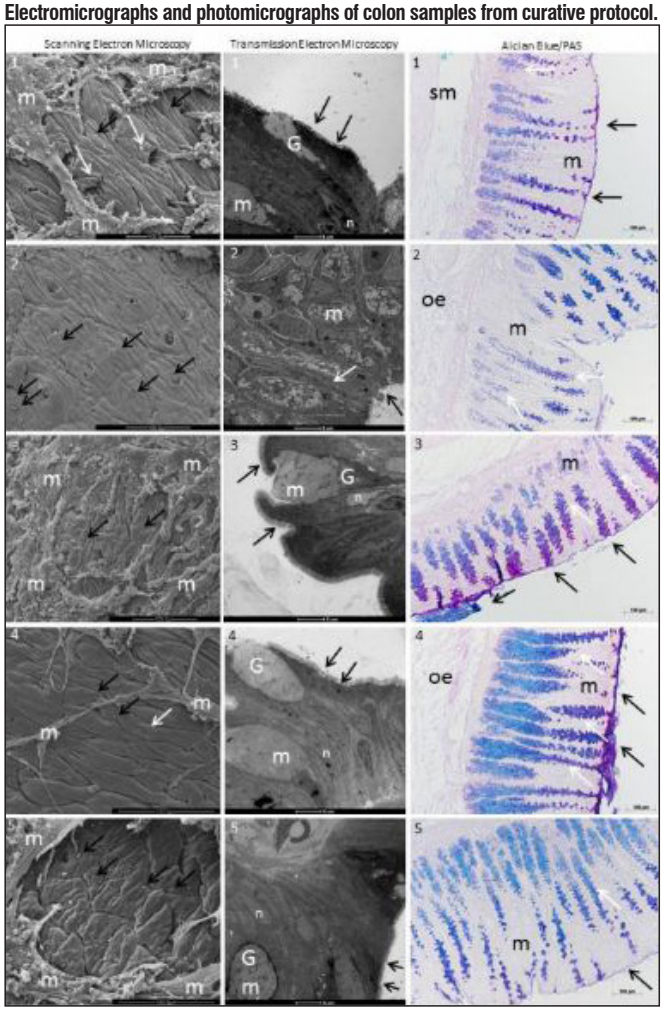
Keywords: Inflammatory Bowel Disease, Bidens pilosa, mucin, supercritical extraction

Effects of Bidens pilosa L. treatments on HSP70, MAPK1 and MUC1 gene expression in colonic samples from curative experiment.



Data are expressed as the mean ± S.E.M. *p<=0.05; ** p<=0.01; *** p<=0.001

Electromicrographs and photomicrographs of colon samples from curative protocol.



Non-colitic group **(1);** TNBS-control group **(2);** 25mg/kg treated group (**3);** 50mg/kg treated group **(4)** and 100mg/kg treated group (**5**). In Scanning Electron Microscopy: crypts *(white arrows)*, goblet cells *(black arrows)* and mucin **(m)**. In Transmission Electron Microscopy: microvilli *(black arrows)*, nucleus **(n)**, goblet cells **(G)**, mucin **(m)** and oedema *(white arrows)*. In PAS/Alcian Blue Staining: Blue staining *(white arrows)*, mucosa **(m)**, submucosa **(sm)** membrane-bond mucins *(black arrow)* and oedema **(oe)**.

PP-124

THE EFFECT OF THE SELECTIVE MC_3 AGONIST PG990 ON HIGH DENSITY HUMAN CHONDROCYTE MICROMASS CULTURES ACTIVATED BY TNF-ALPHA

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Introduction: Osteoarthritis (OA) is a degenerative joint disease partially mediated by the catabolic cytokine TNF-α, leading to progressive and permanent degeneration of cartilage (1). A potential anti-inflammatory and chondroprotective role for melanocortin peptides has been shown (2), although the receptor subtype involved is unclear. This study assesses the chondroprotective and anti-inflammatory effects of the selective melanocortin-3 (hMC_3) receptor agonist PG990 and partially selective agonist [$DTRP^8$]-γ-MSH on TNF-α induced cell death, pro-inflammatory cytokine and matrix metalloproteinase (MMP) release in chondrocyte micromass cultures.

Methods: Micromass cultures of the human chondrocytic cell line C-20/A4 were obtained by seeding cells at a density of 25.0 x 10^6 viable cells/mL into 24-well plates. After 48h micromasses were treated with [$DTRP^8$]-γ-MSH (3.0μg/ml^{-1}) or PG990 (10.0μg/ml^{-1}) for 30mins prior to TNF-α (60pg/ml^{-1}) stimulation for 6h. Micromasses were harvested for Alcian blue staining for sulphated glycosaminoglycan (GAG) content, cell viability and RT-PCR gene expression of MC_1, MC_3, IL-6, IL-8 and MMP-1. Cell free supernatants were analysed for IL-6, IL-8 and MMP-1 release by ELISA. Data are expressed as Mean±SD of n=4 determinations in triplicate. #$p<=0.05$ vs. control or *$p<=0.05$ vs. stimulus.

Results: RT-PCR showed MC_1 and MC_3 expression on micromass C-20/A4 cells. Alcian Blue staining showed an increased GAG accumulation in micromasses treated with PG990 (121.3±2.4 μg/μg protein) when compared to TNF-α (79.3±1.7 μg/μg protein), a similar effect was observed for [$DTRP^8$]-γ-MSH (109±2.9 μg/μg protein). MTT showed an increase in cell viability by PG990 and [$DTRP^8$]-γ-MSH (162.5% and 153% increase respectively compared to TNF-α). IL-6, IL-8 and MMP-1 gene expression was significantly reduced by PG990 compared to cells exposed only to TNF-α (expression normalised to β-actin). IL-6, IL-8 and MMP-1 expression in TNF-α only treated cells was 5.60±0.04, 5.70±0.05 and 6.90±0.09 respectively and all significantly reduced by PG990 to 0.7±0.05 (87.5% reduction), 0.9±0.01 (84.2% reduction) and 1.1±0.04 (84.1% reduction) respectively. [$DTRP^8$]-γ-MSH elicited a similar response (85.7%, 80.7% and 88.4% reduction respectively). A similar response was observed on IL-6, IL-8 and MMP-1 release detected by ELISA for PG990 and [$DTRP^8$]-γ-MSH.

Conclusion: The selective hMC_3 agonist PG990 exhibited chondroprotective and modulation of inflammatory and tissue destructive pathways following TNF-α chondrocyte activation highlighting a role for MC_3 receptor for treatment of OA.

References

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2. Getting SJ, et al. (2006), Mol Pharmacol 70:1850–1855.

Keywords: melanocortins, arthritis, inflammation, chondrocytes, pharmacology

PP-125

THE EFFECT OF OXYGEN AND PENTOXIFYLLINE ON MACROPHAGE AND T CELLS IN HYPOXIC CONDITION

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Objective: Many patients admit the emergency department due to massive hemorrhage, respiratory failure, and further that the experience can fall into shock. Thereafter, it will be damaged by a mechanism such as acute respiratory distress syndrome (ARDS), homeostasis maintenance impossible, multiple organ failure (MOF), immune function reduction and over-expression of inflammation and recovery it can become difficult. In the treatment of shock patients, airway maintenance and oxygen supply are known to be of paramount importance. Therefore, this aim of study was to investigated to effects of oxygen supply and variable medication in hypoxic condtion. We conducted an experiment to determine effect of oxygen and variable medication in iNOs, macrophage migration inhibitory factor (MIF) as an inflammatory cytokine of macrophage, in MTT, IL-2, IL-8 as an immune marker of T cells proliferation and T cells in hyperinflammatory condition by the using coculture.

Methods: The experiments were performed with THP-1 devired macrophage and Jurkat cells. First, macrophage cells put through normoxic state, hypoxic state, oxygen supply and variable medication, and measured the iNOs, MIF by western blots. Second, Jurkat cells were incubated through hypoxic state, oxygen supply and variable medication, and measured MTT, IL-2 and IL-8. Third, in co-culture, after Jurkat cells under hyperinflammatory macrophage cells were incubated through hypoxic state, oxygen supply and variable medication, and measured MTT, IL-2.

Results: 1. In hypoxic state in macrophage cells, iNOs expression and MIF increased when cells were exposed to hypoxia. Pentoxifylline under oxygen supply condition restored iNOs in stimulated macrophage.

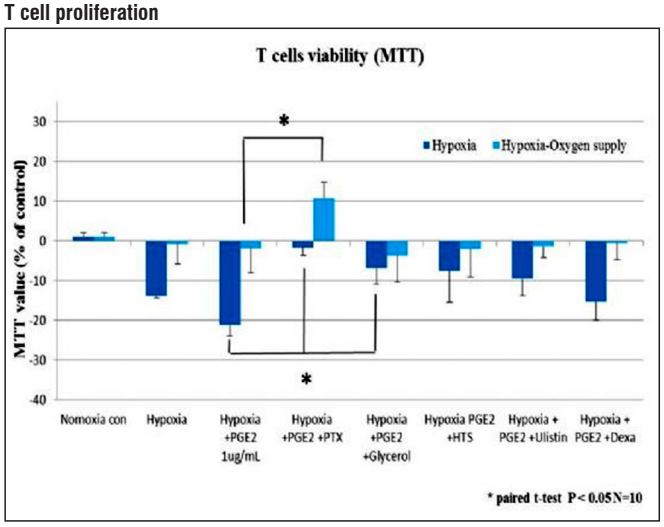
2. T cell viability decreased in hypoxic condition, however pentoxifylline restored T cell viability, regardless of oxygen supply. IL-8, MIF increased in hypoxic condition, however pentoxifylline and steroid restored IL-8, MIF. IL-2 decreased in hypoxic condition,

3. In coculture condition, oxygen supply and pentoxifylline more increased T cell viability, IL-2 than pentoxifylline in hypoxic state,

Conclusions: Hypoxia decreased T cell viability. iNOS, MIF and IL-8 increased in hypoxic state rather than normoxic state. However, PTX restored T cell viability, IL-2 in oxygen supply condition than the hypoxic state.

Keywords: Hypoxia, oxygen, shock

T cell proliferation



T cell viability decreased in hypoxic condition, however pentoxifylline restored T cell viability, regardless of oxygen supply

PP-126

THE NON-ANTIBIOTIC MACROLIDE EM703 IMPROVES SURVIVAL IN A MODEL OF QUINOLONE-TREATED *PSEUDOMONAS AERUGINOSA* AIRWAY INFECTION

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Background: Macrolide antibiotics are increasingly used as anti-inflammatory agents, for example preventing exacerbations in COPD and cystic fibrosis. There are also several studies showing improved outcomes after addition of macrolides to beta-lactam antibiotics when treating severe community-acquired pneumonia. However, a possible beneficial effect from macrolides when treating Gram-negative bacterial airway infections, e.g. caused by *Pseudomonas aeruginosa*, remains to be shown.

Methods: Airway infection was induced in BALB/cJRj mice by nasal instillation of P. aeruginosa. This was followed by treatment with the quinolone levofloxacin in the absence or presence of a macrolide lacking antibiotic activity but with retained anti-inflammatory properties (EM703). Survival, inflammatory responses, and cellular influx to the airways were monitored.

Results: Both pretreatment and parallel administration of EM703 dramatically improved survival in levofloxacin-treated mice with *P. aeruginosa* airway infection. In addition, EM703 decreased the levels of proinflammatory cytokines, increased the number of leukocytes in bronchoalveolar fluid, and reduced the number of neutrophils present in the lungs.

Conclusion: The findings show that the immunomodulatory properties of the novel non-antibiotic macrolide EM703 can be important when treating Gram-negative pneumonia as exemplified by *P. aeruginosa* in this study.

Keywords: Macrolide, EM703, Host defense, anti-inflammatory, Pseudomonas aeruginosa

PP-127

THE IMPACT OF PENTOXYFILLINE IN INFLAMMATORY CELLS INTERACTION

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Objective: Immunity is the state of having sufficient biological defences to avoid infection, trauma, or other unwanted biological invasion. T-cells, macrophage play important role in cell mediated immunity. Pentoxyfilline (PTX) is know that decrease proinflammatory cytokine and TNF-a. However, the effect on immune system was not known well. Aim of this study is to investigate the effect of PTX in inflammation.

Methods: THP-1 derived macrophage were incubated with LPS and/or indicated concentration of PTX for 6hr and wash with PBS to eliminate effect of LPS. In this media, T cells were plated into at trans well plate and co-culture was done at 12hr. The T cell viability was measured by MTT and expression of IL-2 was analyzed by RT-PCR.

Results: PTX inhibit concentration of MIF, TLR4 protein level and mRNA expression of TLR4 in macrophage. However, PTX did not restore in the T cell proliferation with PGE2. In the co-culture study, The T cells viability decreased in the macrophage cells stimulated with LPS. The additional PTX restored the T cells viability. In the same manner, IL-2 expression in the macrophage stimulated with LPS restored in the macrophage cells stimulated with LPS and PTX.

Conclusion: LPS stimulated macrophage cells inhibit the T cell viability in hyperinflammation condition. In this state PTX restore the T cells viability to increase IL-2. PTX influence the cell-cell interaction, therefore, have its immunomodulatory effects.

Keywords: Pentoxyfylline, Macrophage migration inhibitory factor, Macrophage, T cells, Toll like receptor

PP-128

ANTI-INFLAMMATORY ACTIVITY AND INHIBITION OF ICAM-1 EXPRESSION BY AQUEOUS EXTRACT FROM *TRAGIA BENTHAMII* BAK

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Indigenous knowledge will continue to be relevant in the study of crude drugs and biologically active semi-purified as well as purified plant extracts. *Tragia benthamii* (Tbent) commonly called the climbing nettle is a tropical plant claimed to have numerous anti-inflammatory effects in sub-saharan African ethnomedicine which lacks scientific evidence. Aqueous extracts of Tbent were further pre-purified on a RP-C18 parked solid phase system to obtain 100%, 80% and 20% aqueous fractions using 0%, 20% and 80% ddH_2O respectively in solvent B (90% v/v acetonitrile in ddH_2O, 0.05% v/v trifluoroacetic acid). The 20% aqueous fraction was enzymatically and chemically analyzed (by MALDI TOF MS and MS/MS) to contain interesting low molecular weight cysteine-rich stable peptides within the range of 2.5KDa-3.2KDa. Human umbilical vein endothelial cells-immortalized by Telomerase reversed Transcriptase (HUVEC-TERT) were treated with the different fractions of Tbent at a concentration of 100ug/M199. The expression of adhesion molecule (ICAM-1) was evaluated by modified cell ELISA. ICAM-1 antigen was determined by staining monoclonal antibody of ICAM-1 and anti-mouse IGg monoclonal antibody linked with peroxidase from sheep (POX). The 20% aqueous fraction was further tested *in vivo* using carrageenan-induced foot edema (acute inflammation) in 7- day old chicks with Diclofenac as reference drug. The cytotoxicity of the active fraction was investigated using the brine shrimp lethality assay. Result showed that peptide fraction (20% aqueous) comparatively produced a slight reduction in LPS induced ICAM-1 expression relative to other fractions. This would suggest that the crude peptide fraction might have anti-adhesive and anti-inflammatory effect on HUVEC-TERT by inhibiting ICAM-1 surface expression. This may lead to investigate mechanistically the anti-inflammatory effect which might be NFκB-dependent since ICAM-1 is one of its responsive genes. Pre-treatment with the extract (30-300mg/kg, i.p) dose dependently ($P<0.01$) reduced foot edema with maximal inhibition of 0.253 ± 0.180 (84.3%) at 300mg/kg body weight, which was comparable to that of Diclofenac with inhibition ($P<0.05$) of 0.410 ± 0.271 (74.5%) at 10 mg/kg body weight. Therefore, the study has shown that the Cysteine-rich peptide extracts from the ethnomedicinal plant *Tragia benthamii* exhibit anti-inflammatory activity. Thus giving scientific credence to its use for the treatment of inflammation and pain in traditional medicine.

Keywords: Tragia benthamii, Cysteine-rich peptides, HUVEC-TERT, ICAM-1, carrageenan, chicks

PP-129

SCALP TREATMENT WITH LOW LEVEL LIGHT THERAPY/GENTLEWAVES® DOWN-REGULATES INFLAMMATORY BIOMARKERS CD69, AP1 AND MIR21 IN MEN WITH ANDROGENETIC ALOPECIA

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Low-Level Light Therapy (LLLT) is a recent adjunctive technology used for the relief of several cosmetic and dermatological afflictions such as healing, acne, dermal matrix regeneration, post-esthetical inflammatory conditions and hair growth stimulation. While we could confirm the expression of CRY1 and CRY2 circadian genes together with OPN3 and OPN4 photoreceptors in skin and hair cells, it is now widely accepted that the visible light spectra, spanning from blue to near-infrared, activates several cellular functions such as adenosine triphosphate (ATP) and nitric oxide synthesis at least partly through photonic absorption by mitochondrial chromophores. 64 men with androgenetic alopecia were enrolled in a clinical study for evaluating the biological effects of a selected pulsed light source (GentleWaves®) with a major emitting peak in the Yellow/Amber at 590nm and a minor additional peak in the near-infrared (NIR) at 870nm. By using full genome analysis of scalp biopsies, following exposure to LLLT/GentleWaves® during 70s once per day for 3 consecutive days, a significant down-regulation of scalp perifollicular inflammation biomarkers was observed. This was evidenced by an inhibition of both AP-1/FosB mRNA and mir21 transcription together with a disappearance of CD69 mRNA specific to T cells that were found to significantly infiltrate the scalp of about 50% of the 64 studied subjects prior to LLLT/ GentleWaves® treatment. Additionally, illumination of normal human skin keratinocytes in culture in vitro with the same LLLT/GentleWaves® technology confirmed, by using QRT/PCR, the strong down-regulation of several inflammatory biomarkers including IL-1β, MMP-14, CXCL5; CCL22; PTGS2 and ALOX12 mRNAs. Altogether, these observations confirm the potential of LLLT/GentleWaves® as a non-invasive adjunctive technology beneficial for scalp conditions, such as androgenetic alopecia, where a mild perifollicular inflammation is involved.

Keywords: Low level Light therapy, Androgenetic Alopecia, CD69, hair photoreceptors, perifollicular inflammation, full genome analysis

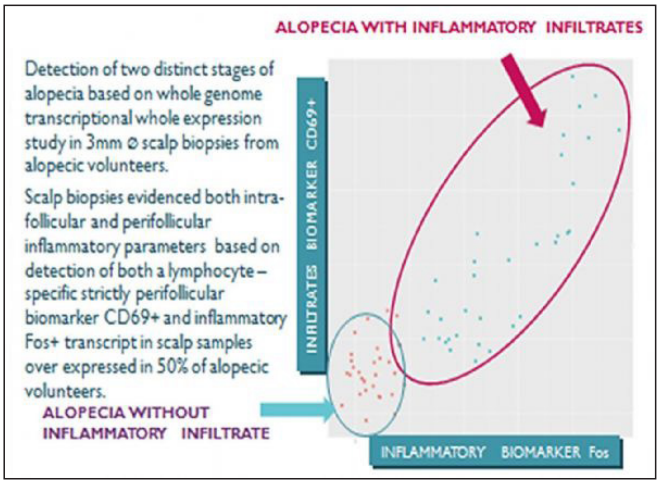

Figure 1. Evidence of inflammatory infiltrates and inflammatory status in scalp in vivo

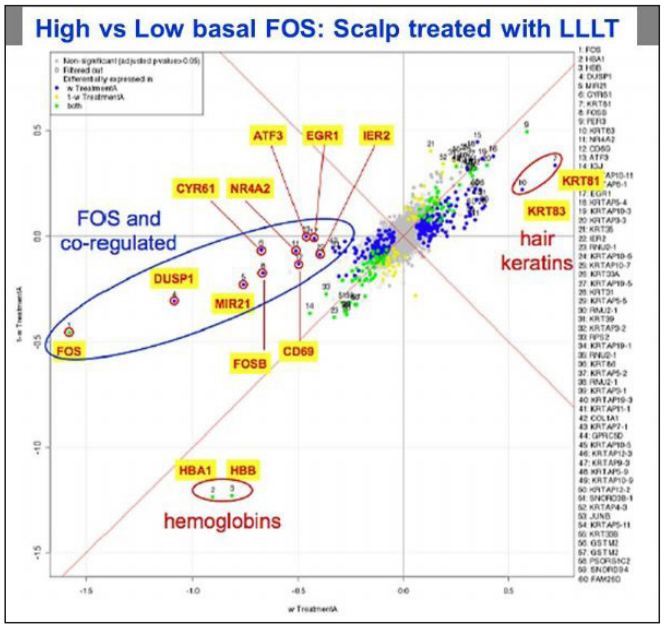

Figure 2. Detection of LLLT/ GentleWaves® down-regulated genes in vivo in scalp from men with androgenetic alopecia based on transcriptional whole expression study.

Left side of the figure indicates down-regulated genes (mostly inflammatory biomarkers) upon LLLT treatment of human scalp in vivo. Right side indicates up-regulated genes.

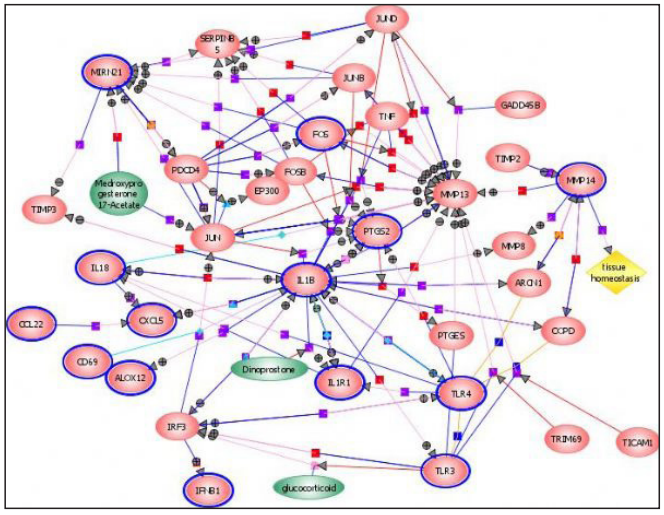

Figure 3. Interactive map of Inflammatory down-regulated genes (circled in blue) by GentleWaves®/LLLT treatment in vitro and in vivo based on transcriptional whole expression study in 3.6 mm Ø scalp biopsies and QRT PCR expression on cultured cells.

From the list of the down-regulated inflammatory genes in response to GentleWaves®/LLLT treatment, we built the shortest paths allowing the functional connection between these inflammatory biomarkers. The links existing between the entities was extracting using a text mining algorithm from public literature followed by a manual curation developed by Altrabio. mRNAs down regulated in vivo: CD69, Fos, mir21, IL-18; mRNAs down regulated in vitro: CCL22; IL-1B; IL-1R1, 12 Lipoxygenase, MMP14, CXCL5, PTGD2S, PTGS2, TLR3, TLR4, INFβ1

Table 1. Comparative expression of photoreceptors in human skin and hair and normal Human Epidermal Keratinocytes (NHEK).

Gene name	Skin biopsy	Scalp biopsy	Plucked Hair	NHEK
CRY1	7,1	7,0	6,6	6,8
CRY2	7,5	7,4	7,1	6,8
GNAT2	5,8	5,9	5,9	5,5
OPN1LW	5,2	5,3	5,6	4,7
OPN1SW	5,3	5,4	5,7	5,2
OPN3	6,8	7,1	6,8	7,6
OPN4	6,6	6,6	6,9	6,2
OPN5	5,5	5,3	5,8	5,0
RGR	5,8	5,8	6,0	5,9
RHO	5,8	5,9	5,9	5,5
RRH	5,6	5,4	5,6	4,5

Expression level: High / Middle / Low

By using genomic analysis with Affymetrix arrays both in vivo of human skin, hair biopsies or plucked hairs and in vitro in Normal Human Epidermal Keratinocytes (NHEK) we were able to establish a fine expression profiling of known potential photoreceptor in both skin and hair keratinized tissues. The highest expressed photoreceptors are CRY1, CRY2, OPN3 and OPN4 both in skin and hair biopsies as well as in NHEK suggesting a preferential epidermal expression of those photoreceptors in skin and hair.

PP-130

PROTECTIN D1$_{N\text{-}3\ DPA}$ AND RESOLVIN D5$_{N\text{-}3\ DPA}$ ARE EFFECTORS OF INTESTINAL PROTECTION

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Introduction. The resolution of acute inflammation is an active process orchestrated by specialized pro-resolving lipid mediators (SPM) that limit the host response within the affected tissue and promote homeostasis. The persistence of inflammatory signals, irrespectively of the etiopathogenesis, is the main feature of chronic inflammatory conditions as inflammatory bowel diseases (IBDs). A breakthrough was recently made with the description of a new biosynthetic pathway for docosapentaenoic acid (n-3 DPA) conversion to novel SPMs by both human and murine leukocytes (Dalli J, Sci Rep. 2014;4:6726). The aim of this study was to investigate presence and effect of n-3 DPA-derived SPM in intestinal inflammation.

Methods. Targeted LC/MS/MS metabololipidomic was used to quantify lipid mediators derived from n-6/n-3 polyunsaturated fatty acids (PUFA) in human colon biopsies. Male C57Bl/6 mice were subjected to colitis (5 days with 2.5% DSS in drinking water followed by 3 days water). Inflammation was assessed by monitoring colon shortening, wall thickness, myeloperoxidase activity and macroscopic/microscopic damage. Mesenteric intra-vital microscopy was performed to assess post-ischemic granulocyte recruitment (30' splanchnic ischemia followed by 90' reperfusion). Human neutrophil-endothelial interactions were assed by flow chamber assay.

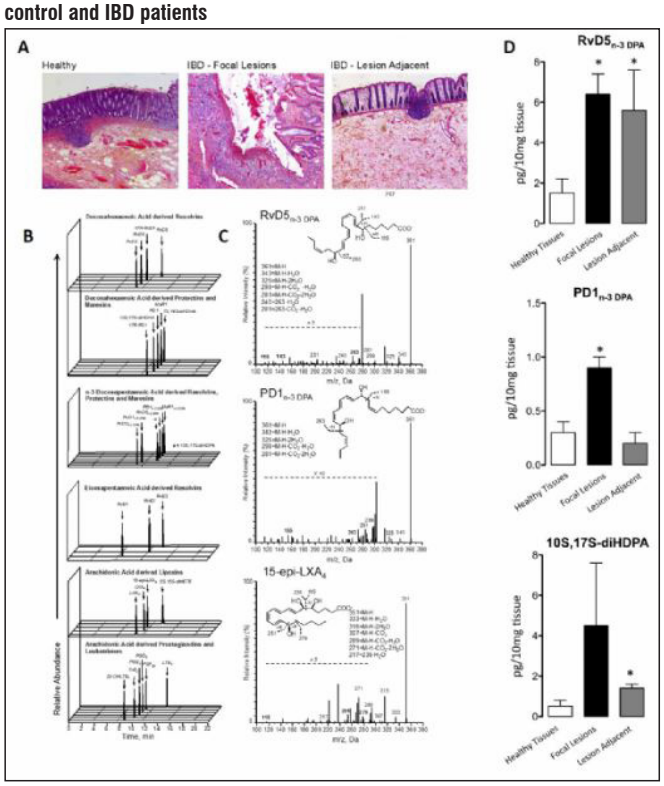
Results. Using lipid mediator profiling we identified and quantified lipid mediators from n-6/n-3 PUFA metabolomes in controls and IBD biopsies. LTB4, PGE2 and TxB2 concentrations

were increased in biopsies harvested from damaged areas of the IBD colon samples as compared to healthy tissues. In these biopsies we also identified SPM from the n-3 DPA metabolome including PD1n-3 DPA and RvD5n-3 DPA. Systemic treatment of mice with PD1n-3 DPA or RvD5n-3 DPA (0.3 μg/mouse i.p. daily for 8 days) significantly prevented colon length reduction and reduced colon wall thickness, MPO activity and macroscopic/microscopic colon damage. Intra-vital microscopy demonstrated that treatment with PD1n-3 DPA or RvD5n-3 DPA (0.1 μg prior to reperfusion) decreased number of adherent and emigrated leukocytes in mesenteric ischemia-reperfusion. The relevance of these results to human was tested assessing the ability of these molecules to regulate human neutrophil-endothelial interactions under flow. Incubation of human neutrophils with of PD1n3 DPA or RvD5n3 DPA (from 10 pM to 100 nM) significantly reduced their adhesion and transmigration onto TNF-α-activated endothelial monolayers compared with cells incubated with vehicle alone.

Conclusion. In the present study we establish the production of n-3 DPA derived mediators in colon inflammation with human tissues. We also found that PD1n-3 DPA and RvD5n-3 DPA, are anti-inflammatory and tissue protective in setting of intestinal inflammation regulating neutrophil and endothelial cell responses to injury/inflammation.

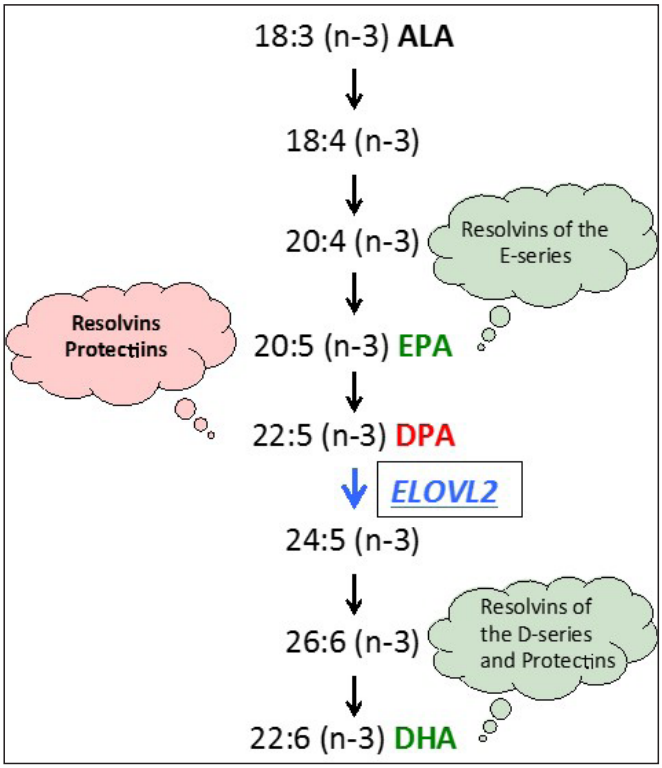
Keywords: specialized pro-resolving mediators, omega-3 fatty acids, resolution of inflammation

Identification of n-3 DPA pro-resolving mediators in colon biopsies from control and IBD patients



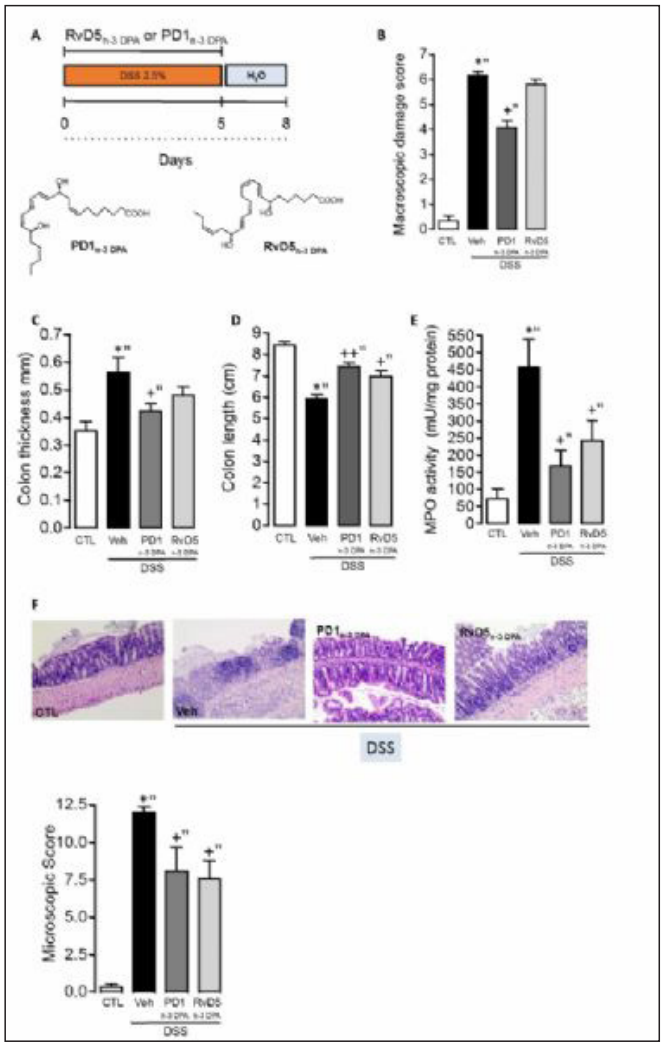
Colon biopsies were obtained from control and IBD patients (A) H&E staining of human colon biopsies. (B-C) LM-SPM profiles were obtained using LC-MS-MS based lipid mediator profiling. (B) Multiple reaction monitoring (MRM) chromatograms for identified mediators (C) Representative MS-MS spectra employed for the identification of n-3 DPA resolvin D5 n-3 DPA protectin D1 and 15-epi Lipoxin A4. Results are representative of n=30 colon biopsies from 21 patients. (D) RvD5n-3 DPA, PD1n-3 DPA and 10S,17S-diHDPA levels. *p < 0.05 IBD (focal lesions or lesion adjacent) versus the healthy tissues, one-way ANOVA followed by Dunnett's post-hoc test.

n-3 PUFA synthesis and metabolism



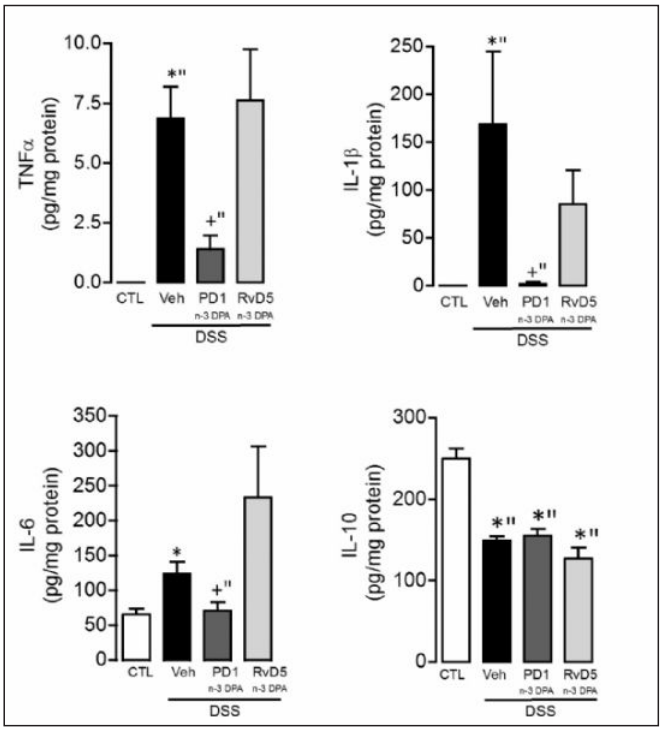
In mammals, alpha-linolenic acid (9Z, 12Z, 15Z-octadecatrienoic acid; ALA) is converted via elongation and desaturation to eicosapentaenoic acid (EPA) and subsequently to docosahexaenoic acid (DHA). An intermediate in the conversion of EPA to DHA is n-3 docosapentaenoic acid (7Z,10Z,13Z,16Z,19Z-docosapentaenoic acid) or n-3 DPA, which carries 22 carbons and contains five double bonds (the first on carbon 7). E serie Resolvins are produced from EPA, D serie Resolvins, Protectins and Maresins from DHA. ELOVL2 controls the elongation process of PUFA with 22 carbons to produce 24 carbon precursors for DHA formation in vivo.

PD1n-3 DPA and RvD5n-3 DPA protect mice



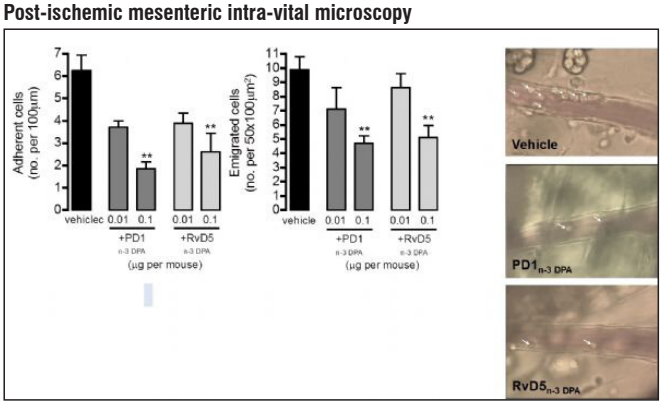
(A) C57bl6 mice had access to drinking water with or without 2.5% DSS for 5 days, then all switched to normal water for further 3 days prior to colon collection for analyses (day 8). Animals were treated i.p. with vehicle (100 μl PBS 0.01% EtOH) or with a dose of 0.3 μg PD1n-3 DPA or RvD5n-3 DPA daily from day 0 to 5. Chemical structure for PD1n-3 DPA and RvD5n-3 DPA. Control mice (CTL) received only drinking water. Day 8 analyses: **(B)** macroscopic damage, **(C)** wall thickness and **(D)** colon length. **(E)** Intestinal MPO activity. **(F)** Histological score (performed on colon sections stained with H&E) and images for representative colon sections. Data are reported as means ± SEM of n=6 mice per group. *P < 0.05 versus CTL. +P < 0.05,++P < 0.01 versus DSS vehicle-treated group, one-way ANOVA followed by Dunnett's post-hoc test.

PD1n-3 DPA reduces the production of pro-inflammatory cytokines following DSS-induced colitis



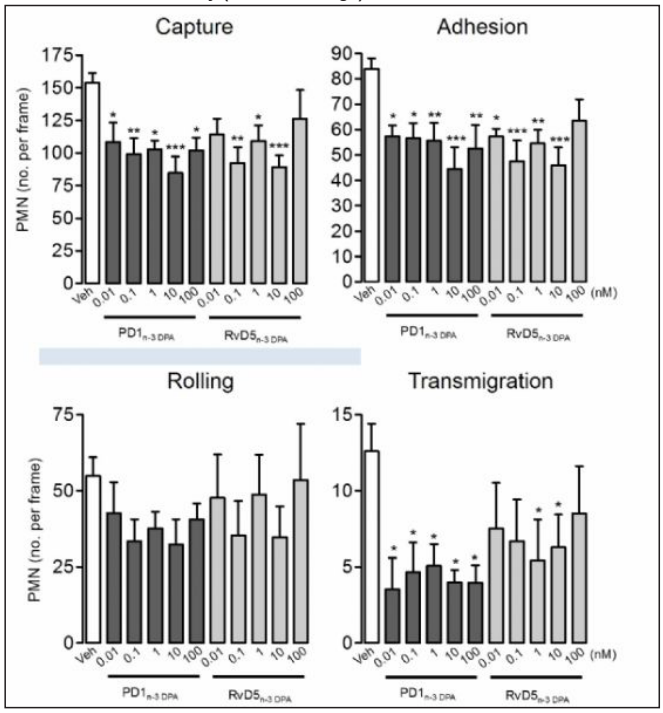
Cytokines (TNF-a, IL-1b, IL-6 and IL-10) levels were determined in colon homogenates using ELISA. Data are reported as means ± SEM of n=6 mice per group. *P < 0.05 versus CTL. +P < 0.05,++P < 0.01 versus DSS vehicle-treated group, one-way ANOVA followed by Dunnett's post-hoc test.

PD1n-3 DPA and RvD5n-3 DPA regulate neutrophil-endothelial interactions - Post-ischemic mesenteric intra-vital microscopy



C57bl6 mice were subjected to intestinal ischemia. Animals were treated i.v. prior to reperfusion with 0.01 or 0.1 μg PD1n-3 DPA, RvD5n-3 DPA or vehicle (100 μl PBS 0.01% EtOH). Post-capillary venules were imaged and recorded for offline quantitation of white blood cell interaction with the endothelium. Data are mean ± SEM of 6 mice per group. **P < 0.01 vs respective vehicle value, one-way ANOVA followed by Dunnett's post-hoc test.

PD1n-3 DPA and RvD5n-3 DPA regulate neutrophil-endothelial interactions- In vitro flow chamber assay (human settings)



Human PMNs were incubated with vehicle (PBS 0.1% EtOH), PD1n-3 DPA or RvD5n-3 DPA (0.01 to 100 nM) for 15 min at 37°C. Cells were then perfused over TNFa-stimulated endothelial cell monolayers at 1 dyne/cm2 for 8 min, and the extent of cell capture, adhesion, rolling and transmigration were quantified in 6 frames per treatment using Image Pro-plus software analysis. Results are mean ± SEM of n=6 donors, *P < 0.05,**P < 0.01,***P < 0.001 vs vehicle, one-way ANOVA followed by Dunnett's post-hoc test.

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REBAMIPIDE REGULATES GOBLET CELL DIFFERENTIATION AND ALLEVIATES RADIATION INDUCED COLITIS

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Background & aims: Radiation therapy application to abdominal region can cause a clinical problem called acute radiation enteritis. Goblet cells are columnar epithelial cells that specialize in secretion of high molecular weight called mucins that serve as protective and healing functions in the gut. The secretory mucin 2 (MUC2), which is the predominant structural component of the intestinal mucus layer, is expressed by goblet cells in the colon and related to inflammation. Rebamipide is a cytoprotective agent that is already in clinical used as anti-gastric ulcer drug. It is known that the application of topical rebamipide increases both the proliferation of goblet cells and the mucin secretion in ophthalmic disorders. In this study, we investigated whether rebamipide regulates MUC2 synthesis and is involved in alleviation of radiation-induced colitis.

Methods: Whole abdomen was single irradiated with total a dose of 13 Gy. Mice were treated with rebamipide (200, 400 mg/kg/day, oral administration) after irradiation for 6 days. Histology, bacteria translocation, expression of inflammatory cytokines, Muc2, Trefoil factor 3 (Tff3), Hairy and enhancer of split 1 (Hes1), and mouse atonal homolog 1 (Math1) gene was evaluated.

Results: Irradiated mouse showed massive infiltration of inflammatory cells, destruction of epithelium, and increased bacterial translocation. Otherwise, mouse treated with rebamipide reduced histological damage and improved barrier function with decrease bacteria translocation and anti-inflammatory effects. Also, we identified that expression of MUC2 positive goblet cells in irradiated colon was reduced, while the rebamipide treated intestine upregulated synthesis of MUC2. In mRNA levels, goblet cell differentiation makers (MUC2, TFF1) increased in rebamipide treated group. Also, we found that transcription factors (Hes1, Math1) related to goblet cell differentiation altered by rebamipide application.

Conclusion: The increase of MUC2 synthesis during acute inflammation by application of rebamipide may provide a new therapeutic strategy to manage radiation-induced intestinal injury.

Keywords: Rebamipide, radiation, colitis, goblet cell, MUC2

PP-132

TRANSGENIC MICE EXPRESSING HUMAN PROTEINASE 3 EXHIBIT SUSTAINED NEUTROPHIL-ASSOCIATED PERITONITIS

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Proteinase 3 (PR3) is a myeloid serine protease expressed in neutrophils, monocytes and macrophages. PR3 has a number of well-characterized pro-inflammatory functions including cleaving and activating chemokines, controlling cell survival and proliferation and when presented on the surface of apoptotic neutrophils, PR3 can disrupt the normal anti-inflammatory reprogramming of macrophages following the phagocytosis of apoptotic cells. To better understand the function of PR3 in vivo, transgenic mice were generated expressing human PR3 (hPR3Tg). During zymosan-induced peritonitis, hPR3Tg mice displayed an increased accumulation of neutrophils within the peritoneal cavity compared to WT control mice with no difference in the recruitment of macrophages, B or T lymphocytes. Mice were also subjected to cecum ligation and puncture, a model used to induce peritoneal inflammation through infection. hPR3Tg mice displayed decreased survival rates in acute sepsis, associated with an increased neutrophil extravasation. This decreased survival and increased neutrophil accumulation was associated with the cleavage of Annexin A1 (AnxA1), a powerful anti-inflammatory protein known to facilitate the resolution of inflammation. Additionally, neutrophils from hPR3Tg mice displayed enhanced survival during apoptosis compared to controls and this may also contribute to the increased accumulation observed during the later stages of inflammation. Taken together, our data suggests

that hPR3 plays a pro-inflammatory role during acute inflammatory responses by affecting both neutrophil accumulation, survival and the resolution of inflammation.

Keywords: Proteinase 3, Neutrophils, Annexin A1, Inflammation, Sepsis

PP-133

REBAMIPIDE INHIBITS MMP9 AND ATTENUATES THE BARRIER DISRUPTION IN RADIATION ENTERITIS

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Intestinal barrier disruption was usually seen as the early stage of radiation enteritis in cancer patient with radiation therapy. The integrity of the epithelial barrier is regulated by junctional complexes of intestinal epithelial cells. However, MMP9 induced by pro-inflammatory cytokines degrades junctional complexes during inflammation. The goal of this study was to determine the effect of rebamipide on inflammation and barrier function of small intestine after radiation exposure.

C57BL/6 mice were exposed to 13 Gy X-ray at the abdominal area. Subsequently, irradiated mice were treated with rebamipide for experimental periods. Intestinal barrier function was analyzed using bacterial translocation assay. The irradiation group presented significantly elevated bacterial translocation to mesenteric lymph nodes had reached a maximum on day 6 and regenerated on day 10 post irradiation. Increased mRNA levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 and decreased junctional molecules such as occludin and claudin-3 were observed in small intestine after irradiated 6 days. Rabamipide treated mice revealed dramatic recovery of intestinal barrier function and junctional molecules. The increased mRNA levels of pro-inflammatory cytokines were suppressed in rebamipide treated mice. Moreover, MMP-9, an enzyme involves in degradation of junctional complexes was also suppressed in the small intestinal tissues of rebamipide-treated groups.

In conclusion, rebamipide could ameliorate inflammation in the small intestine and improve the tight junctional structure between epithelial cells in radiation enteritis.

Keywords: Barrier function, inflammation, Intestine, MMP9, Radiation

PP-134

INHIBITION OF INFLAMMASOME ACTIVATION IMPROVES LUNG ACUTE INJURY INDUCED BY CARRAGEENAN IN A MOUSE MODEL OF PLEURISY

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The NLRP3 inflammasome is a molecular pathway activated by a wide range of cellular 'danger' to elicit innate immune defences through the activation of Caspase-1 and the maturation of pro-inflammatory cytokines as IL-1β and IL18. The expression of NRLP3 is abnormally elevated in numerous human inflammatory diseases, including pulmonary diseases. An injection of carrageenan into the pleural cavity triggered an acute inflammatory response: tissue damage, inflammatory exudates, leukocyte infiltration and increased MPO activity. Additionally, carrageenan led to the expression of nuclear factor-κB (NFκB), large increase of ATP release and generation of reactive oxygen species (ROS). All these stimuli assembled the NRLP3/ASC/Caspase-1 complex and the released of the pro-inflammatory cytokines IL-1β and IL18. The aim of this study was to assess the effect of BAY 11-7082 (30 mg/Kg i.p.) or Brilliant Blue G (BBG 45.5 mg/Kg i.p.), two inflammasome blocking agents, in a mouse model of carrageenan-induced pleurisy. Moreover, carrageenan injection modulated the expression of anti-oxidant genes and enzymes such as NRF2 and MnSOD, up-regulated inducible nitric oxide synthase (iNOS), nitrotyrosine levels, induced poly-ADP-ribosyl polymerase (PARP), as well as triggered apoptosis (Bax and Bcl-2 expression) in the lung tissues. Treatments with BAY 11-7082 or BBG 1 hour after carrageenan injection attenuated pulmonary membrane thickening and polymorphonuclear leukocytes infiltration, reduced NFkB translocation in the nucleus and inhibited the assembly of the NRLP3/ASC/Caspase-1 complex. BAY 11-7082 or BBG administrations also down-regulated iNOS, nitrotyrosine and PARP expression and inhibited carrageenan-induced apoptosis. In conclusion we demonstrate that treatments with inflammasome blocking agents significantly reduce the development of acute lung injury carrageenan-induced.

Keywords: BAY 11-7082, BBG, pathway

PP-135

PEA/PLD ASSOCIATION, REDUCE INFLAMMATORY PROCESS ASSOCIATED TO EXPERIMENTAL MOUSE MODEL OF ATHEROSCLEROSIS, INDUCED BY CAROTID ARTERY LIGATION

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Atherosclerosis, a disease of the large arteries, is the primary cause of heart disease and stroke. In westernized societies, it is the underlying cause of about 50% of all deaths. Increasing evidence has highlighted the roles of oxidative stress and inflammation in the promotion of atherosclerotic disease. Palmitoylethanolamide (PEA), an endogenous fatty acid amide belonging to the N-acyl ethanolamine family, has anti-inflammatory and neuroprotective effects. However, PEA lacks direct capacity to prevent formation of free radicals. Polydatin (PLD) that is a natural precursor of resveratrol has antioxidant activity. Thus, the combination of PEA and PLD could have beneficial effects on inflammatory process and oxidative stress. The aim of this study was to investigate the effect of PEA in association with PLD in murine model of atherosclerosis induced by carotid artery ligation. This model shows that 14 days after carotid artery ligation there is a significant structural change within the vessel, and that there is an important involvement of the inflammatory pathway in the progression of this disease. In this study we demonstrated that PEA/ PLD association treatment reduces atherosclesosis lesion, adhesion molecules (ICAM-1 (vehicle 7.5 ± 0.7 treatment 2 ± 0.1, p< 0.05), V-CAM (vehicle 6.5 ± 0.7 treatment 2.5 ± 0.7, p< 0.05)) expression, proinflammatory cytokines (TNF-α (vehicle 6 ± 0.1 treatment 2 ± 1.41, p< 0.05), IL-1β (vehicle 7 ± 0.1 treatment 1.5 ± 0.7, p< 0.05)) production, iNOS (vehicle 23914,89 ± 1063,071 treatment 14762,48 ± 3955,329, p< 0.05) and PAR formation (vehicle 7 ± 1.41 treatment 2 ± 0.1, p< 0.05), NF-kB expression (vehicle 23631,95 ± 7257,002 treatment 275,028 ± 106,1056, p< 0.05) and apoptosis (BAX (vehicle 6 ± 0.1 treatment 1.5 ± 0.7, p< 0.05), Fas-L (vehicle 22853,57± 959,0419 treatment 12836,61 ± 915,4346, p< 0.05)) activation. Our results show that treatment with PEA/PLD 30 mg/Kg is able to reduce vascular damage and attenuates the inflammatory process

Keywords: Palmitoylethanolamide, Polydatin, Atherosclerosis, Inflammation

PP-136

LIPID MEDIATOR CLASS-SWITCHING DOWNSTREAM OF PGE_2 DETERMINES THE OUTCOME OF INFLAMMATION RESOLUTION *IN VIVO*

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Rationale and hypothesis: Successful inflammation resolution is critical to prevent chronic inflammatory disease and permanent tissue damage. Few treatments are available for inflammatory disease. Therefore, there is a need for new understanding of the molecular mechanisms and signalling pathways involved in inflammation resolution to allow us to exploit them therapeutically. Inflammation resolution is tightly regulated, including by signalling between neutrophils and macrophages. Neutrophils can either die at inflammatory sites by apoptosis and be cleared by macrophages, or can be removed from inflammatory sites by altered migratory patterns (reverse migration). We hypothesised that lipid mediator signalling molecules, produced through the arachidonic acid pathway and acting on leukocytes determine the outcome of inflammation in response to tissue injury.

Objectives: The objectives were to study the effects of anti-inflammatory lipid mediators, PGE_2, Alox12 and Alox15, and assess gene expression during inflammation in an *in vivo* zebrafish injury model, to elucidate how lipid mediators regulate neutrophil removal from the wound site.

Methodology: Tail transection on transgenic zebrafish larvae (3 days post-fertilisation) was performed, recruiting neutrophils and macrophages to the wound site. Larvae were treated with prostaglandin E2 (PGE_2) or lipoxinA4 (LXA4), a lipoxygenase product. Neutrophil counts and reverse migration assays were subsequently performed. Inhibitors for PGE_2 receptor EP4 and Alox15 were used to elucidate potential signalling pathways. Gene expression via qPCR/*in situ* analysis was performed on tail-transected larvae at 2, 4, 6 hours post-injury to assess LOX expression.

Findings: Signalling by the eicosanoid PGE_2, through EP4 receptors, is necessary and sufficient to drive inflammation resolution, decreasing neutrophil numbers at the wound site. Inhibition of the anti-inflammatory lipoxygenase Alox15 significantly delays inflammation resolution, with neutrophils persisting at wound sites 24 hours after injury. qPCR reveals an increase in *alox12* following tail transection. Furthermore, a downstream product of *alox12*, LXA4, induces earlier reverse migration of neutrophils away from the wound site. These data indicate that PGE_2 produced following injury increases Alox12 production and therefore elevated LXA4 levels, may allow neutrophils at the wound site to migrate away from the inflammatory site, and that lipid mediator pathways are highly important in successful inflammation resolution. This pathway may be amenable to therapeutic manipulation for the treatment of inflammatory conditions.

Keywords: Inflammation, lipid-mediators, zebrafish, PGE2, lipoxinA4

PP-137

ROLE OF S-NITROSOGLUTATHIONE REDUCTASE (GSNOR) ON INFLAMMATION

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It has been known that inflammatory stimuli such as Lipopolysaccharide (LPS) induce inducible nitric oxide (NO) synthase (iNOS) in various tissues and iNOS can sequentially induce inflammation. One of the actions of NO is S-nitrosylation of proteins, a covalent attachment of NO moiety to reactive cysteine thiols, which can affect functions of many proteins. S-nitrosoglutathione (GSNO) reductase (GSNOR) is an enzyme which decomposes GSNO and downregulates protein S-nitrosylation. We have already reported that GSNOR knockout (KO) mice have a more severe myocardial dysfunction after LPS challenge. However, the role of GSNOR on inflammation has not been fully elucidated.

In this study, we examined the influence of GSNOR on inflammation. First, we investigated in a mouse sepsis model. LPS (10 mg/kg) injected intraperitoneally in wild type (WT) and KO mice, and after 6 hr, expression levels of cytokines in various tissues were determined. GSNOR in WT mice was expressed mainly in liver, kidney and heart, but lower levels of GSNOR also was detected in brain, lung and small intestine. WT mice treated with LPS resulted in reduction of GSNOR expression along with a strong induction of inflammatory cytokines, TNF-α, IL-1β and IL-6 in liver, kidney and heart. In liver, LPS-induced TNF-α, IL-1β expression were significantly decreased in KO mice compared with WT mice, whereas IL-6 expression was not affected by GSNOR deficiency. In kidney, LPS-induced IL-1β expression was only decreased in KO mice compared with WT mice. Next as ex vivo study, we elucidated the role of GSNOR in bone marrow derived macrophages (BMM) form WT and KO mice in more detail. BMM from KO mice inhibited LPS-induced TNF-α, IL-1β and IL-6 expression, as compared with WT mice. Consistently, knockdown of GSNOR by siRNA inhibited cytokine expression induced by LPS, in cultured RAW264.7 cells. On the other hand, we also found that IL-6 expression was predominantly induced in heart of LPS-treated WT mice, and further induction was observed in LPS-treated KO mice. Moreover, GSNOR deficiency increased cardiac protein S-nitrosylation levels after LPS challenge. These results indicate that S-nitrosylation of some proteins is involved in induction of heart failure in sepsis and GSNOR deficiency might cause deterioration of inflammation through further upregulation of protein S-nitrosylation in heart. Our findings identify GSNOR as a potential molecular target in heart failure. Importantly, GSNOR deficiency in macrophages, however, also plays an important role in suppression of inflammation. Although GSNOR inhibitor is considered as a therapeutic drug for inflammation disease such as asthma, it is important to provide drug delivery system having excellent drug migration properties to the appropriate tissue.

Keywords: Nitric Oxide, S-nitrosylation, S-nitrosoglutathione reductase, Cytokine, inflammation

PP-138

ENHANCED EXPRESSION OF NLRP3 INFLAMMASOME, INTERLEUKIN-1B, AND INTERLEUKIN-18 IN SJÖGREN'S SYNDROME

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Backgroud: Sjögren's syndrome is a systemic autoimmune disease characterized by lymphocyte infiltration and subsequent dysfunction of exocrine glands, such as the salivary gland, lacrimal gland, and other glands in the skin, vagina, and gastrointestinal system, finally leading to dryness in the exocrine glands and dysfunction in the affected organs and tissues. The precise mechanism of Sjögren's syndrome remains unclear.

Objective: The aim of this study was to identify the association of NLRP3 inflammasome-induced inflammation with disease activity and damage in Sjögren's syndrome.

Methods: A total of 25 female patients with Sjögren's syndrome and 25 sex-matched, healthy controls were consecutively enrolled. The mRNA expression levels of NLRP3, ASC, caspase-1, interleukin-1β (IL-1ββ, and IL-18 in peripheral blood mononuclear cells (PBMCs) were measured, as well as serum IL-1β and IL-18 protein expression levels. The EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) and Sjögren's Syndrome Disease Damage Index (SSDDI) were also evaluated.

Results: Patients with Sjögren's syndrome group showed higher expression of mRNA IL-1β and IL-1β at the protein level than controls ($p < 0.001$ and $p = 0.001$, respectively). The mRNA levels of caspase-1 and ASC were significantly increased in patients with Sjögren's syndrome compared to controls ($p = 0.021$ and $p = 0.008$, respectively). The mRNA levels of IL-1β and IL-18 were positively correlated with the mRNA level of NLRP3 ($r = 0.549$, $p < 0.001$ and $r = 0.298$, $p = 0.036$, respectively). Additionally, serum IL-β1 protein levels were positively associated with mRNA levels of caspase-1 in patients with Sjögren's syndrome ($r = 0.311$, $p = 0.028$). Based on the SSDDI scores, patients with damage (SSDDI >= 1) had higher IL-1β and NLRP3 mRNA expression compared to patients without damage (SSDDI = 0) ($p = 0.005$ and $p = 0.016$, respectively). Patients with disease activity (ESSDAI >= 1) showed higher IL-18 mRNA expression than inactive patients ($p = 0.023$).

Conclusion: This study confirmed that NLRP3 inflammasome-mediated inflammation might be implicated in the pathogenesis of Sjögren's syndrome.

Keywords: Sjögren's syndrome, NLRP3, Inflammasome, Interleukin-1β, Interleukin-18

PP-139

THE ANTIOXIDANT ACTIVITY OF PISTACHIOS REDUCES CARDIAC TISSUE INJURY OF ACUTE ISCHEMIA/REPERFUSION (I/R) IN DIABETIC STREPTOZOTOCIN (STZ)-INDUCED HYPERGLYCAEMIC RATS

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Diabetes mellitus represents one of the most important risk factors for the development of heart pathology. By itself it represents a source of vascular and heart dysfunction through formation of reactive oxygen species (ROS) and can compromise the recovery from cardiovascular diseases.

Myocardial infarction is the leading cause of death occurring after prolonged ischemia of the coronary arteries. Restore of blood flow is the first intervention against heart attack, although the process of restoring blood flow to the ischemic myocardium could induce additional injury. This phenomenon, termed myocardial ischemia-reperfusion (MI-R) injury, is characterized by the formation of oxygen radicals. Pistachios are excellent sources of antioxidants, such as lutein, β-carotene, and γ-tocopherol in addition to selenium, flavonoids, and proanthocyanidins. Pistachios have important glucose- and insulin-lowering effects and can improve the inflammatory profile by downregulating both the expression and the circulating levels of several metabolic risk markers.

The present study is aimed to evaluate the antioxidant properties of pistachios treatment on the inflammatory process associated to myocardial ischemia/reperfusion injury (I/R) in diabetic rats.

Left anterior descending (LAD) ligation has been used to induced MI-R injury in diabetic rats. Occlusion of coronary artery was prolonged for 30 min followed by 2 h reperfusion. Rats were pre-treated with either raw or roasted salted pistachios (30 mg/kg) 18h prior to the experimental procedure.

Here we demonstrated that treatment with raw pistachios reduced myocardial tissue injury (I/R 3.5±0.3 vs raw pistachios 2.0±0.2 p <=0.05), neutrophil infiltration (I/R 35.0±3.0 vs raw pistachios 22.0±2.0 p <=0.05), adhesion molecules (ICAM-1, (I/R 7.0±0.3 vs raw pistachios 3.0±0.2 p <=0.05) P-selectin (I/R 5.0±0.1 vs raw pistachios 2.0±0.1 p <=0.05)) expression, proinflammatory cytokines (TNF-α, I/R 11.0±2.0 vs raw pistachios 4.0±0.2 p <=0.05)IL-1β (I/R 12.0±0.2 vs raw pistachios 6±0.2 p <=0.05)) production, nitrotyrosine and PAR formation, NF-kB expression (I/R 23361.6 ± 3656.0 vs raw pistachios 1911.95 ± 770.0 p <=0.05)) and apoptosis (Bax, (I/R 29235.2 ± 3394.0 vs raw pistachios 2301.37 ± 869.1 p <=0.05)) Bcl-2) activation. The effect of raw pistachios was higher compared with roasted pistachios (results not shown).

This data have clearly showed a modulation of the inflammatory process, associated with MI-R injury, following administration of pistachios.

Keywords: Antioxidants, Cardiac Ischemia, Hyperglicemia

PP-140

ORAL ADMINISTRATION OF LINOLEIC ACID INDUCES NEW VESSEL FORMATION AND IMPROVES SKIN WOUND HEALING IN DIABETIC RATS

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Introduction: Impaired wound healing has been widely reported in diabetes. Linoleic acid (LA) accelerates the skin wound healing process in non-diabetic rats. However, LA has not been tested in diabetic animals.

Objectives: We investigated whether oral administration of pure LA improves wound healing in streptozotocin-induced diabetic rats.

Methods: Dorsal wounds were induced in streptozotocin-induced type-1 diabetic rats treated or not with LA (0.22 g/kg b.w.) for 10 days. Wound closure was daily assessed for two weeks. Wound tissues were collected at specific time-points and used to measure fatty acid composition, and contents of cytokines, growth factors and eicosanoids. Histological and qPCR analyses were employed to examine the dynamics of cell migration during the healing process.

Results: LA reduced the wound area 14 days after wound induction. LA also increased the concentrations of cytokine-induced neutrophil chemotaxis (CINC-2αβ), tumor necrosis factor-α (TNF-α) and leukotriene B4 (LTB4), and reduced the expression of macrophage chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 (MIP-1). These results together with the histological analysis, which showed accumulation of leukocytes in the wound early in the healing process, indicate that LA brought forward the inflammatory phase and improved wound healing in diabetic rats. Angiogenesis was induced by LA through elevation in tissue content of key mediators of this process: vascular-endothelial growth factor (VEGF) and angiopoietin-2 (ANGPT-2).

Conclusions: Oral administration of LA hastened wound closure in diabetic rats by improving the inflammatory phase and angiogenesis.

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Keywords: fatty acids, inflammation, cytokines, tissue repair, VEGF, transcription factors

PP-141

REGULATION OF BONE METABOLISM BY RESOLVIN D1

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Purpose: Resolvin-D1 (RvD1) is a derivative of omega-3 fatty acids and a potent anti-inflammatory agent synthesized during the resolution phase of inflammation. In human cartilage, we recently reported that RvD1 strongly inhibited a number of factors involved in inflammation, catabolism, oxidative stress, and apoptosis. Thus, the overall objective of this study is to further investigate its effects on bone metabolism.

Methods: First, murine macrophages RAW267.4 were used to assess osteoclasts (OC) recruitment and bone resorption. RAW264.7 cells were incubated with 50 ng/ml LPS with or without RvD1 (0 - 10 μM) for 48 hours. Cell viability was verified with the MTS test. OC phenotype markers, namely TRAP and cathepsin-K, were assessed by western blot, enzymatic staining and immunocytochemistry. Levels of TNF-α, IL-1β, IL-6, IL-10, were measured by ELISA, and PGE2 levels by EIA. NO release was measured by Greiss reaction.

To investigate bone resorption, RAW264.7 cells were seeded in hydroxyapatite plates, then treated with 50 ng/ml LPS with or without RvD1 (0.5 and 1 μM) for 48 hours. Plot formation was assessed by Von Kossa staining.

Second, human osteoblasts (Ob) were obtained from post-surgery discarded trabecular bone of osteoarthritic (OA) patients who underwent total knee arthroplasty. First passage human OA Ob were treated either with RvD1 (0.1 - 1 μM) alone, or with 20 nM VitD3 with or without RvD1 (0.1 - 1 μM), for 48 hours. Cell viability was evaluated with the MTS test. Alkaline phosphatase (PAL) activity and osteocalcin (OCN) release was determined by colorimetric reaction and ELISA, respectively.

Results: In RAW264.7 cells, our results clearly show that RvD1 strongly reduces OC recruitment and activation as indicated by the inhibition of TRAP and cathepsin K expression as well as TNF-α, IL-1β, IL-6, PGE2 and NO release, as well as the concurrent enhancement of IL-10 levels. Besides, RvD1 decreases bone resorption through the inhibition of plots formation in hydroxyapatite matrix. In human OA Ob, RvD1 partially decreases VitD3-induced PAL activity, while it maintains OCN expression at control levels.

Conclusions: Our in vitro results clearly show that RvD1 may play an important role in the regulation of bone metabolism. Additionally to our previous data, our findings suggest that RvD1 presents a novel and original perspective to musculoskeletal and bone diseases therapy.

Keywords: Resolvin D1, bone resorption, osteoclast, osteoblast

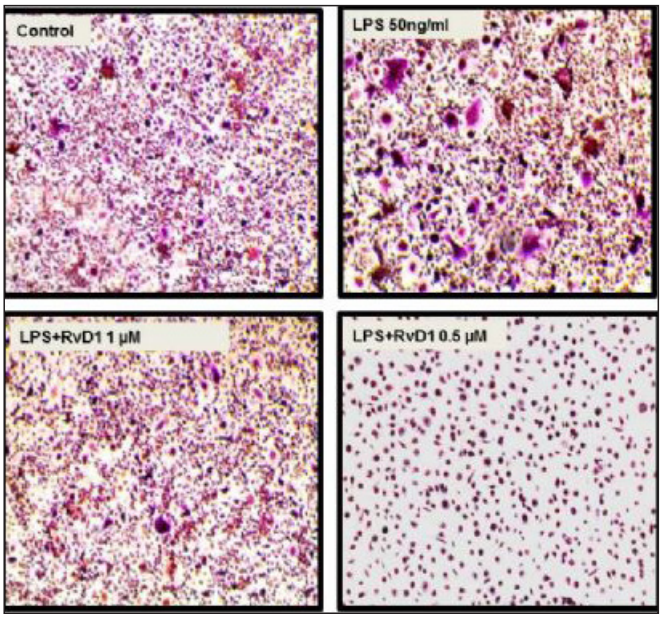

Figure 1. RvD1 inhibits osteoclasts recruitment

PP-142

EFFECTS OF CORTICOSTEROID AND ANTI-TNF IN MURINE COLLAGEN-ANTIBODY INDUCED ARTHRITIS

Dominic Poulin, Cornicelli Joe, Young Alan, Rashid Harunor, Lafond Jean François, Bédard Agathe, Samadfam Rana

Charles River Laboratories

The purpose of this study was to compare the efficacy of the TNF alpha blockers (Etanercept and Humira) with a corticosteroid (dexamethasone) in a murine model of collagen-antibody induced arthritis (CAIA). Etanercept is derived by introducing human DNA into Chinese hamster ovary cells, creating a genetically engineered protein. On the other hand, Humira uses fully human proteins and phage display technology to produce monoclonal antibodies. Both Etanercept and Humira are directed against TNF-α, a key player in rheumatoid arthritis.

Female BALB/C mice were assigned to naive or to CAIA groups. CAIA animals were further assigned to control, dexamethasone or anti-TNF treatments. Dexamethasone was given orally, daily after disease onset at 0.3mg/kg, Etanercept was given subcutaneously, twice weekly at 10 mg/kg and Humira was given intraperitoneally, every 3 days at 3 mg/kg. Clinical score and paw inflammation were evaluated. At necropsy, serum and paw samples were collected for cytokine analysis, and/or histopathology. Histopathologic assessment of the sampled paws was performed on all animals. Cytokine analysis was performed only for the Etanercept.

In the CAIA animals, clinical score and paw inflammation was observed starting on Day 5, peaked on Days 11 to 13 and then slowly decreased towards the end of the study (Days 21 to 22). Increases in serum cytokines were limited to IFN-γ, IL-1β, IL-5 and TNF-α. All cytokines (as well as CRP) analyzed in the paw extract samples were increased from 34 to 450%. TNF-α and IL-1β, were especially elevated. Histopathological changes associated with arthritis (inflammation, erosion, synovial hyperplasia, bone degeneration and periostal changes) were observed in CAIA control animals.

Etanercept reduced clinical score and paw inflammation by 25 to 50%, while dexamethasone reduced the same parameters by 50 to 75%. Humira had a slight effect (16% reduction) on paw volume from Day 14 onwards. Etanercept reduced all cytokines and CRP levels in the paw tissue, while the only serum cytokine affected by Etanercept was IL-1β. Humira, Etanercept and dexamethasone also reduced the total composite histopathological score by 44, 67 and 84%, respectively.

We conclude that both corticosteroid and anti-TNF therapies are effective in ameliorating the arthritic response in the CAIA model, albeit to varying extents.

Keywords: Arthritis, corticosteroid, anti-TNF, collagen-antibody

PP-143

EFFECTS OF CORTICOSTEROID IN THE RAT OVALBUMIN PULMONARY INFLAMMATION MODEL

Dominic Poulin, Alan Young, Joe Cornicelli, Harunor Rashid, Rana Samadfam

Charles River Laboratories

The Brown Norway Rat lung inflammation model is an established allergen dependent pharmacokinetic/pharmacodynamic (PK/PD) model that can be used to investigate the basic pathology of disease and to assess the in vivo efficacy of anti-asthma drugs.

Rats were assigned to naive or to ovalbumin (OVA) sensitized groups. The OVA animals were further assigned to vehicle or corticosteroid treatment. Rats were treated with betamethasone (0.003 to 3mg/kg, PO), dexamethasone (0.003 to 3mg/kg, PO) or budesonide (3mg/kg, PO). Two weeks following sensitization, animals were challenged with an aerosol of OVA by whole-body inhalation. Lungs were lavaged 6 to 48 hours after challenge and Broncho Alveolar Lavage Fluid (BALF) assessed for differential cell counts and inflammatory mediators (IFN, IL-1, IL-4, IL-5, IL-6, IL-10, IL-13, KC/GRO and TNF, MSD multiplex platform).

Significant increases in neutrophils (30 fold from 6 to 24 hours) and eosinophils (7 to 10 fold at 48 hours) were observed in the BALF of OVA challenged animals compared to control animals. Betamethasone and dexamethasone dose-dependently inhibited neutrophils (up to 91%) and eosinophils (up to 83%). Budesonide reduced neutrophils by 70% and eosinophils by 91%. Following OVA challenge, increases (up to 6 fold) in all mediators were seen in the BALF apart from KC/GRO (no increase), and TNF (80-fold increase). Dexamethasone at 3mg/kg decreased the cytokines levels in the BALF samples and were generally comparable to non-OVA levels.

Based on this data, it is considered treatment with budesonide, betamethasone and dexamethasone are efficacious in the Brown Norway Rat OVA-induced pulmonary inflammation model and maybe used as clinically relevant reference agents.

Keywords: Ovalbumin, corticosteroid, pulmonary inflammation

PP-144

GENISTEIN EFFECTS ON PRIMARY RAT CHONDROCYTES: POSSIBLE USE IN OSTEOARTHRITIS

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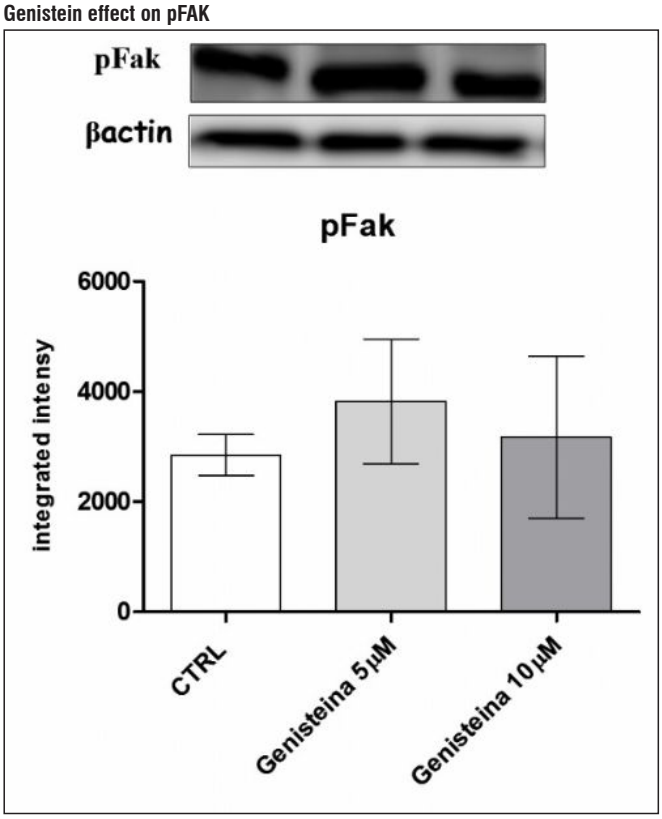
Osteoarthritis is characterized by a progressive degeneration of cartilages with consequent deterioration of subchondral bone. Wnt proteins affect cellular homeostasis by regulating cell proliferation, cell fate determination, and differentiation. Wnt5a is a representative of the Wnt family that activates the β-catenin-independent pathway. It has been shown that the articular joint development, including cartilage, bone, and joint cavities, are highly dependent on Wnt signaling. Genistein is an isoflavone with potential anti-inflammatory activity and is currently used to improve bone mass following menopause.

Rat primary chondrocytes have been obtained from the long bones of C57Bl6/J embryos and cultured in DMEM with different doses of genistein (5 and 10 μM) for 24hrs. Cells have been then used for western blot and real-time qPCR analysis. Genistein significantly affected the TGF-β/FAK/SMAD pathways and the expression of Wnt-5a at the dose of 5 μM.

The results suggest a role for this isoflavone for treating osteoarthritis.

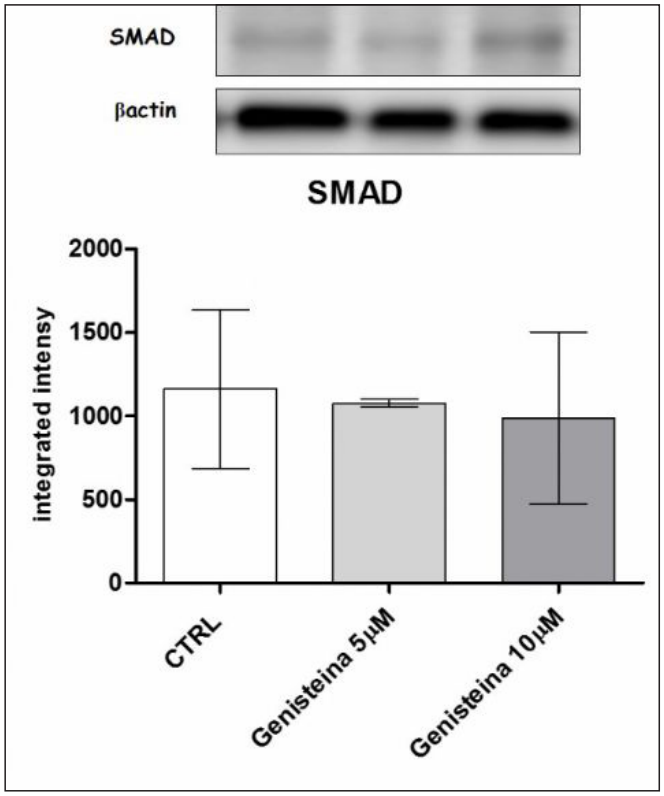
Keywords: chondrocyte, genistein, wnt-5a

Genistein effect on pFAK



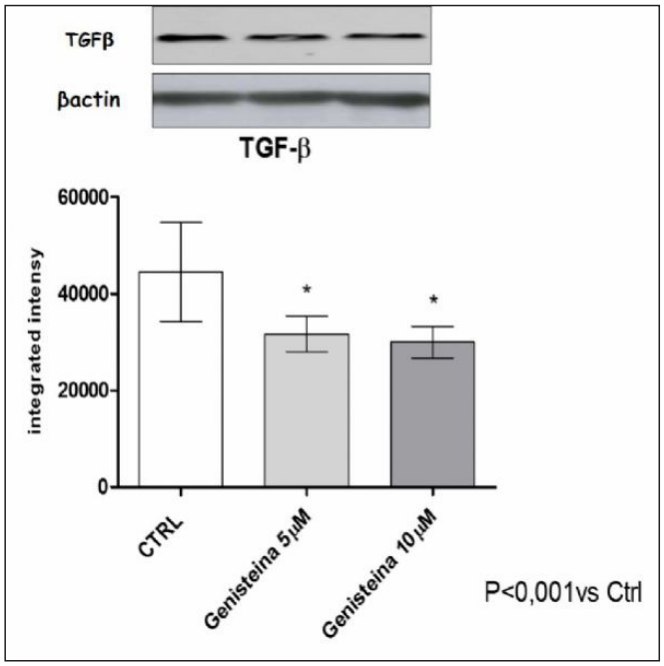
Western blot results obtained from chondrocytes treated with genistein. Bars represents mean and SD of 3 experiments.

Genistein effect on SMAD



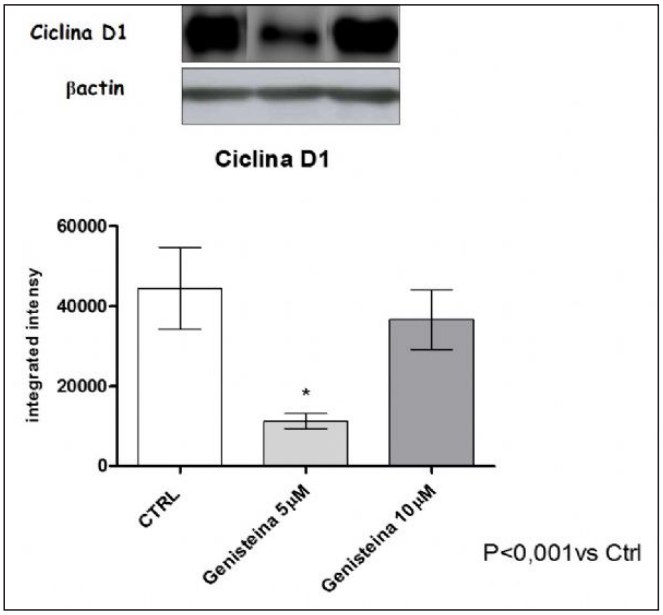
Western blot results obtained from chondrocytes treated with genistein. Bars represents mean and SD of 3 experiments.

Genistein effect on TGF-b



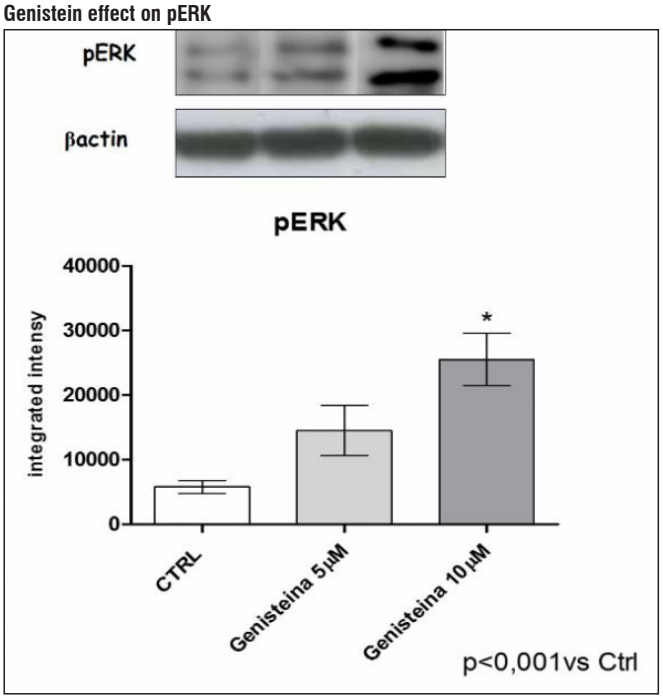
Western blot results obtained from chondrocytes treated with genistein. Bars represents mean and SD of 3 experiments.

Genistein effect on cyclinD1



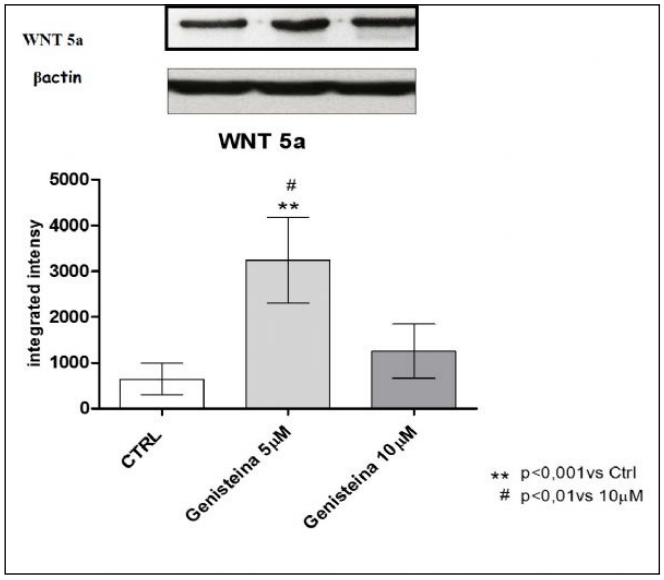
Western blot results obtained from chondrocytes treated with genistein. Bars represents mean and SD of 3 experiments.

Genistein effect on pERK



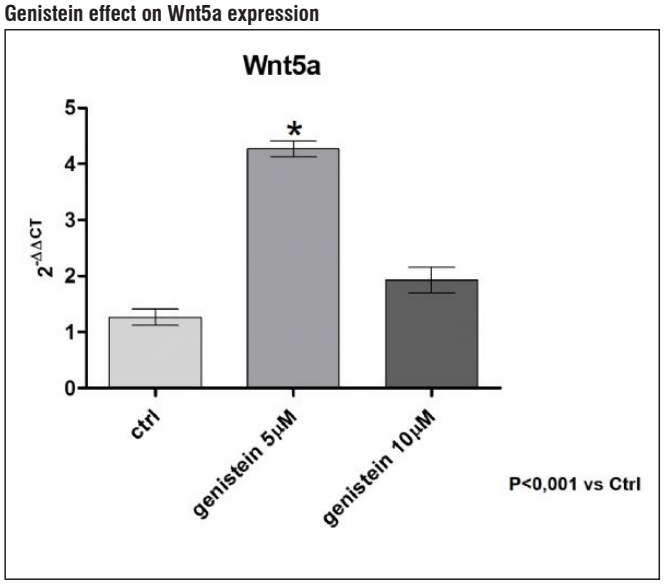
Western blot results obtained from chondrocytes treated with genistein. Bars represents mean and SD of 3 experiments.

Genistein effect on Wnt5a



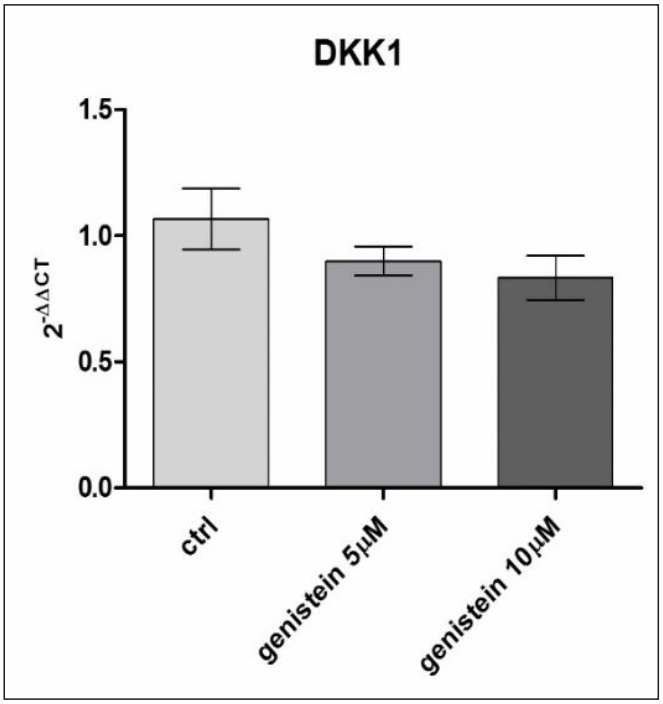
Western blot results obtained from chondrocytes treated with genistein. Bars represents mean and SD of 3 experiments.

Genistein effect on Wnt5a expression



RT-qPCR results obtained from chondrocytes treated with genistein. Bars represents mean and SD of 3 experiments.

Genistein effect on DKK1 expression



RT-qPCR results obtained from chondrocytes treated with genistein. Bars represents mean and SD of 3 experiments.

PP-145

PODOPLANIN EXPRESSION BY MESENCHYMAL STROMAL CELLS PROMOTES MIGRATORY CAPACITY

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[2]*Institute of Cardiovascular Sciences, College of Medical & Dental Sciences, University of Birmingham, Birmingham, United Kingdom*

Introduction: Podoplanin (gp38) is a mucin-type protein that is expressed by tissue resident stromal cells at sites of inflammation, in disorders such as rheumatoid arthritis and cancer. Recent evidence suggests that podoplanin can mediate anti-inflammatory functions at these sites in the presence of its ligand CLEC-2. Here we examined the expression pattern of podoplanin on mesenchymal stromal cells from umbilical cord (MSC) and the mechanism by which it influences MSC function.

Methods: Human MSC were obtained from healthy donors and screened for the expression of podoplanin using immunohistochemistry, flow cytometry and qPCR. To determine the effects of podoplanin on MSC migration and proliferation, cells were treated with siRNA before seeding on 8μm pore Transwell filters or 12-well plates respectively. In some experiments, MSC were treated with inhibitors against RhoA, ROCK, and Rac1 signalling over the course of the migration assay.

Results: Podoplanin was differentially expressed by MSC in a donor dependent manner. Three patterns of expression were observed: podoplanin negative MSC; podoplanin positive MSC; bimodal samples with a proportion of cells positive and a proportion expressing little or no podoplanin.

Functionally, podoplanin positive MSC migrated through 8μm pores more efficiently than podoplanin negative MSC. This trend was recapitulated by podoplanin knockdown in positive MSC, where siRNA reduced podoplanin gene and protein expression by 61% and 34% respectively at 72h post-transfection. Podoplanin siRNA transfection had no effect on MSC proliferation compared to controls. However, podoplanin siRNA significantly reduced MSC trans-filter migration compared to controls.

RhoA and ROCK inhibition reduced MSC trans-filter migration similarly for podoplanin positive or negative MSC. Interestingly, Rac1 inhibition had no effect on the podoplanin negative MSC but selectively reduced the migratory capacity of the positive cells.

Conclusion: Thus podoplanin expression plays an regulatory role in the migration of MSC, mediated through the Rac1 signalling pathway. Physiologically, expression of podoplanin could enhance the localisation of MSC to sites of tissue damage and inflammation, where they could exert their reparative and immunomodulatory effects.

Keywords: migration, podoplanin, gp38, stroma, mesenchymal stromal cells

PP-146

MONITORING OF PNEUMOCYSTIS JIROVECII ACQUIRED PNEUMONIA BY SPECIALIZED PRO-RESOLVING MEDIATORS

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It is now well described that ending inflammation is not a passive phenomenon only due to catabasis of pro-inflammatory mediators. Stopping circuits are controlled by the synthesis of new mediators, called lipoxins, resolvins, protectin and maresins. Those mediators are issued from polyunsaturated fatty acids and are actively involved in terminating inflammation and return to homeostasis. If specialized pro-resolving mediators are not produced, the good ending of the inflammatory process won't occur. Uncontrolled inflammation could then be due to a defect of resolution.

Nothing is known on these mediators during Pneumocystis pneumonia (PCP), even if inflammation plays a major role in the pathophysiology of PCP. While necessary for the control and elimination of Pneumocystis jirovecii (the causative opportunistic fungus for PCP), the host's inflammatory response can therefore lead to lung damage and may explain PCP severe prognosis with high mortality rates.

We describe here the development of a methodology using liquid chromatography-tandem mass spectrometry (LC/MS-MS). This technic allows concomitant quantification of low-level of inflammatory and resolutive lipid bioactive mediators (e.g. PGE2, LxA4, RvD1), inactive products (e.g. TxB2) and pathway biomarkers (e.g. 5-HETE).

We have applied this methodology to follow the resolution in severe patients developing PCP.

Among the seven evaluated patients, inflammatory PGE2 was quantifiable in 4 patients, detected in 2 patients (under the limit of quantification (LOQ)) and not detected in 1 patient (under the limit of detection (LOD)). Lipoxin B4 (and its isomer), a specialized pro-resolving mediator, was quantifiable in 2 patients and under the LOQ but detectable in the 5 remaining patients, suggesting that LXB4 is synthesised during PCP. RvD1 and RvD2, two others mediators known for actively stopping inflammation, were undetectable in all the patients.

Results of this pilot clinical study might thus orientate towards a defect of the control of inflammation during PCP due to a clear lack of synthesis of D1 and D2 resolvins.

Keywords: Inflammation, resolution, lung infection, biomarkers, specialized proresolving mediators, lipidomic

PP-183

EMERGING ROLE OF GABA IN GUT INFLAMMATION

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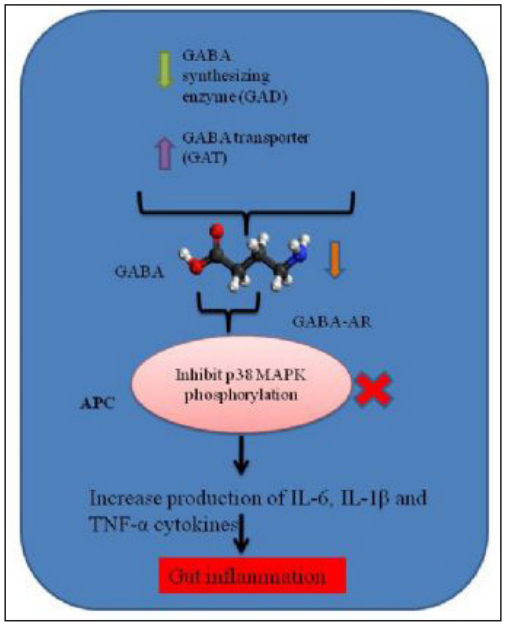
Recent evidences indicate that enteric nervous system (ENS) contributes in gut disorders. Neuropeptides produced by Enteric Nervous System (ENS) regulate inflammatory process via interaction between ENS and enteric immune System (EIS). Role of γ-aminobutyric acid (GABA) has been implicated in autoimmune diseases like multiple sclerosis, type1 diabetes and rheumatoid arthritis where they modulate the immune response but role in gut inflammation has not been defined. Ulcerative colitis (UC) and diarrhoeal predominant irritable bowel syndrome (IBS-D) both involve inflammation of gastrointestinal tract. UC is a chronic, relapsing and idiopathic inflammation of gut. IBS is a common functional gastrointestinal disorder characterised by abdominal pain, discomfort and alternating bowel habits. Mild inflammation is known to occur in IBS-D. Aim: Aim of this study was to investigate the role of GABA in UC as well as in IBS-D.

Materials-Methods: Blood and biopsy samples from UC, IBS-D and controls were collected. ELISA was used for measuring level of GABA in serum of UC, IBS-D and controls. RT-PCR analysis was done to determine GABAergic signal system in colon biopsy of UC, IBS-D and controls. RT-PCR was done to check the expression of proinflammatory cytokines. CurveExpert 1.4, Graphpad prism-6 software were used for data analysis. Statistical analysis was done by unpaired, two way student`s t-test. All sets of data were represented as mean± SEM. A probability level of $p < 0.05$ was considered statistically significant. Results and Conclusion: Significantly decreased level of GABA and altered GABAergic signal system was detected in UC and IBS-D as compared to controls. Significantly increased expression of proinflammatory cytokines was also determined in UC and IBS-D as compared to controls. Hence we conclude that insufficient level of GABA in UC and IBS-D leads to overproduction of proinflammatory cytokines which further contributes to inflammation. GABA may be used as a promising therapeutic target for treatment of gut inflammation or other inflammatory diseases.

Keywords: diarrheal predominant irritable bowel syndrome, γ-aminobutyric acid (GABA), inflammation, ulcerative colitis

Altered GABAergic signalling contributes to gut inflammation



Decreased expression of GAD and increased expression of GABA transporter resulting into low level of GABA. Dimnished level of GABA may not be able to inhibit p38 MAPK phosphorylation that further leads to uncontrolled production of proinflammatory cytokines and chronic inflammation

Signalling molecules and pathways

PP-147

ROLE OF TSPO LIGANDS ON GPCR AND TLR-4 PATHWAYS ON NEUTROPHILS

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TSPO (Translocator 18KDa; tryptophan-rich sensory protein oxygen sensor) is a constitutive outer mitochondrial membrane protein overexpressed in inflammatory cells during local or systemic processes. Nevertheless the role of the TSPO on inflammation is not understood. Here we investigated the role of TSPO ligands on neutrophil functions elicited by two different inflammatory pathways. Peritoneal neutrophils were isolated from male Balb-C mice, treated with the TSPO ligands diazepam, Ro5-486 or PK11195 (1,100 or 1000nM; 2 hours) and further stimulated with lipopolyssacharide from Escherichia coli (LPS), a binding of Toll-Like Receptor-4 (TLR4), or leukotriene B4 (LTB4), a ligand of G-protein coupled receptor (GPCR). LPS treatment did not overexpress TSPO on neutrophils, and pre-treatments with any TSPO ligand altered the secretion of cytokines, adhesion molecules expression, and the production of reactive oxygen and nitrogen species caused by LPS stimulation. Conversely, all TSPO ligands impaired the LTB4 actions, visualized by reductions on L-selectin shedding, β2 integrin overexpression, neutrophil chemotaxis, and actin filaments assembly. Nevertheless, the mechanisms of TSPO ligands on LTB4 induced neutrophil locomotion are distint, as diazepan treatment enhanced cofilin and did not modify Arp2/3 expressions, and Ro5-486 and PK11195 treatments reduced both cofilin and Arp2/3 expressions. Together, our data exclude a direct role of TSPO ligands on TLR-4 elicited pathways, and point out that TSPO activation inhibits GPCR inflammatory pathways on neutrophils, with relevant role on neutrophil inlfux into inflammatory sites.

Keywords: chemotaxis, cytokines, LPS, LTB4, cofilin, Arp2/3

PP-148

FERRIC CARBOXYMALTOSE DOES NOT INDUCE APOPTOSIS IN HUMAN ENDOTHELIAL CELLS: IMPLICATIONS IN CHRONIC KIDNEY DISEASE

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Introduction: Several iron preparations are clinically delivered intravenously in chronic kidney disease to prevent anaemia. A major cause of death in chronic kidney disease patients is accelerated cardiovascular complications. While toxic blood components are known to be involved, the treatments (including iron preparations) are known to cause damage to the endothelial cells lining the blood vessels. This could exacerbate the vascular damage. A variety of iron agents are used in the clinic with iron sucrose the most common one. More recently, ferric carboxymaltose (FCM) has been successfully introduced and there is evidence this is less toxic to vascular cells.

Objectives: This project aimed to measure functional effects of iron sucrose and FCM on human endothelial cells in vitro. Apoptosis was determined by TUNEL assay and annexin V staining. Mechanisms involved were explored by quantifying phosphorylation of p38 MAP kinase and expression of anti-apoptotic Bcl-2 and pro-apoptotic Bax.

Methods: Human umbilical vein endothelial cells (HUVEC) were seeded at $2x10^5$ cells per well in a 6 well plate and stimulated with 50 μg/ml of either iron sucrose or FCM over a 24 hour time course. Apoptosis was quantified by TUNEL and annexin V assay. p38 MAP kinase phosphorylation, Bcl-2 and Bax expression was quantified by western blotting.

Results: Following both TUNEL and annexin V assays, apoptosis was induced by iron sucrose (24h, 50μg/ml) whereas FCM did not stimulate a significant increase in apoptosis compared to untreated control. Both iron sucrose and FCM induced phosphorylation of p38 MAP kinase with maximum phosphorylation observed at 60-120 min treatment. The apoptosis detected following iron sucrose treatment was significantly reduced when cells were pre-treated with the specific p38 inhibitor, SB203580.

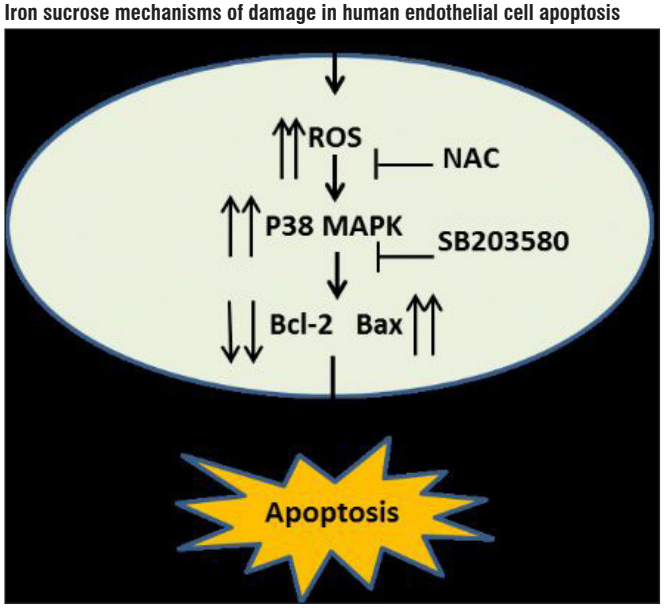
Expression of Bcl-2 protein expression was reduced at 24 hours following treatment with iron sucrose, whereas Bax expression was significantly increased following the same treatment. No changes were observed in either protein following FCM treatment. Pre-treatment with SB203580 partially reversed the changes in expression observed following iron sucrose treatment.

Conclusions: The data presented confirms that clinical treatments to ameliorate anaemia can cause damage to

endothelial cells lining the blood vessels in vitro. From the data obtained, there are clear benefits to using FCM compared to iron sucrose. These effects need to be confirmed on human kidney blood vessels in vitro and in vivo but indicate usage of FCM may be a more beneficial treatment for anaemia in chronic kidney disease patients in the clinic.

Keywords: chronic kidney disease, endothelial cells, apoptosis, p38 MAP kinase, bcl-2, bax

Iron sucrose mechanisms of damage in human endothelial cell apoptosis



This schematic indicates that p38 MAP kinase is upstream of changes in bcl-2/bax expression which contribute to apoptosis induced by iron sucrose in human umbilical vein endothelial cells

PP-149

YGHJ (SSLE), A SECRETED LIPOPROTEIN OF NEONATAL SEPTICEMIC *E. COLI* INDUCES TLR2 MEDIATED INFLAMMATION IN MACROPHAGES WITH THE INVOLVEMENT OF NFKB AND MAP KINASE SIGNALING

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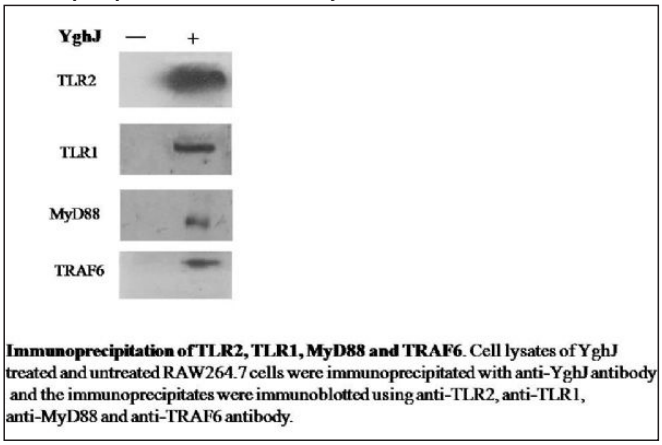
Division of Pathophysiology, National Institute of Cholera and Enteric Diseases (NICED), Kolkata, India

YghJ, also known as SslE of diverse *E. coli* pathotypes is a cell surface associated and secreted lipoprotein harbouring M60 metalloprotease domain. Importantly, YghJ was identified as a potential vaccine candidate for extraintestinal pathogenic *E. coli* which provided nearly complete protection from sepsis in a mouse model. Sepsis is characterized by the overproduction of diverse mediators such as proinflammatory cytokines and so far no factor other than LPS is known to stimulate the secretion of these cytokines. In our earlier study we first showed that YghJ from neonatal septicemic *E. coli* (NSEC) can trigger the production of a wide array of cytokines in murine macrophages which are essentially implicated in the pathogenesis of sepsis. Furthermore we found that YghJ can cause extensive tissue damage in mouse ileum. However, the signaling pathway leading to YghJ mediated proinflammation is still unexplored. In this study, we first report that YghJ induces overexpression and activation of both TLR2 and TLR1 on mouse macrophage RAW264.7 cells. Immunoprecipitation shows that YghJ specifically binds to TLR2/TLR1 heterodimer and also activates MyD88 and TRAF6. Moreover, YghJ induces phosphorylation of ERK1/2, JNK1/2 and p38 and nuclear translocation of p50-p65 to trigger the MAP Kinase and NFκB signaling pathways. Pretreatment of macrophages with specific inhibitors against each signal molecule showed the involvement of ERK1/2, JNK1/2 and NFκB in the secretion of IL-1 (IL-1α and IL-1β) cytokines and involvement of p38 and ERK1/2 in the secretion of TNF-α. To further confirm the prerequisite of TLR2 in YghJ mediated proinflammation, RAW264.7 cells were transfected with TLR2 siRNA. Interestingly, YghJ induced activation of both NFκB and MAP Kinase signaling pathways was completely abrogated in TLR2 siRNA-transfected cells. Moreover, significant reduction of YghJ stimulated cytokine secretion was found in TLR2 knockdown cells. In addition, our study also demonstrates that YghJ enhances cellular ROS level, the overproduction of which is associated with inflammatory diseases. The secreted cytokines and chemokines from activated macrophages play important role in discrimination of M1 and M2 polarization of macrophages as well as attract and activate T lymphocytes. The cytokines (IL-1α, IL-1β, TNF-α) induced by YghJ are representative of type I polarization (M1). In our present study we further validate the M1 polarization of macrophages by investigating the chemokine profile induced by YghJ. Interestingly, our results demonstrate that YghJ stimulate the production of chemokines representative of M1 polarization such as MIP-1α, MIP-1β, RANTES, CXCL9 and CXCL10. In conclusion, YghJ, the secreted lipoprotein of NSEC activates TLR2/TLR1 dependent proinflammation in mouse macrophages with the specific involvement of NFκB and MAP kinase signaling pathways. The cytokine and chemokine profile illustrates that YghJ induces M1 polarization.

Keywords: YghJ (SslE), Toll-like receptor2 (TLR2), proinflammation, NFκB, MAP kinase, Macrophages

Immunoprecipitation of TLR2, TLR1, MyD88 and TRAF6



Cell lysates of YghJ treated and untreated RAW264.7 cells were immunoprecipitated with anti-YghJ antibody and the immunoprecipitates were immunoblotted using anti-TLR2, anti-TLR1, anti-MyD88 and anti-TRAF6 antibody.

PP-150

BOERHAVIA DIFFUSA ATTENUATES INFLAMMATION AND BONE DAMAGE THROUGH INHIBITING THE ACTIVATION OF NUCLEAR FACTOR-KB IN WISTAR RATS WITH ADJUVANT-INDUCED ARTHRITIS

Ritu Karwasra, Surender Singh, Yogendra Kumar Gupta
All India Institute of Medical Sciences, New Delhi

Background: *Boerhavia diffusa* Linn. belongs to family Nyctaginaceae is an herbaceous plant and known as 'Punarnava' in Hindi, used as herbal medicine for pain relief and various other infirmities. It is used as green leafy vegetable in many Asian and African countries and traditionally documented as one of the important ingredient in many herbal formulations for treatment of inflammatory disorders.

Objective: The present study was carried out to investigate the effects of *Boerhavia diffusa* root extract on joints swelling, arthritic index, production of inflammatory mediators and nuclear factor-κB (NF- κB) activation in rats with adjuvant-induced arthritis (AIA).

Materials-Methods: *Boerhavia diffusa* root extract (50, 100 and 200 mg/kg) and indomethacin (3mg/kg) were administered orally, daily during the study period of 21 days and their effect on joint dysfunction was evaluated by measuring joint diameter, levels of GSH, MDA, SOD and tissue histology in Complete Freund's Adjuvant model. In order to reveal the immunological influences of *Boerhavia diffusa*, the serum levels of TNF-α was measured and the histopathological changes in the joints of AIA rats were investigated. Furthermore, the involvement of the NF-κB, VEGF, TNF-R1, IL-1, IL-6, IL-10 in the effect of *Boerhavia diffusa* was examined by immunohistochemical examination in ameliorating Rheumatoid arthritis. Joint dysfunction was evaluated by radiographic analysis and the damage to subchondral bone was evaluated by radiographic scoring.

Results: *Boerhavia diffusa* at a dose of 200 mg/kg significantly decreased the joint diameter ($p<0.001$) and arthritic index ($p<0.01$) in CFA-induced rats. It also markedly decreased the serum level of TNF-α as compared to CFA-control group. It was observed that the root extract of Boerhavia diffusa at a dose of 200 mg/kg effectively down regulate the expression of NF-κB and angiogenesis marker VEGF along with decrease in overexpression of pro-inflammatory cartilage cytokines (TNF-R1, IL-1, IL-6). *Boerhavia diffusa* significantly ($p<0.001$) restored the imbalance by decreasing pro-oxidant (MDA) and increasing anti-oxidant (SOD and GSH) levels in the tissue indicating its role in inhibition of damage caused by ROS. The histopathological and radiographic analysis, particularly of metatarsal, phalanges and ankle joint in *Boerhavia diffusa* (200 mg/kg) group showed protective effect against adjuvant-induced arthritis

Conclusion: The tuberous roots of *Boerhavia diffusa* attenuates the inflammation development through inhibiting the NF-κB-mediated proinflammatory cytokines production in AIA rats. Thus, our study provides the ground evidence of *Boerhavia diffusa* in treatment of Rheumatoid arthritis to be used clinically.

Keywords: Cytokines, Boerhavia diffusa, Angiogenesis, Experimental arthritis, NF-κB pathway

PP-151

6-HYDROXY-5,7-DIMETHOXY-FLAVONE SUPPRESSES THE NEUTROPHIL RESPIRATORY BURST VIA SELECTIVE PDE4 INHIBITION TO AMELIORATE ACUTE LUNG INJURY

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Over-activated neutrophils produce enormous oxidative stress and play a key role in the development of acute and chronic inflammatory diseases. 6-Hydroxy-5,7-dimethoxy-flavone (UFM24), a flavone isolated from the Annonaceae Uvaria flexuosa, showed inhibitory effects on human neutrophil activation and salutary effects on lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice. UFM24 potently inhibited superoxide anion generation, reactive oxidants, and CD11b expression, but not elastase release, in N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLF)-activated human neutrophils. However, UFM24 failed to scavenge superoxide anion and inhibit the activity of subcellular NADPH oxidase. fMLF-induced phosphorylation of protein kinase B (Akt) was inhibited by UFM24. Noticeably, UFM24 increased cyclic adenosine monophosphate (cAMP) concentration and protein kinase (PK) A activity in activated human neutrophils. PKA inhibitors significantly reversed the inhibitory effects of UFM24, suggesting that the effects of UFM24 were through cAMP/PKA-dependent inhibition of Akt activation. Additionally, activity of cAMP-related phosphodiesterase (PDE)4, but not PDE3 or PDE7, was significantly reduced by UFM24. Furthermore, UFM24 attenuated neutrophil infiltration, myeloperoxidase activity, and pulmonary edema in LPS-induced ALI in mice. In conclusion, our data demonstrated that UFM24 inhibits oxidative burst in human neutrophils through inhibition of PDE4 activity. UFM24 also exhibited significant protection against endotoxin-induced ALI in mice. UFM24 has potential as an anti-inflammatory agent for treating neutrophilic lung damage.

Keywords: acute lung injury, 6-hydroxy-5, 7-dimethoxy-flavone, neutrophil, oxidative stress, phosphodiesterases 4

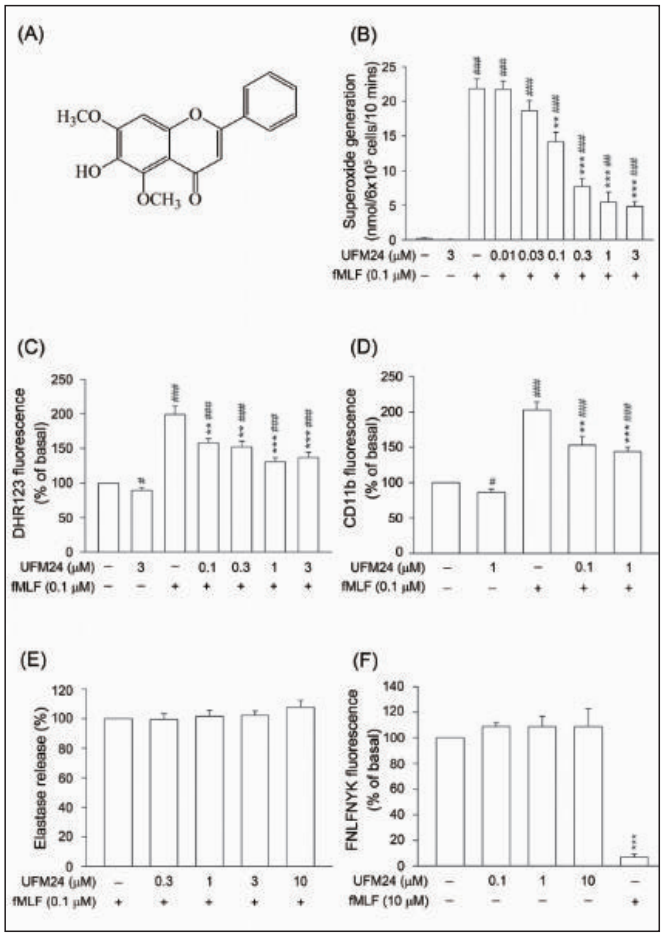

Figure 1. UFM24 significantly inhibits superoxide anion release, intracellular reactive oxidant formation, and integrin expression by fMLF-stimulated neutrophils.

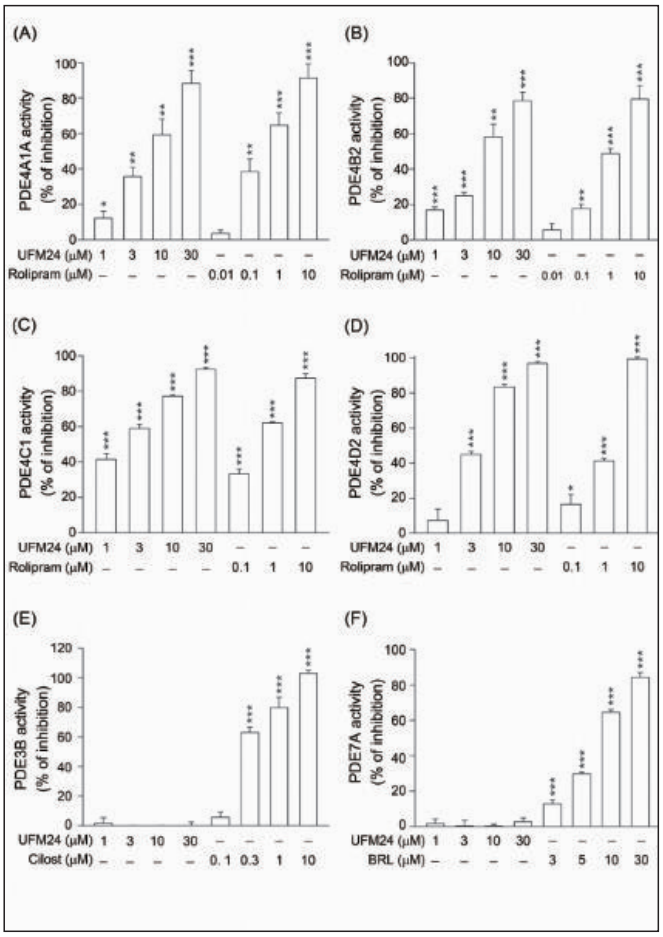

Figure 3. UFM24 selectively inhibits the enzymatic activities of PDE4 in a survey of neutrophilic cAMP-specific PDE subtypes.

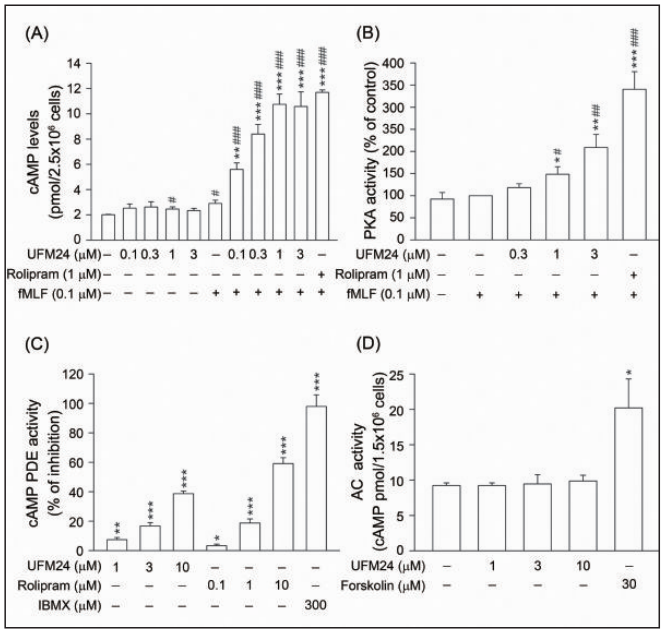

Figure 2. UFM24 increases cAMP levels and PKA activities as well as inhibits cAMP-specific PDE activities in neutrophils.

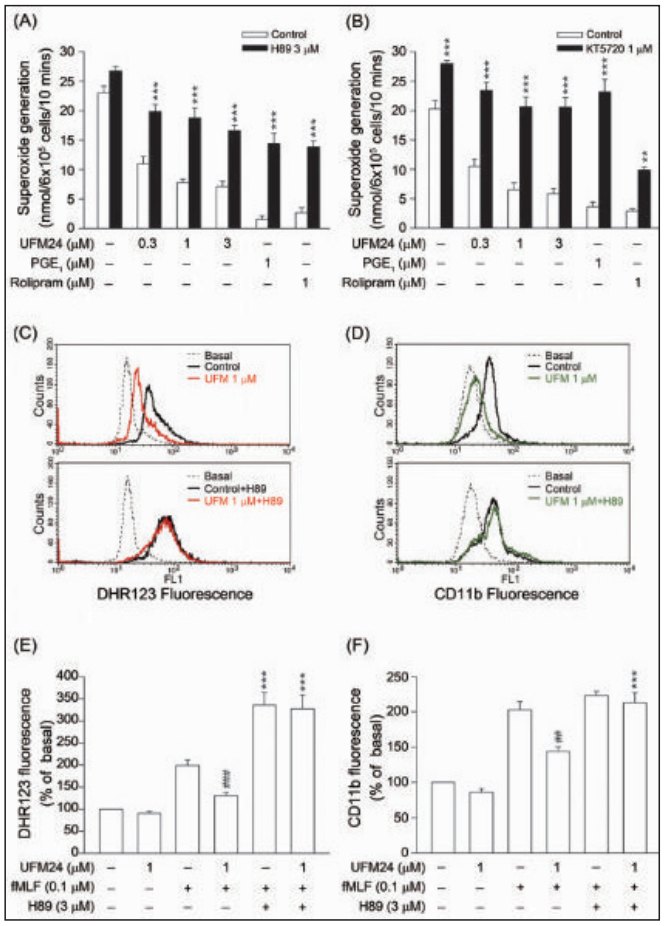

Figure 4. PKA signal regulates the inhibitory effects caused by UFM24.

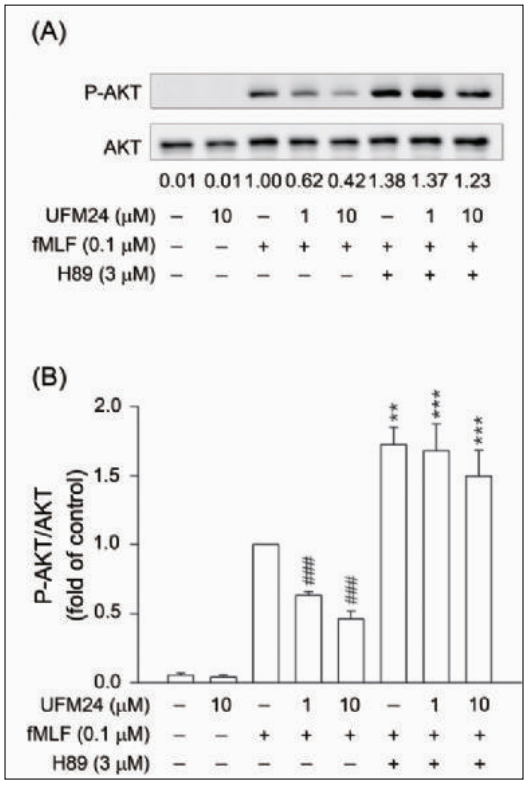

Figure 5. UFM24 inhibits the phosphorylation of Akt in fMLF-activated human neutrophils.

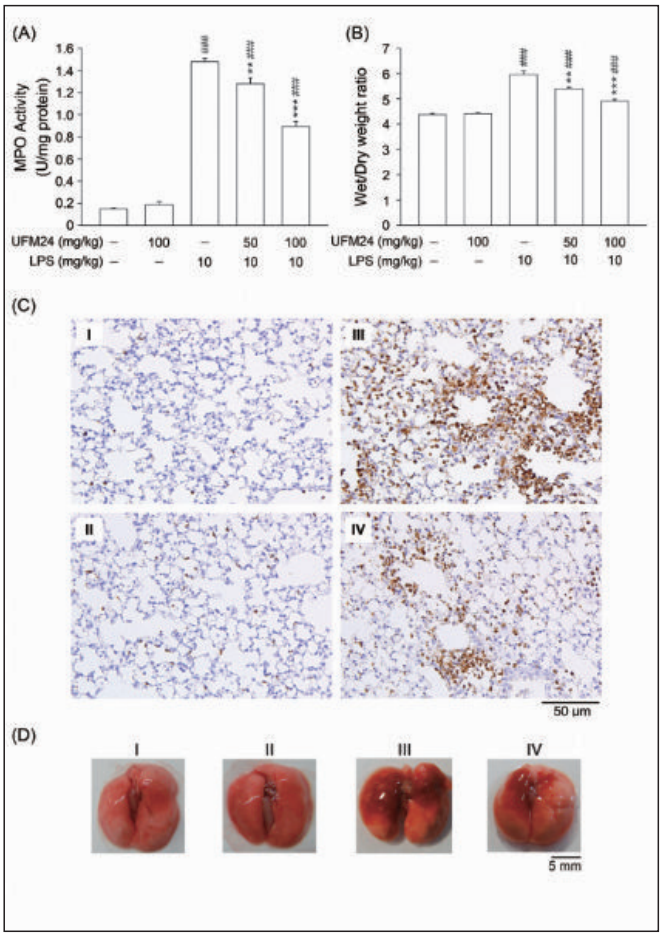

Figure 6. Effects of UFM24 on MPO activities, wet-to-dry-weight ratios, and histopathologic examination of mouse lungs in LPS-induced ALI.

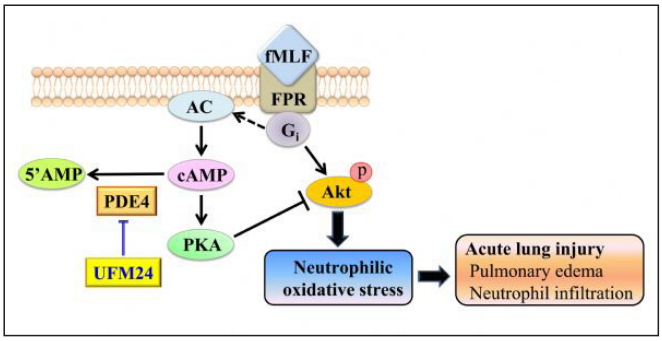

Figure 7. A diagram showing that UFM24 inhibits O2•- generation, reactive oxidant formation, and integrin expression in fMLF-activated neutrophils and protects against LPS-induced acute lung injury. UFM24 is a specific inhibitor of PDE4. The anti-inflammatory effects of UFM24 are mediated by inhibiting PDE4 and then enhancing cAMP/PKA-dependent inhibition of Akt activation. UFM24 ameliorates LPS-induced acute lung injury in mice.

PP-152

AMPK-RELATED KINASE MPK38/MELK STIMULATES GPR120-MEDIATED ANTI-INFLAMMATORY PATHWAY BY PHOSPHORYLATING GPR120 AT SER226

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G-protein coupled receptor 120 (GPR120) functions as a receptor for omega-3 fatty acids (ω-3 FAs), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and plays a crucial role in anti-inflammatory response. Here, we show that murine protein serine-threonine kinase 38/maternal embryonic leucine zipper kinase (MPK38/MELK), a member of the AMP-activated protein kinase (AMPK)-related family of kinases, physically interacts with and phosphorylates GPR120 at Ser226. The phosphorylated GPR120 causes an anti-inflammatory effect by inhibiting NF-kB transcriptional activity in monocytic RAW 264.7 cells. The importance of GPR120 Ser226 phosphorylation by MPK38/MELK in GPR120-mediated anti-inflammatory pathway was also confirmed using CRISPR/Cas9-mediated GPR120 (S226A) knockin cells. These results raise the possibility that MPK38/MELK may be a therapeutic potential target in inflammatory diseases including obesity and type 2 diabetes.

Keywords: G-protein coupled receptor 120 (GPR120), anti-inflammation, murine protein serine-threonine kinase 38/maternal embryonic leucine zipper kinase (MPK38/MELK)

PP-153

TERMINALIA CHEBULA SUPPLEMENTATION WITH TACROLIMUS ATTENUATES THE OVEREXPRESSION OF PRO-INFLAMMATORY CARTILAGE CYTOKINES AND MODULATES ANTIOXIDANT STATUS IN ADJUVANT ARTHRITIC RATS

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Backgrund: *Terminalia chebula* Retz. commonly known as "Harad" in Hindi and "Black Myrobalan" in English, belongs to family Combretaceae has been reported to possess antinflammatory and antiarthritic activities. Tacrolimus has also been shown to suppress inflammation and has been approved for rheumatoid arthritis in various countries like Canada, Europe etc. The major side effect associated with tacrolimus is its nephrotoxicity.

Objective: The present study was designed to evaluate the combinatory effect of tacrolimus (TAC) and *Terminalia chebula* (TC) on the progression of adjuvant-induced arthritis in rats.

Material-Methods: Arthritis was induced in rats by a single injection of 0.1 ml of Complete Freund's adjuvant into the subplantar surface of left hind paw of rat. Rats were treated with TAC (3 mg/kg) daily, TC (200 mg/kg) daily, and combination of TAC and TC daily for a period of 21 days. The changes in paw swelling, histopathological, immunohistochemical and radiographic analysis was assessed to evaluate the antiarthritic effect of TAC in combination with TC. Lipid peroxidation and antioxidant enzyme activities in joint tissue homogenate were performed to observe the modulation of antioxidant status along the expression of different pro-inflammatory cartilage cytokines like IL-1, IL-6, TNF-R1, VEGF and NF-κB. Level of TNF-α was assessed using enzyme-linked immunosorbent assay.

Results: The combination of TAC and TC significantly ($p<0.001$) decreases arthritic index and paw diameter of hind paw on day 21, as compared to CFA injected rats. Significant decrease in lipid peroxidation (MDA) and increase in antioxidant (GSH, SOD) was noted in combination group. Moreover, histopathological examination demonstrated that combination of TAC and TC significantly reduced the synovial hyperplasia and inflammatory cells invasion in joints tissues. Combination of TAC and TC significantly ($p<0.001$) decrease serum TNF-alpha level and modulate the expression of pro-inflammatory cytokines, evaluated immunohistochemically.

Conclusion: TAC significantly attenuates arthritis, however, higher doses of TAC possess significant adverse effects such as nephrotoxicity so to reduce the dose dependent side effects, we evaluate the combinatory effect in which TC significantly alleviates rheumatoid arthritis and reduces side effects owing to decline in levels of oxidative markers. Therefore, TC can serve as a useful adjuvant and promote the safe use of TAC in the management of Rheumatoid arthritis.

Keywords: Anti-oxidant, Inflammation, Rheumatoid arthritis, Terminalia chebula

PP-154

ATTENUATION OF TACROLIMUS INDUCED NEPHROTOXICITY BY POMEGRANATE VIA DOWN REGULATING PRO-INFLAMMATORY CYTOKINE AND INHIBITING APOPTOSIS IN WISTAR RATS

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Background: Pomegranate (*Punica granatum* L. Punicaceae) is one of the oldest known edible fruit, represents a phytochemical reservoir with a high potential medicinal value. This fruit, grown mainly in the mediterranean region, has been used for centuries to treat many ailments such as parasitic and microbial infections, ulcers, diarrhea, hemorrhage and dysentery. It is traditionally documented as one of the important ingredient in many herbal formulations for treatment of nephrotoxicity and urinary disorders.

Objective: The aim of the study was to investigate the nephroprotective activity of pomegranate hydroalcoholic extract in tacrolimus induced kidney injury model. Second, if so, could the attenuation of overexpression of proinflammatory cytokines be the plausible mechanism in ameliorating experimental nephrotoxicity via modulating inflammatory signaling pathway.

Materials-Methods: Wistar rats (n=6) were allocated into six groups constituting normal control, tacrolimus-induced, pomegranate rind extract in doses 50, 100 and 200 mg/kg and pomegranate per se group, administered orally for a period of 4 weeks. Subcutaneous injection of tacrolimus (5 mg/kg) was administered daily to all groups, except normal control and pomegranate per se group. On day 28, tacrolimus resulted in substantial nephrotoxicity in Wistar rats with significant ($p<0.001$) elevation in serum creatinine and blood urea nitrogen, decline in the concentrations of reduced glutathione and superoxide dismutase, elevation in TNF-α level in renal tissues. Pathological changes in renal tissues were examined by histopathology and rate of apoptosis (caspase-3, Bcl-2, Bax) or

expression of pro-inflammatory cytokines (TNF-R1, IL-1, IL-6) was detected by immunohistochemical analysis.

Results: Pomegranate at a dose of 200 mg/kg b.w significantly ($p<0.001$) ameliorates increased serum creatinine and BUN. In parallel to this, it also exhibits anti-apoptotic activity through reduction of active caspase-3 in renal tissues. Pomegranate treatment also reduced serum TNF-α level, oxidative stress and expression of pro-inflammatory markers (TNF-R1, IL-1, IL-6).

Conclusion: Findings of the study indicate that pomegranate is effective in mitigating tacrolimus-induced nephrotoxicity possibly, in part through its antioxidant activity.

Keywords: Apoptosis, Pomegranate, Inflammation, Nephrotoxicity, Oxidative stress

PP-155

P300 AND C/EBPB-REGULATED IKKB/NF-KB ACTIVATION ARE INVOLVED IN THROMBIN-INDUCED IL-8/CXCL8 EXPRESSION IN HUMAN LUNG EPITHELIAL CELLS

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Asthma and chronic obstructive pulmonary disease (COPD) are common chronic lung inflammatory diseases. Thrombin and interleukin (IL)-8/C-X-C chemokine ligand 8 (CXCL8) play critical roles in lung inflammation. Our previous study showed that c-Src-dependent IκB kinase (IKK)/IκBα/nuclear factor (NF)-κB and mitogen-activated protein kinase kinase kinase 1 (MEKK1)/extracellular signal-regulated kinase (ERK)/ribosomal S6 protein kinase (RSK)-dependent CAAT/enhancer-binding protein β (C/EBPβ) activation are involved in thrombin-induced IL-8/CXCL8 expression in human lung epithelial cells. In this study, we aimed to investigate the roles of p300 and C/EBPβ-reliant IKKβ/NF-κB activation in thrombin-induced IL-8/CXCL8 expression. Thrombin-induced increases in IL-8/CXCL8-luciferase activity and IL-8/CXCL8 release were inhibited by p300 small interfering (siRNA). Thrombin-caused histone H3 acetylation was attenuated by p300 siRNA. Stimulation of cells with thrombin for 12 h resulted in increases in IKKβ expression and phosphorylation in human lung epithelial cells. However, thrombin did not affect p65 expression. Moreover, 12 h of thrombin stimulation produced increases in IKKβ expression and phosphorylation, and IκBα phosphorylation, which were inhibited by C/EBPβ siRNA. Finally, treatment of cells with thrombin caused increases in p300 and C/EBPβ complex formation, p65 and C/EBPβ complex formation, and recruitment of p300, p65, and C/EBPβ to the IL-8/CXCL8 promoter. These results together with our previous results imply that p300-dependent histone H3 acetylation and C/EBPβ-regulated IKKβ expression and NF-κB activation contribute to thrombin-induced IL-8/CXCL8 expression in human lung epithelial cells. Results of this study will help clarify C/EBPβ signaling pathways involved in thrombin-induced IL-8/CXCL8 expression in human lung epithelial cells.

Keywords: chronic lung inflammatory diseases, Thrombin, IL-8/CXCL8, p300, C/EBPβ, NF-κB

PP-156

A STRUCTURE-BASED INTERACTION ANALYSIS OF THE CYTOPLASMIC REGULATOR OF THE CHEMOKINE RECEPTOR FROUNT AND AN ANTI-INFLAMMATORY COMPOUND

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Introduction: Leukocyte chemotaxis is induced when chemokines bind to their receptors during the inflammation response. This phenomenon plays an important role in immune responses for biological protection, and is thus associated with the occurrence of various diseases, such as rheumatoid arthritis and cancer. We previously identified the cytoplasmic regulator, FROUNT, which binds to the membrane proximal C-terminal region (Pro-C) of the chemokine receptor CCR2 [1]. We determined the NMR structure of the chemokine receptor-binding domain (FNT-C) of FROUNT, which is located in the C-terminal region, and the NMR structures of Pro-C in the membrane-bound and FROUNT-bound forms [2]. We also obtained a compound that inhibits the FROUNT–CCR2 interaction and exerts an anti-inflammatory effect. The aim of this study is to reveal the underlying inhibitory mechanism of the compound against the FROUNT–CCR2 interaction and to optimize the chemical structure of the compound, based on the FROUNT and CCR2 structures.

Methods: NMR titration analyses with ^{1}H–^{15}N HSQC spectra were performed using ^{15}N- or $^{13}C/^{15}N$-labeled FNT-C proteins and 0.25, 0.5, 1 and 2 equivalents of the compound or its first metabolite. The spectra were recorded on a 600 MHz spectrometer equipped with a CryoProbe (Bruker BioSpin).

Results and Discussion: Upon the titration of the compound to FNT-C, many NMR signals from free FNT-C were diminished, while the signals from FNT-C complexed with the compound appeared in a slow-exchange manner on the NMR time scale. A much wider region was affected by the titration than that expected from the chemical structure of the compound, which indicates that a large conformation change of FNT-C is induced upon compound binding. Upon the titration of the first metabolite of the compound, negligible signal changes were observed, indicating that the compound loses the inhibitory effect at the first step of its metabolism. The region with drastic chemical shift changes was in close proximity to the CCR2 Pro-C-binding region of FROUNT, which we previously identified. Upon the titration of Pro-C to the FROUNT–compound complex, negligible signal changes were observed. These results indicated that the compound induces an allosteric inhibition of the FROUNT–CCR2 interaction. Backbone ^{15}N relaxation experiments indicated that the millisecond time scale exchange dynamics were different between FNT-C and the FNT-C—compound complex, not only in the direct compound-binding site but also in part of the Pro-C binding site on FNT-C, thus supporting the allosteric inhibition hypothesis. We will optimize the regulatory compounds, based on the three-dimensional structures of FNT-C and CCR2 Pro-C, to contribute to the development of highly specific anti-inflammatory drugs.

References

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2. Esaki, K., et al. FEBS J. 281, 5552–5566 (2014)

Keywords: FROUNT, CCR2/CCR5, interaction analysis, NMR, structure, anti-inflammatory compound

PP-157

DELETION OF THE PROSTAGLANDIN D2 RECEPTOR DP1 EXACERBATES AGING-ASSOCIATED AND INSTABILITY-INDUCED OSTEOARTHRITIS

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CRCHUM and Department of Medecine

Objective: The D prostanoid receptor 1 (DP1), a receptor for prostaglandin D2 (PGD2), plays important roles in inflammation and cartilage metabolism. However, its role in the pathogenesis of osteoarthritis (OA) remains unknown. We undertook this study to explore the roles of DP1 in the development of OA and to evaluate the efficacy of a DP1 selective agonist in the treatment of OA.

Methods: We compared the development of aging-associated OA and destabilization of the medial meniscus (DMM)-induced OA in DP1-deficient (DP1-/-) and wild-type (WT) mice. The progression of OA was assessed by histology, immunohistochemistry, and microcomputed tomography (micro-CT). Cartilage explants from DP1-/- and WT mice were treated with interleukin-1α (IL-1α) ex vivo, to evaluate proteoglycan degradation. The effect of intra-peritoneal administration of the DP1 selective agonist BW245C on OA progression was evaluated in WT mice.

Results: Compared to WT mice, DP1-/- mice had exacerbated cartilage degradation in both models of OA and this was associated with increased expression of MMP-13, and ADAMTS-5. In addition, DP1-/- mice demonstrated enhanced subchondral bone changes. Cartilage explants from DP1-/- mice showed enhanced proteoglycan degradation following treatment with IL-1α. Intraperitoneal injection of BW245C attenuated the severity of DMM-induced cartilage degradation and bony changes in WT mice.

Conclusion: These findings indicate a critical role for DP1 signaling in OA pathogenesis. Modulation of DP1 functions may constitute a potential therapeutic target for the development of novel OA treatments.

Keywords: PGD2, inflammation, cartilage, Osteoarthritis

PP-158

CRITICAL ROLE OF N-TERMINAL DOMAIN AND MIDDLE-DOMAIN ON CHAPERONE ACTIVITY AND STABILITY OF CLPL

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In streptococcus pneumoniae, ClpL (Hsp100 family) of chaperones is important to survive under harsh environmental stress conditions. Other members of Hsp100, M-domain is known to control cochaperon interaction, disaggregation, and controlling the Hsp100 activity. N-terminal domain, on the other hand, is responsible for binding to the aggregated protein. However, the roles of M and N-terminal domains was unkown, especially in ClpL. In this study, the ClpL M-domain and N-terminal domain deletion mutation was designed to describe their role in these chaperone functions. In result, M-domain deletion mutant still retained ATPase, foldase, holdase, disaggregation activity compared to the ClpL wild type. However, it was not a terminal domain mutation. Interestingly, the M-domain deletion decreased hexamer stability when ATP existed, and the N-domain deletion greatly inhibited ATPase and chaperone activities. In conclusion, N-terminal domain of the ClpL is assume to have an important role to regulate chaperone activity.

Keywords: ClpL, N-terminal Domain and Middle-Domain, Chaperone Activity

PP-159

HUMAN NEUTROPHILS EXPRESS 15-LIPOXYGENASE-2 AND METABOLIZE FATTY ACIDS AND THE ENDOCANNABINOID ANANDAMIDE THROUGH THIS PATHWAY

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Context: Although neutrophils are key players in host defense, they are also involved in causing the tissue damage that is observed in inflammatory diseases. Understanding how they exert their functions is essential in order to dampen their negative effects while promoting their beneficial effects. In this regard, neutrophils produce a wide array of inflammatory lipids such as leukotriene B4. They synthesize these mediators using several precursors such as arachidonic acid and endocannabinoids. While studying these pathways, we noticed that neutrophils biosynthesize 15-lipoxygenase (LO) metabolites. Therefore, the aim of this study was to define the metabolic pathway involved.

Methods: Human neutrophils and eosinophils were isolated from the peripheral blood of healthy and rhinitic volunteers. Bioactive lipids produced upon cell stimulation with arachidonic acid or endocannabinoids were measured by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

Results: Our immunoblot analyses show that human neutrophils express 15-LO-2, in sharp contrast with eosinophils which only express 15-LO-1. When incubated with arachidonic acid, both neutrophils and eosinophils synthesized 15-hydroxyeicosatetraenoic acid (15-HETE). Moreover, neutrophils metabolized the endocannabinoid anandamide (AEA) into 15-HETE-EA and the fatty acids linoleic acid, eicosapentaenoic acid and docosahexaenoic acid into 13-HODE, 15-HEPE and 17-HDHA, respectively. This 15-LO biosynthetic activity of neutrophils was rapid (maximal after ~15 seconds) and dependent on substrate concentration. While successfully blocking the synthesis of 15-HETE in arachidonic acid-stimulated eosinophils, selective 15-LO-1 inhibitors did not alter the production of 15-LO metabolites in neutrophils. This indicates that these lipids are originating from the 15-LO-2 rather than the 15-LO-1 pathway.

Conclusion: Our data shows that human neutrophils express 15-LO-2 and have the ability to synthesize several 15-LO derivatives of fatty acids and endocannabinoids. This pathway may contribute to the regulation of inflammation and host defense, notably by controlling the levels of endocannabinoids and their metabolites.

Keywords: Neutrophil, lipid mediators, lipoxygenase, endocannabinoid

PP-160

CURCUMIN POTENTIATES THE ANTI-INFLAMMATORY ACTIVITY OF FLAVOCOXID AT A POST-TRANSCRIPTIONAL LEVEL IN HUMAN CHONDROCYTES WITH AN INFLAMMATORY PHENOTYPE

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Both flavocoxid, a catechin and baicalin mixture, and curcumin exert anti-inflammatory activity in several experimental in vivo and in vitro paradigms of inflammation. We investigated the effects of both compounds in an experimental in vitro model of arthritis based on the use of human articular chondrocytes triggered with lipopolysaccharide (LPS).

Human articular chondrocytes were stimulated with LPS (2 ug/ml; Escherichia coli serotype 055:B5) alone or in combination with different treatments: flavocoxid 16 ug/ml and 32 ug/ml, curcumin 5 ug/ml and 10 ug/ml or a drug combination of flavocoxid and curcumin. Four hours after treatment, total RNA was isolated from the cells to evaluate the mRNA expression of Nuclear Factor Kappa B (NF-κB, both p50 and p65 subunits), interleukin 1 beta (IL-1β), IL-13 and the metalloproteinases (MMP) 1 and 3. Total protein content of IL-1β, IL-13, MMP-1 and 3 were also evaluated in the cell lysates. LPS prompted the mRNA expression of pNF-κB, IL -1β, IL-13, MMP-1 and 3. Both doses of flavocoxid did not change the inflammatory phenotype induced by LPS in chondrocytes, whereas both doses of curcumin partially blunted the inflammatory phenotype. A drug combination at both doses markedly reduced the mRNA expression of pNF-κB, IL-1β, IL-13, MMP-1 and 3 and the effect was significantly greater ($p<0.01$) than both doses alone. Overlapping results were observed in the protein expression of the several inflammatory markers.

The results suggest that curcumin potentiates flavocoxid antinflammatory activity and that curcumin in combination with flavocoxid has a greater effect than curcumin alone, thus strongly suggesting the potential for a dual combination of the two compounds for the management of osteoarthritis.

Keywords: Chondrocytes, lipopolysaccharide, inflammation, flavocoxid, curcumin

PP-161

LIPOPOLYSACCHARIDE POTENTIATES PLATELET RESPONSES VIA TOLL-LIKE RECEPTOR 4-STIMULATED AKT-ERK-PLA2 SIGNALLING

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Lipopolysaccharide (LPS) from the cell envelope of Gram-negative bacteria is a principal cause of the symptoms of sepsis. LPS has been reported to modulate the function of platelets although the underlying mechanisms of LPS action in these cells remain unclear. Platelets express the Toll-like receptor 4 (TLR4) which serves as a receptor for LPS, although the potential role of TLR4 in controlling platelet responses to LPS has not been explored. In this study, we therefore investigated the actions of LPS prepared from different strains of Escherichia coli on platelet function, the underlying signalling mechanisms, and the potential role of TLR4 in orchestrating these. We report that LPS increased the aggregation of washed platelets stimulated thromboxane (U46619) or GPVI collagen (CRP-XL) receptor agonists, effects that were prevented by with a TLR4 antagonist. Associated with this LPS enhanced fibrinogen binding, P-selectin exposure and reactive oxygen species (ROS) release. Increased ROS was found to be important for the actions of LPS on platelets, since these were inhibited in the presence of superoxide dismutase (SOD) and catalase. The effects of LPS were associated with phosphorylation of Akt, ERK1/2 and PLA2 in stimulated platelets, and inhibitors of PI3K, Akt and ERK1/2 reduced significantly LPS enhanced platelet function and associated ROS. Furthermore, inhibition of platelet cyclooxygenase or the thromboxane (TP) receptor, revealed an important role for thromboxane A2. We therefore conclude that LPS increases human platelet activation through a TLR4-PI3K-Akt-ERK1/2-PLA2 -dependent pathway that is dependent on ROS and TXA2 formation.

Keywords: Platelets, TLR4, lipopolysaccharide, reactive oxygen species

PP-162

ACUTE INCREASE IN *O*-GLCNAC IMPROVES SURVIVAL IN MICE WITH LPS-INDUCED ENDOTOXEMIA

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The attachment of *O*-linked β-N-acetylglucosamine (*O*-GlcNAc) is a common post-translational modification controlled by two enzymes: *O*-GlcNAc transferase (OGT) and β-N-acetylglucosaminidase (OGA). Acute increases in *O*-GlcNAc reduce release of pro-inflammatory mediators and regulate inflammatory processes by decreasing NF-κB activation. We postulated that acute increases of *O*-GlcNAc reduce endotoxemia-associated mortality, release of pro-inflammatory mediators and cardiovascular changes (hypotension and vascular refractoriness to constrictor stimuli). C57BL6/J mice received lipopolysaccharide (LPS) injections to produce mild (LPS-M, 10 mg/Kg, i.p.) or severe (LPS-S, 20 mg/Kg, i.p.) endotoxemia. Mice received glucosamine (substrate for UDP-GlcNAc production; GlcN 300 mg/Kg, i.v.), Thiamet-G (OGA inhibitor; ThG, 150 μg/Kg i.v.), or vehicle, 30 min (GlcN) or 12 h (ThG) before the LPS administration. Mice were euthanized 6 h later. Bone marrow-derived macrophages (BMDM) from male C57BL6/J mice were incubated with vehicle, GlcN (5 mM, 30 min) or ThG (1 μM, 12 h) and then stimulated with LPS (1 μg/mL, 6 h). GlcN treatment increased survival in mice with LPS-induced endotoxemia (LPS-M= 20%, LPS-S= 50%, $p<0.05$). GlcN treatment reduced serum levels (pg/mL) of IL-1β [LPS-M= 286.3±14.5, LPS-M+GlcN= 212.9±3.1, LPS-S= 343.9±29.1, LPS-S+GlcN= 128.4±11], IL-6 [LPS-M= 448.0±11.5, LPS-M+GlcN = 241.2±11.6, LPS-S= 508.6±21.7, LPS-S+GlcN= 451.6±8.9] and TNF-α [LPS-M= 311.3±15.7, LPS-M+GlcN= 76.5±5.5, LPS-S= 354.2±32.1, LPS-S+GlcN= 136.2±10.2] as well as aortic

mRNA expression (2-ΔΔCT) of IL-1β and TNF-α ($p<0.05$). GlcN treatment attenuated, but did not normalize, LPS-induced hypotension [(mmHg) Naive= 108±9.0, Naive+GlcN= 112±10.0, LPS-M= 72±6.0, LPS-M+GlcN= 92±8.5, LPS-S= 59±4.5, LPS-S+GlcN= 88±7.5]. LPS-treated mice exhibited decreased vascular reactivity to phenylephrine (PhE, $p<0.05$) and treatment with GlcN increased vascular responses to PhE ($p<0.05$). Treatment of mice with ThG produced similar results, i.e. ThG increased survival, reduced serum levels of cytokines, attenuated LPS-induced hypotension and vascular refractoriness to PhE ($p<0.05$). Treatment of BMDM with GlcN and ThG decreased LPS-induced NF-kB-p65 translocation into the nucleus, as well as decreased release of inflammatory cytokines (IL-1β, TNF-α and IL-6) by these cells ($p<0.05$). In conclusion, acute increases in *O*-GlcNAc-modified proteins reduce LPS-induced inflammatory and cardiovascular events, making this pathway a potential target for therapeutic intervention in conditions associated with systemic inflammatory responses. Financial support: FAPESP (CRID 2013/08216-2), CAPES and CNPq, Brazil.

Keywords: O-GlcNAc, inflammation, vascular, macrophage

Translational/Drug discovery

PP-163

PHARMACOKINETIC INTERACTION OF WATER EXTRACT OF ANDROGRAPHIS PANICULATA AND IBUPROFEN IN RABBIT

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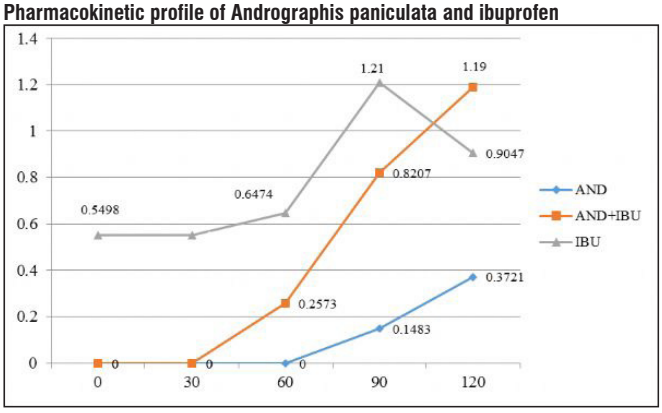
Andrographolide is the main bioactive component of Andrographis paniculata (Burm. F.) Nees) which has been traditionally used as pain reducer in Indonesia. Previous studies showed that water extract of Andrographis paniculata, calculated as andrographolide, administered in healthy rabbits, was fastly absorbed from the stomach (tmax 1 hour), distributed in the circulation system (tmax 1.5 hours) and metabolized in the liver (tmax 2 hours), in subsequent process. Furthermore, the chromatogram profile of andrographolide in A23187 induced-New Zealand rabbits' urine and faeces showed that andrographolide was not detected in the urine while compounds with higher polarity were observed at 1.5 to 3 minutes. Andrographolide was still detected in faeces along with a more nonpolar compound. The pharmacokinetic interaction of water extract of Andrographis paniculata and ibuprofen in rabbit showed a higher Cmax of andrographolide when the herb extract was administered altogether with ibuprofen.

Keywords: antiinflammation, cyclooxygenase, drug metabolism, inflammation, NSAIDs

Pharmacokinetic profile of Andrographis paniculata and ibuprofen



PP-164

TARGETING OF VIRAL INTERLEUKIN-10 USING AN ANTIBODY FRAGMENT SPECIFIC TO DAMAGED ARTHRITIC CARTILAGE IMPROVES ITS THERAPEUTIC POTENCY

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Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by joint inflammation, which results in progressive joint damage. The current systemic treatment using disease modifying anti-rheumatic drugs (DMARD) is associated with systemic side effects, since such treatment does not deliver pharmacologically active molecules solely to the site of disease activity within the joints.

Objective: The aim of this project is to specifically target an anti-inflammatory cytokine to the inflamed joint, which will result in high local concentrations and low systemic concentrations, increasing efficacy whilst minimising side-effects. We chose collagen type II (CII) modified by reactive oxygen species (ROS), namely anti-ROS-CII, as a target as it is uniquely present in damage cartilage. As a targeting unit we used a single chain fragment variable (scFv) specific to ROS-CII.

Methods: We developed a panel of human single chain fragment variables (scFv) that bind specifically to collagen type II post-translationally modified by oxidants present in arthritic joints (anti-ROS-CII) which: i) binds specifically to arthritic cartilage; ii) localises in the arthritic joint in vivo in mouse model of inflammatory arthritis or osteoarthritis following systemic administration of labelled anti-ROS-CII.

We have fused the viral interleukin-10 (vIL-10), a major anti-inflammatory cytokine, to anti-ROS-CII scFv (1-11E) to create 1-11E/vIL-10 fusion via a matrix-metalloproteinase (MMP) cleavable linker, so that the anti-inflammatory cytokine is released in the inflamed knee, where MMPs are upregulated. The specific binding of 1-11E/vIL-10 to ROS-CII was determined by ELISA, western blotting and by immunostaining of arthritic cartilage, while vIL-10 bioactivity was evaluated in vitro using an MC-9 cell proliferation assay. We also assessed the in vivo localisation and therapeutic efficacy 1-11E/vIL-10 by employing the mouse model of antigen-induced arthritis (AIA).

Results: We were able to demonstrate the specific binding of 1-11E/vIL-10 to damaged arthritic cartilage. Interestingly, the in vitro IL-10 activity in the fusion protein was observed only after cleavage with MMP-1. We observed that 1-11E/vIL-10 systemically administered to arthritic mice, localised specifically to the arthritic knee, with peak accumulation observed after 3 days. Moreover, 1-11E/vIL-10 reduced inflammation significantly quicker than vIL-10 fused to the control anti-hen egg lysozyme scFv (C7/vIL10).

Conclusion: Here we describe that vIL-10 fused to the human antibody fragment specific for damaged arthritic cartilage is a valid targeted anti-inflammatory therapy in the treatment of mouse model of arthritis. Our results further support the hypothesis that targeting bio-therapeutics to arthritic joints may be extended to include anti-inflammatory cytokines which lack efficacy when administered systemically.

Keywords: rheumatoid arthritis, osteoarthritis, collagen II, IL-10, scFv, reactive oxidants

PP-165

ANTIBODIES TO POSTTRANSLATIONALLY MODIFIED INSULIN IN TYPE 1 DIABETES

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Introduction: Insulin is the most specific beta-cell antigen and a potential primary auto-antigen in type 1 diabetes. Insulin autoantibodies (IAA) are the earliest marker of beta-cell autoimmunity, however, only slightly more than 50% of children and even fewer adults newly-diagnosed with type 1 diabetes are IAA positive.

Aim/hypothesis: the aim of this study is to investigate the effect of oxidative posttranslational modifications (oxPTM) by reactive oxidants (ROS) associated with islet inflammation in inducing neo-antigenicity in type 1 diabetes.

Methods: oxPTM of insulin was generated using ribose and various reactive oxidants (ROS). Modifications were analyzed by Native-PAGE, 3-dimensional fluorescence and mass spectrometry. Binding to native and oxPTM insulin by ROS was evaluated by ELISA and Western Blotting using as probes sera from 116 patients with newly diagnosed T1D, 82 type 2 diabetes (T2D) and 68 healthy subjects.

IAA was measured by the gold standard radiobinding assay (RBA).

Results: Native PAGE, 3D fluorescence and mass spectrometry show the effect of the oxPMT on insulin.

Significant higher binding to oxPTM-INS vs native-insulin was observed in type 1 diabetics, with 84% sensitivity compared to 61% sensitivity for RBA. oxPTM-INS autoantibodies and IAA co-existed in 50% of type 1 diabetics. Importantly 34% IAA-negative diabetics were oxPTM-INS-positive. Altogether, 95% of type 1 diabetics presented autoimmunity to insulin by RBA, oxPTM-INS or both. Binding to oxPTM-INS was directed toward oxPTM-INS fragments with slower mobility than native insulin.

Conclusion: These data suggest that oxPTM-INS is a potential autoantigen in new-onset type 1 diabetics and that oxPTM-INS autoantibodies may be a novel diagnostic biomarker for type 1 diabetes.

Keywords: post-translational modifications, insulin, insulin autoantibodies, oxidative stress, type 1 diabetes, bio-marker

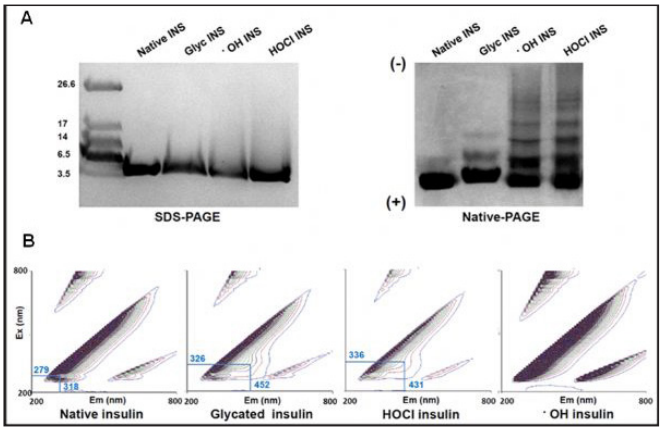

Figure 1. Analysis of native and oxPTM insulin.
A. The native PAGE showed a clear reduction in mobility of the insulin after glycation by ribose as the appearance of two bands with slower mobility. Exposure of Insulin to the HOCl and •OH-generating systems induced the appearance of additional and slower mobility bands, and a smear of protein through the entire line suggesting fragmentation. **B.** 3Dfluorescence profile of modified and native insulin. Modification of insulin with HOCl or ribose resulted in a substantial shift in the Emmax and Exmax wavelength. Modification with •OH resulted in loss of the native fluorescence and increased in light scattering suggesting aggregation of the native molecules.

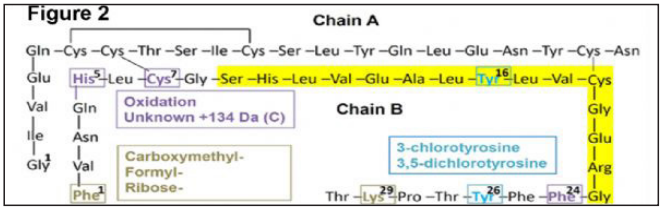

Figure 2. Mass spectrometry analysis of oxPTM insulin.
Mass spectrometry of oxPTM-INS identified chlorination of Tyr16 and Tyr26; oxidation of His5, Cys6 and Phe24 and glycation of Lys29 and Phe1 in B-chain.

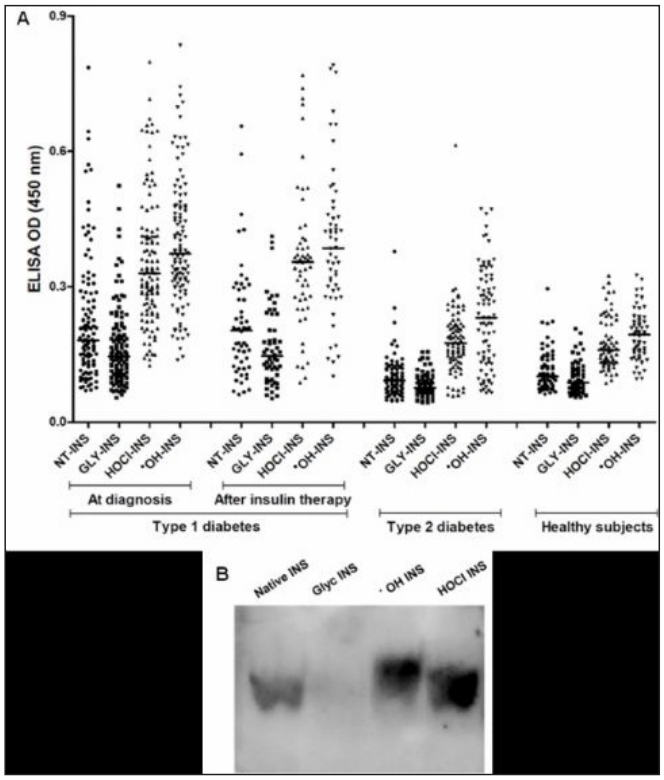

Figure 3. Binding of serum to oxPTM-INS

A. Reactivity to oxPTM-insulin (oxPTM-INS) modified by ribose (GLY-INS), HOCl (HOCl-INS) and •OH (•OH -INS) were significantly higher in type 1 diabetes compared with controls ($p<0.001$). Binding to oxPTM-INS was significantly higher than to native-INS ($p<0.001$). 79% of T1D sera showed reactivity to oxPTM-INS compared to 34% to native insulin (79.31% sensitivity, 97.14% specificity). **B.** Binding to native and oxPTM-INS as detected by western blot. Binding to native insulin and a stronger intensity binding to a smear of smaller mobility fragment of •OH-INS and HOCl-INS was observed.

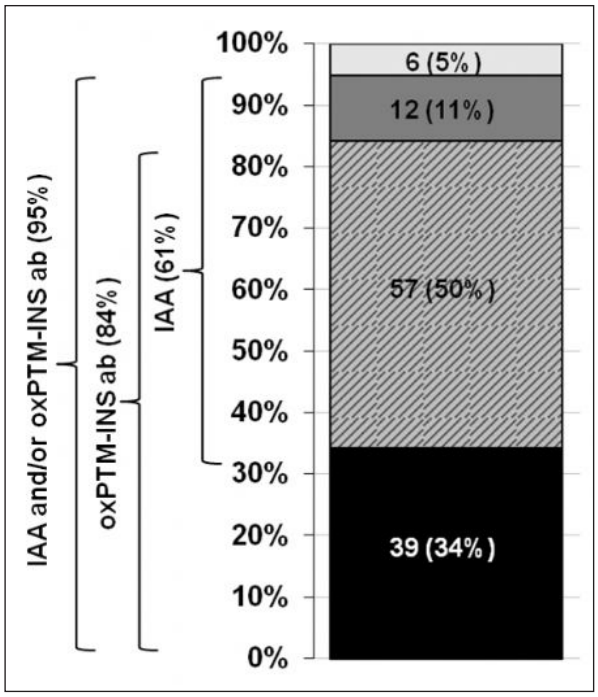

Figure 4. Serum binding specificity to oxPTM-INS.

Pre-incubation of type 1 diabetes serum samples with oxPTM-INS, but not with native insulin (NT-INS), strongly inhibited binding to oxPTM-INS, indicating the presence of antigen-binding sites specific to oxPTM-INS. Data for insulin modified by •OH as example of oxPTM-INS are shown.

PP-166

SYNTHESIS AND *IN VITRO* EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF IMIDAZOPYRIDINE DERIVATIVES

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The balance of inflammation is of extreme importance in the mediation of innate immune response, being accountable for the control and development of several immune-related diseases, including periodontitis, atherosclerosis, rheumatoid arthritis, inflammatory bowel disease and cancer. These usually arise from chronic inflammation, by excess of pro-inflammatory signaling which encompass different pathways and signaling molecules. A major role is played by cytokines, including TNF-alpha, which usually functions as an expander of pro-inflammatory signaling. Its deregulation is largely associated to the development of such pathologies. TNF-alpha inhibitors have emerged as a treatment alternative, but have encountered difficulties in specificity, large-scale production and efficiency.

With an unfavorable cost/benefit ratio, a demand for new treatment methods came to light. The production of small molecule inhibitors of cytokines has been shown to be a viable and promising alternative for therapeutic treatment. Among various classes of compounds, imidazopyridines show a very broad application spectrum, taking in a noticeable anti-inflammatory activity. Fifteen different imidazopyridines derivatives were designed and synthetized with the aim of being tested for TNF-alpha inhibitory activity. Our goal is to verify the action of these derivatives on lipopolysaccharide-stimulated inflammatory response and elucidate which intracellular pathways involving TNF-alpha are altered with the use of these molecules. A few of these compounds also present fluorescent characteristics, allowing the monitoring of their metabolization, for example, using confocal microscopy, allowing for a better search of its actions and intracellular localization. In our observations, LPS treatment of U-937 cells and peritoneal macrophages induced an increase in TNF-alpha expression and release, which were inhibited by treatment with compounds 4-B, 4-D and 4-J in a dosedependent manner. Other pro and anti-inflammatory cytokines also had their production altered after treatment. Currently we are working on elucidating the intracellular pathways actually involved in these changes. These results not only indicate that imidazopyridines can be used as therapeutic agents for inflammation-related diseases, they open up horizons for studies on the architecture of such molecules and for the development of promising compounds for the treatment of pathologies involving inflammation.

Keywords: inflammation, small molecules, TNF-alpha, imidazopyridines, LPS

PP-167

THE NOVEL ALDEHYDE TRAP, ADX-102, REDUCES INFLAMMATION-MEDIATED LUNG INFILTRATE IN A MOUSE MODEL OF LPS-INDUCED ACUTE LUNG INJURY

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Clinical and preclinical data implicate aldehydes as pro-inflammatory mediators. ADX-102, a novel aldehyde sequestering agent, has been shown to inhibit the NFkB pathway and fibrotic changes in cardiac myofibroblasts; modulate inflammatory responses in animal models of inflammation; and diminish inflammation in Phase 2 clinical trials in noninfectious anterior uveitis and allergic conjunctivitis. In addition, increased malondialdehyde levels have been observed in lipopolysaccharide (LPS)-induced models of acute respiratory distress syndrome (ARDS), which are characterized by protein and cell infiltrates, expression of inflammatory mediators, and decreased lung function. Thus, ADX-102 was tested in a mouse model of LPS-induced lung injury, which may mimic conditions observed in human ARDS, a severe inflammatory disease.

Mice (n = 8 per group) were challenged intranasally with 10 μg of LPS to induce pulmonary inflammation. Animals were administered intraperitoneal ADX-102 (100 mg/kg, 2 hours before, and 6, 22, 30, and 46 hours after, LPS challenge), vehicle (same dosing schedule as ADX-102-treated animals), or dexamethasone (3 mg/kg, 2 hours before, and 22 and 46 hours after, LPS challenge). Animals receiving intranasal saline only served as controls. After the last treatment, animals were anaesthetized, blood collected, and lung function measured. Animals were then sacrificed, bronchoalveolar lavage (BAL) was performed, and BAL fluid collected and analyzed.

LPS challenge resulted in a perturbed pressure-volume relationship in the lung (decreased compliance and increased elastance), indicative of lung stiffness. BAL fluid from untreated animals showed increases in: macrophages, neutrophils, lymphocytes and eosinophils; total protein, indicating increased vascular permeability; and several cytokines and chemokines. In addition, FACS analysis showed increases in activated macrophage populations.

IP administration of ADX-102 resulted in statistically significant reductions in total cells, macrophages, lymphocytes and eosinophils in BAL fluid, with little or no effect on relative proportions of the cell types; and significant reductions in populations of activated macrophages [(F4/80+, MHC-II+) and (F4/80+, CD86+)], relative to vehicle control. Lung compliance and elastance also improved, although these were not statistically significant. ADX-102 treatment resulted in statistically significant reductions in IFNγ, IL-6, IL-12p40, IL-12p70, IL-17, LIF, LIX, KC, MIP-1α, MIP-1β, and TNFα in BAL fluid. ADX-102 also resulted in a statistically significant decrease in total protein in BAL fluid, consistent with reduced infiltration of inflammatory cells and the presence of chemokines and cytokines in BAL fluid, suggesting reduced vascular permeability in lung.

The data show that ADX-102 can significantly reduce inflammation in LPS-induced lung injury, and build on existing evidence that aldehyde sequestration represents a novel anti-inflammatory therapy.

Keywords: aldehydes, inflammation, lung, cytokines, chemokines, macrophages

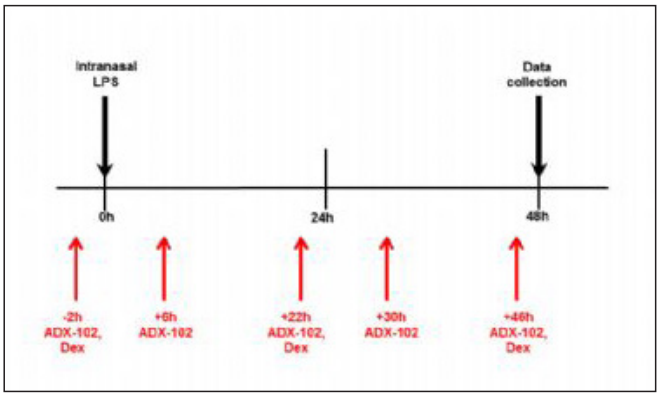

Figure 1. Study Design

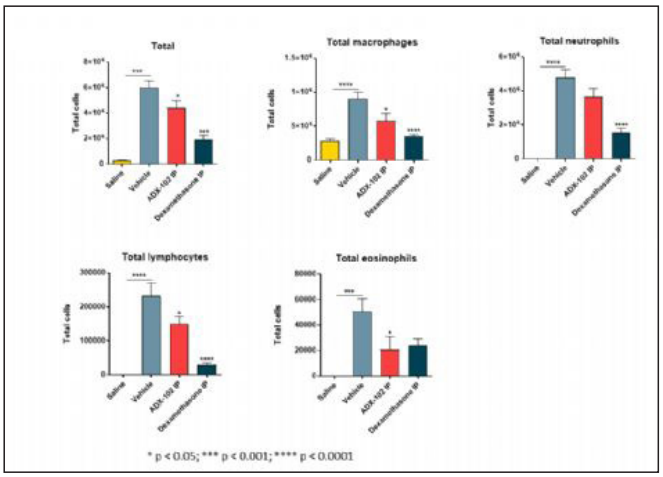

Figure 2a. BALF Total and Differential Counts

Cells recovered from the BALF were counted and stained prior to conducting a differential cell count. Data represent group means +/- SEM, and statistically significant differences between groups were determined by one-way ANOVA with Dunnett's multiple comparisons post-test, compared to vehicle control group.

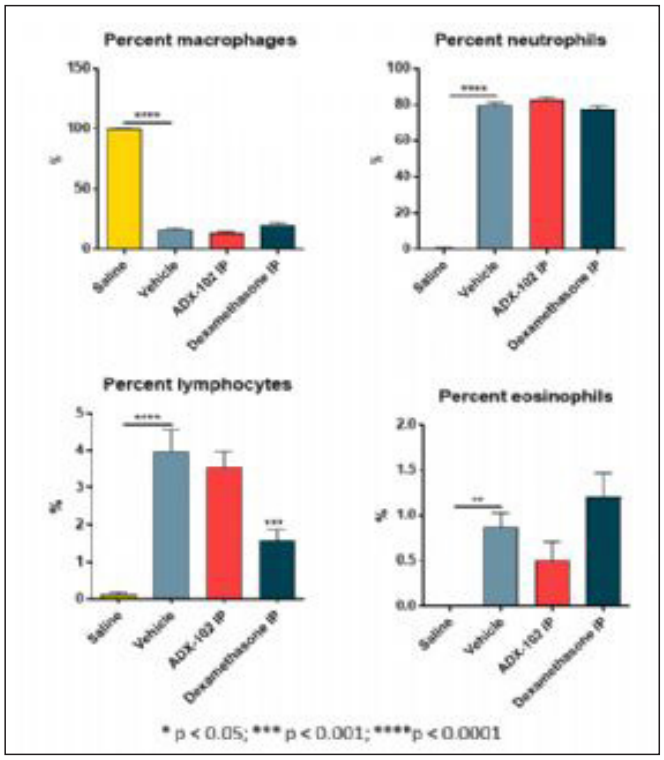

Figure 2b. BALF Percentage of Cells

Data represent group means +/- SEM, and statistically significant differences between groups were determined by one-way ANOVA with Dunnett's multiple comparisons post-test, compared to vehicle control group.

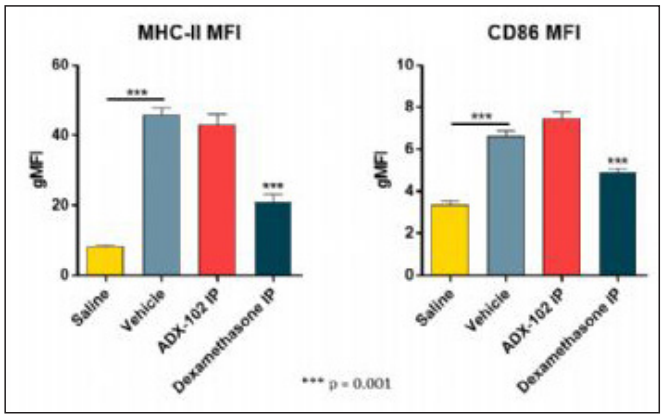

Figure 3b. Macrophage Activation

Macrophage activation was determined by measuring mean fluorescence intensity (MFI), for each of the activation markers, MHC-II and CD86. Data represent group means +/- SEM, and statistically significant differences between groups were determined by one-way ANOVA with Dunnett's multiple comparisons post-test, as compared to saline control group.

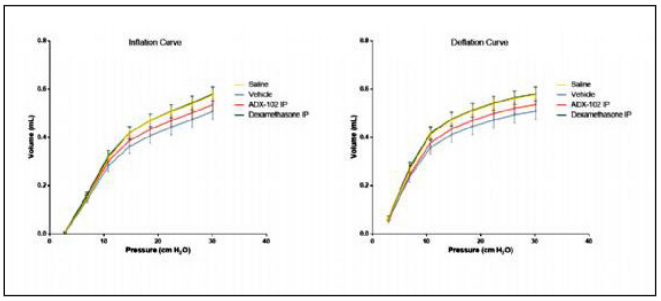

Figure 4. Pressure-Volume Loops

Lungs were inflated and deflated using a pre-calculated, stepwise (quasi-static) model. Volume required to inflate lungs to a pressure of 30 cm of water was calculated for both inhalation and exhalation.

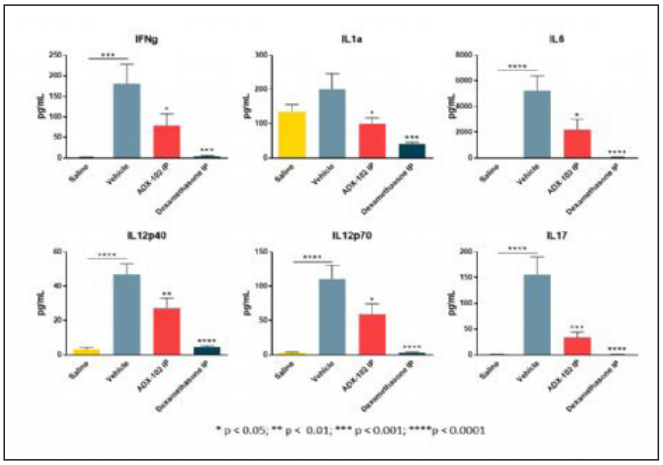

Figure 5a. BALF Cytokines

A panel of cytokines was used to analyze BALF for inflammatory mediators. Data represent group means +/- SEM, and statistically significant difference between groups were determined by one-way ANOVA with Dunnett's multiple comparisons post-test, compared to vehicle control group.

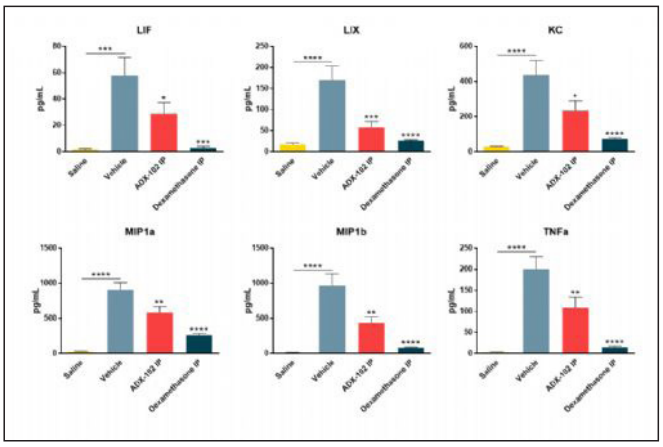

Figure 5b. BALF Chemokines

A panel of chemokines was used to analyze BALF for inflammatory mediators. Data represent group means +/- SEM, and statistically significant difference between groups were determined by one-way ANOVA with Dunnett's multiple comparisons post-test, compared to vehicle control group.

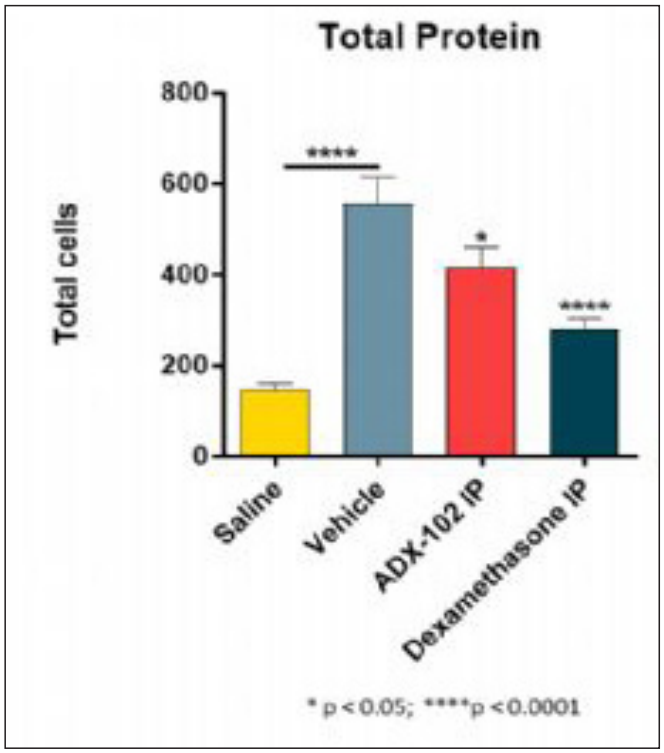

Figure 6. Total Protein in BALF

BALF supernatant was assayed for total protein content by BCA. Data represent group means +/- SEM, and statistically significant differences between groups were determined by one-way ANOVA with Dunnett's multiple comparisons post-test, compared to vehicle control group.

PP-168

COMPREHENSIVE SCREENING OF COMPOUNDS THAT MODULATE HUMAN MACROPHAGE POLARIZATION

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[2]Institute of Biochemistry I, Faculty of Medicine, Goethe University, Frankfurt am Main, Germany

Under pathophysiologic conditions, macrophages (Mθ) undergo classical (M1) or alternative (M2) activation (Wang N *et al.* 2014). Spatio-temporal regulation of the M1 to M2 phenotype shift is required for resolution and dysregulation is often associated with chronic inflammatory diseases (Murray PJ and Wynn TA 2011). Given the relevance of phenotypic plasticity in Mθ subsets to disease progression, they constitute a strategic therapeutic target. Among drugs for the treatment of chronic inflammatory diseases, however, effects on Mθ polarization are mostly unknown.

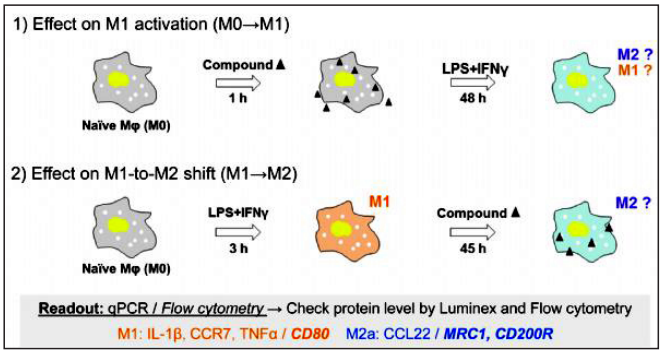
To identify compounds that modulate Mθ polarization, we established a comprehensive screening system using human peripheral blood monocyte-derived Mθ (PBMC Mθ). As previously described (Shiratori H *et al.* in revision), PBMC Mθ were obtained by cultivation of PBMCs isolated from human buffy coats in RPMI1640 medium supplemented with 2.5% human plasma for 7 days. The effects of limited incubation with compounds on "M1 activation" or "M1-to-M2a transition" were adjudged from changes in protein level of previously characterised (Shiratori H *et al.* in revision), M1 (IL-1β, CCR7, TNFα, CD80) and M2a (CCL22, MRC1, CD200R) markers upon pre-incubation of Mθ with a drug for 1 h, followed by M1 stimulation (50 ng/ml LPS + 20 ng/ml IFNγ) for 48 h or M1 stimulation of Mθ for 3 h and subsequent exposure to a drug for 45 h, respectively.

We examined seven drugs used clinically for chronic inflammatory disease therapy. The reference compound, azithromycin (25-50 μM) (Parnham MJ *et al.* 2014) as well as tofacitinib (2.5-5 μM) induced downregulation of M1 markers and upregulation of some M2a markers during both M1 activation and M1-to-M2 shift. Hydroxychloroquine treatment (2.5-10 μM) also inhibited M1 polarization and promoted M1-to-M2 shift. Pioglitazone treatment (15-60 μM) suppressed the expression of M1 markers during both M1 activation and M1-to-M2 shift. Lovastatin treatment downregulated M1 marker genes during M1 activation and promoted M1 activation by costimulation of Mθ with LPS and IFNγ. Methotrexate (1.25-6 μM) and IL-38 (10-100 ng/ml) treatment did not alter expression of any markers. IL-1β and CCL22 proved robust markers for M1 and M2 Mθ in combination with CD80.

Our screening system is able to identify novel compounds or repurposed drugs for anti-inflammatory applications.

Keywords: human, macrophage polarization, polarization marker, drug screening, chronic inflammatory disease, M1-M2

Workflow



PP-169

IDENTIFICATION OF ANTI-ANGIOGENIC POTENTIAL AND CELLULAR MECHANISM OF NAPYRADIOMYCIN A1 ISOLATED FROM THE MARINE-DERIVED STREPTOMYCES SP. YP127

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Angiogenesis is a process of new blood vessel formation. Excessive angiogenesis is a critical factor in the progression of cancer, macular degeneration, and other chronic inflammatory diseases. During investigations of the effects of crude

extracts of cultured marine microorganisms, an extract of the cultured Streptomyces sp. YP127 strain was found to inhibit human umbilical vein endothelial cell (HUVEC) tube formation. Bioassay-guided fractionation and spectroscopic data analyses led to the identification of napyradiomycin A1 (1) as an anti-angiogenic compound from the extract. Compound 1 inhibited HUVEC tube formation in a dose-dependent manner. It inhibited endothelial cell proliferation but did not affect human dermal fibroblast proliferation. Compound 1 also suppressed migration and invasion of vascular endothelial cells. In addition, compound 1 suppressed VE-cadherin expression and increased cell permeability of endothelial cell membrane. Therefore, these results suggested that compound 1 modulates cell permeability and inhibits the angiogenesis of endothelial cells.

Keywords: natural product, drug, angiogenesis, inhibitor

PP-170

WOUND HEALING ACTIVITY OF PURE NATURAL COMPOUNDS FROM AYURVEDIC MEDICINAL PLANTS USED SINCE THE 3RD AND 4TH CENTURY

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Ayurveda has long been studied for wound healing potential to provide new leads towards drug discovery. However, with publicised cases of toxicity from adulterated or contaminated herbs and negligible documented evidence of safety and efficacy, Ayurveda lacks global recognition. Following a systematic approach, pure ubiquitous compounds identified from Ayurvedic medicinal plants were investigated for wound healing activity. Plants common to those used in ancient Western herbal medicine for treating wound healing conditions were identified from English translations of the Sanskrit 3rd and 4th century Ayurvedic manuscripts "Sushruta Samhita" and "Charaka Samhita". Relational databases such as the Pharmacopoeia of India, Indian Herbal Pharmacopoeia and NAPRALERT® were explored to classify ubiquitous pure chemicals present in selected medicinal plants (*Achillea millefolium, Boerhaavia diffusa, Inula helenium, Plantago ovata, Potentilla reptans* and *Ruta graveolens*). From over 300 plants, those documented to be safe and widely known for early medieval use across continents were selected whereas, endangered, toxic or harmful plants were rejected. These selected six plants were assessed for potential to accelerate cell migration-dependent wound closure of early passage (2-6) Mitomycin-C (300 μM) treated human dermal fibroblasts (HDF) and human umbilical vein endothelial cells (HUVEC). Wound closure was assessed by standard scratch wound assay. Metabolic activity was assessed by MTT assay, and cell replication by Cyquant® cell proliferation. Experiments were repeated three times. Six compounds aucubin, allantoin, ferulic acid, salicylic acid, β-sitosterol and quercetin were selected from 150 compounds present in all six plants based on documented evidence of some pharmacological activity of relevance to wound healing. Of these, β-sitosterol (1-50 μM) strongly inhibited HDF and HUVEC cell MTT activity. Only aucubin (10μM) and salicylic acid (1 μM) induced a significant ($P<0.01$) increased DNA content in HUVEC and all compounds showed no effect on HDF. Aucubin (1-5μM) and salicylic acid (1-10μM) accelerated wound closure at 12h, compared to vehicle control (0.2% ethanol) in both HUVEC and HDF. This investigation of Ayurvedic plants used traditionally for superficial wound healing indicates aucubin and salicylic acid that are prominent in *Plantago ovata* and *Achillea millefolium* respectively may provide an explanation for their efficacy as reflected in global use down through the centuries. Our results correlate with the literature for anti-inflammatory activity of aucubin in carrageenan-induced hind paw edema model and wound healing activity of salicylic acid in dermal wounds. Our approach to identify anti-inflammatory phytochemicals from historic texts coupled with classic *in vitro* wound healing assays suggests a novel paradigm for the discovery from sustainable natural resources.

Keywords: Ayurvedic, Wound, Scratch, Pure compounds, Human dermal fibroblasts, Human umbilical vein endothelial cells

PP-171

ANTI-INFLAMMATORY EFFECTS OF SILVER NANOPARTICLES STABILIZED IN SOLUTION BY SODIUM ALGINATE

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In connection with the increasingly intensive use in medicine of nanoparticles of various materials, which are applied both as therapeutic drugs and as transport systems for diagnostic and medicinal means, the study of their pro-inflammatory and anti-inflammatory effects is becoming increasingly important. Among the most investigated particles of nanometals, the silver nanoparticles can be distinguished, which attract attention due to their marked antibacterial properties.

The aim of the work was to study the effects of silver nanoparticles (linear particle size is 10-20 nm) stabilized in solution by sodium alginate at different means of introduction to intact rats and in the modeling of inflammatory processes.

The object of the experimental study was white male rats of the Wistar line (n=169) with a body mass of 180-210 g, which were kept in standard vivarium conditions. The effect of silver nanoparticles on intact tissues of the lungs, abdominal cavity and stomach lining during long oral administration, a single-dose endotracheal and intraperitoneal introduction, and on the development of acute inflammatory process in the modeling of pneumonia, peritonitis, and ulcerative necrotic lesions of the gastric mucosa was studied. As a marker for the formation of inflammatory changes, the reactions of nonspecific proteinases and their inhibitors at a systemic (blood serum) and local level (bronchoalveolar and peritoneal lavage, homogenate supernatant of gastric mucosa) were investigated, as well as morphological studies were carried out.

The obtained results showed that the effect of silver nanoparticles stabilized in solution by sodium alginate on the intact tissues of rats is characterized by minimal reactions of components of the proteinase-inhibitor system, both at a systemic and local level, which indicates the absence or minimal pro-inflammatory activity of silver nanoparticle solution. Modeling of inflammatory processes in the lungs, abdominal cavity, stomach lining against the background of silver nanoparticle introduction results in a significant decrease of the activation degree of nonspecific proteinases, preservation of the inhibitory potential marked mainly at a local level. The inactivation of proteinases, preserving proteolytic enzyme inhibitor activity and decreasing intensity of the

morphological signs of inflammation while using silver nanoparticles suggest the presence of local anti-inflammatory effects of silver nanoparticles, which requires further research to justify the possibility of their practical application.

Keywords: silver nanoparticles, pneumonia, peritonitis, proteinases

PP-172

PR013 REDUCES SYMPTOMS OF ALLERGIC CONJUNCTIVITIS IN THE MURINE CAC™ MODEL AND DEMONSTRATES EXCELLENT SAFETY IN REPEAT-DOSE OCULAR TOLERABILITY STUDIES

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Background: PR013 is a novel formulation of high concentration hypochlorous acid (HOCl) currently in development for ocular inflammation, including allergic conjunctivitis (AC). PR013, while in development for AC generally, may hold particular promise as an exciting alternative therapy for patients suffering from more moderate to severe, or chronic AC requiring steroids, and those who are refractive to current standard therapies (antihistamines +/- mast cell stabilizers), including pediatric patients. The goals of this study were to examine the effect of PR013 at 0.01%, 0.05% and 0.1% on symptoms of AC in the murine conjunctival allergen challenge (CAC™) model, and determine the tolerability of PR013 in repeat dose studies in the rabbit and canine models. The CAC™ model reproduces the signs and symptoms of allergic conjunctivitis by replicating the natural disease process and has been widely used pre-clinically to evaluate the efficacy of AC treatments.

Experimental: Murine CAC™ Model: Symptoms of allergic conjunctivitis were induced by i.p. injection and ocular application of short ragweed allergen (SRW) in Balb/C mice. On days 21-24, PR013 was instilled into the eyes of mice followed 60 minutes later by instillation of SRW (challenge). Ocular hyperemia was evaluated at baseline, and 18 minutes after ocular challenge. Prednisolone (1%) and olopatidine (0.1%) were used as positive controls.

Ocular Tolerability: Repeat dose tolerability studies were conducted in beagle dogs and rabbits by instilling PR013 at various concentrations into the eyes for 5 and 10 days, respectively. The animal corneas and conjunctivae were examined for clinical symptoms.

Results: Dosing prior to allergen challenge did not result in any hyperemia, indicating that PR013 was well tolerated in the murine model. Treatment with high concentrations (0.05% and 0.1%) of PR013 (after allergen challenge) resulted in significant, dose dependent, reduction in hyperemia in the murine CAC™ model compared to vehicle. Results demonstrated consistent, better control of hyperemia than olopatidine (0.1%) and similar efficacy to prednisolone (1%) without concomitant weight loss in the animals. The 0.01% concentration of PR013 did not demonstrate significant reduction in hyperemia, further supporting the concept that therapeutic efficacy of the molecule is only seen in high concentrations. Repeat dose tolerability studies indicated that PR013 was well tolerated in the canine and rabbit models when dosed over 5 and 10 days.

Keywords: Inflammation, allergic conjunctivitis, allergen challenge, tolerability

PP-173

AN *IN-VIVO* APPROACH TO TESTING ANTI-INFLAMMATORY AGENTS BASED ON LIVE IMAGING OF GRANULOCYTE-MACROPHAGE DYNAMICS AND MARKER ASSESSMENTS IN ZEBRAFISH

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The development of the innate immune system is well conserved among vertebrates, so the cells involved in the innate immune response share structurally and functionally a high degree of homology between teleosts and mammals. Zebrafish (*Danio rerio*) embryos and larvae are widely used model organisms, which allow the study of innate immune cells *in vivo* in real-time, isolated from any interference by adaptive immunity. Monocyte as well as granulocyte-specific transgenic reporter lines of zebrafish have been developed for intravital imaging of these cells and for their behavior during inflammation and wound-healing. Successful applications of such lines in screening approaches have demonstrated the merits of zebrafish early life-stages for the study of anti-inflammatory drugs. We are establishing a chemical- and wound-induced inflammation screen with embryos and larvae [<= 120 hours post-fertilization (hpf)] of wild-type and transgenic (*mpeg*:GFP and *lyz*:dsRed) zebrafish for compound testing within drug repurposing strategies. Several reference compounds with known anti-inflammatory activities have been tested for effects on neutrophil and macrophage recruitment. Short-term exposure of 72 hpf embryos to the highly fish-toxic metal, copper (as $CuSO^4$) or the ototoxic antibiotic, neomycin induced an inflammatory response in the neuromast cells, displayed by the consecutive recruitment of neutrophils and macrophages. Similarly, caudal fin ray transection (fin-cut) provoked the migration of these cells to the wound site. Modulation of these responses by drugs was quantified based on fluorescence readouts (cell number, intensity, velocity). Additionally, the macrophage polarization was monitored by measuring transcript levels of *nos2a/b* (*nitric oxide synthase 2a/b*), *arg2* (*arginase 2*) as well as *il1b* (*interleukin 1, beta*), *il6* (*interleukin 6*) and *tnfb* (*tumor necrosis factor b*). Significant differences in the neutrophil/macrophage spatial distribution and gene expression patterns were found in chemically and mechanically injured larvae after exposure, for instance, to pioglitazone and azithromycin. Further reference compounds are currently being tested to optimize and validate the assay. It can be concluded that an anti-inflammatory zebrafish embryo-larval assay based on intravital real-time immune cell imaging, represents an efficient enabling tool for an in-vivo screening assessment of drugs without the limitations of animal experiments.

Keywords: zebrafish embryos, drug screening, macrophages, neutrophils, in-vivo imaging

PP-174

PHARMACOLOGICAL MODULATION OF CIRCULATING CATHELICIDIN-RELATED ANTIMICROBIAL PEPTIDE (CRAMP) IN THE MOUSE MODEL OF IMIQUIMOD-INDUCED PSORATIC SKIN

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Background/Aims: Topical application of the TLR-7 agonist imiquimod (IMQ) induces rapid development of psoriasiform skin lesions in mice. There are striking pathophysiological similarities between IMQ-induced lesions and human psoriasis, which make the IMQ model a tool of choice for developing new therapies. However, clarifying some aspects of the model would further reinforce its face validity. Published studies have highlighted the contribution of antimicrobial peptides to the development of autoimmune disorders. The cathelicidin antimicrobial peptide LL-37, secreted by innate immune cells (neutrophils, macrophages) and keratinocytes, appears to play a critical role in the pathogenesis of psoriasis. LL-37 binds to self-DNA released by damaged cells, forming a stable activator ligand to TLR-9 in plasmacytoid dendritic cells, thereby leading to the production of type I interferons and further inflammatory pathway activation. In the clinic, psoriatic skin expresses increased levels of LL-37. Similarly, IMQ-treated lesional skin expresses elevated levels of the murine LL-37 orthologue, CRAMP. Patients with psoriasis have elevated circulating levels of LL-37, which seem to correlate with lesion severity. The goal of this work was to determine whether circulating CRAMP levels were also elevated in the IMQ model and if modulation with clinical standards was possible.

Methods: Male BALB/cByJ mice received daily topical applications of 61.5 mg Aldara™ cream (5% IMQ) on shaved back skin for 7 days. Control mice were topically treated with 61.5 mg of Vaseline®. Dexamethasone (0.1, 0.3, 1 mg/kg) or vehicle (0.5% MC) were dosed once daily, by oral gavage, 1 hour before IMQ applications. Severity of psoriatic lesions was determined daily by using a 12-point scoring system that takes into account intensity of erythema, skin thickness (micrometer) and presence of scaling. CRAMP plasma levels were determined by ELISA.

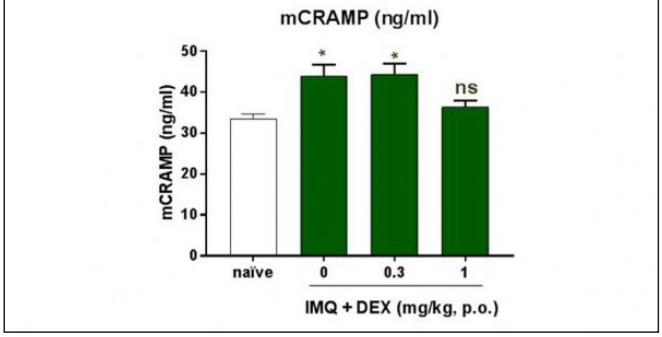
Results: Lesion severity scores in vehicle-treated mice increased steadily starting one day after initiating IMQ treatment, to achieve 9.1 ± 1.0 on day 7. DEX dose-dependently reduced severity scores. The effects of the 0.3 and 1 mg/kg doses were significant on Day 6, and from Day 4 to 7, respectively, whereas that of the 0.1 mg/kg dose did not reach significance. CRAMP plasma levels at day 7 were significantly elevated in mice treated with the vehicle and 0.3 mg/kg DEX compared to naïve mice (43.9 ± 2.8 and 44.4 ± 2.6, vs. 33.5 ± 1.1 ng/ml, respectively, $p < 0.05$, Dunnett's). However, no significant differences were detected in mice treated with 1 mg/kg DEX (36.4 ± 1.5, $p = 0.96$).

Conclusion: The results of this study suggest that IMQ treatment increases circulating CRAMP levels in addition to inducing psoriasis-like skin lesions. Both effects could be modulated pharmacologically. Monitoring of circulating CRAMP levels throughout IMQ-induced psoriasis studies may be a valuable additional tool for an objective measure of treatment efficacy.

Keywords: imiquimod, psoriasis, model, cathelicidin, CRAMP, biomarker

Effects of dexamethasone on severity scores and CRAMP plasma levels in the IMQ mouse model

Severity scores	Baseline	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Vaseline	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
IMQ + VEH (5 ml/kg, p.o.)	0 ± 0	0.5 ± 0.2	3.2 ± 0.2	4 ± 0.1	6.9 ± 0.2	7.5 ± 0.3	9.2 ± 0.2	9.1 ± 0.3
IMQ + DEX (0.1 mg/kg, p.o.)	0 ± 0	0.5 ± 0.2	3.2 ± 0.3	4.8 ± 0.3	7.8 ± 0.4	7.5 ± 0.4	8.3 ± 0.3	8.6 ± 0.2
IMQ + DEX (0.3 mg/kg, p.o.)	0 ± 0	0.3 ± 0.2	3.2 ± 0.2	4.5 ± 0.3	6.9 ± 0.4	6.9 ± 0.3	7.7 ± 0.2**	8.9 ± 0.4
IMQ + DEX (1 mg/kg, p.o.)	0 ± 0	0.2 ± 0.1	2.6 ± 0.2	4 ± 0.3	5.5 ± 0.4**	5.8 ± 0.4***	6.5 ± 0.3****	7.9 ± 0.3*



Repeated topical application of imiquimod on mouse back skin increased CRAMP plasma levels. Daily oral administration of the corticosteroid dexamethason dose-depentenly reduced severity scores of skin lesions and decreased CRAMP plasma levels.

PP-176

DRAMATIC ELEVATION IN URINARY AMINO TERMINAL TITIN FRAGMENT EXCRETION QUANTIFIED BY IMMUNOASSAY IN DUCHENNE MUSCULAR DYSTROPHY PATIENTS AND IN DYSTROPHIN DEFICIENT RODENTS

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Enzyme-linked and electrochemiluminescence immunoassays were developed for quantification of amino (N-) terminal fragments of the skeletal muscle protein titin (N-ter titin) and qualified for use in detection of urinary N-ter titin excretion. Urine from normal subjects contained a small but measurable level of N-ter titin (1.0 ± 0.4 ng/ml). A 365-fold increase (365.4 ± 65.0, $P = 0.0001$) in urinary N-ter titin excretion was seen in Duchene muscular dystrophy (DMD) patients. Urinary N-ter titin was also evaluated in dystrophin deficient rodent models. *Mdx* mice exhibited low urinary N-ter titin levels at 2 weeks of age followed by a robust and sustained elevation starting at 3 weeks of age, coincident with the development of systemic skeletal muscle damage in this model; fold elevation could not be determined because urinary N-ter titin was not detected in age-matched wild type mice. Levels of serum creatine kinase and serum skeletal muscle troponin I (TnI) were also low at 2 weeks, elevated at later time points and were significantly correlated with urinary N-ter titin excretion in *mdx* mice. Corticosteroid treatment of *mdx* mice resulted in improved exercise performance and lowering of both urinary N-ter titin and serum skeletal muscle TnI concentrations. Low urinary N-ter titin levels were detected in wild type rats (3.0

± 0.6 ng/ml), while *Dmd*^{mdx} rats exhibited a 556-fold increase (1652.5 ± 405.7 ng/ml, P = 0.002) (both at 5 months of age). These results suggest that urinary N-ter titin is present at low basal concentrations in normal urine, and increases dramatically coincident with muscle damage produced by dystrophin deficiency. Urinary N-ter titin has potential as a facile, non-invasive and translational biomarker for DMD.

Keywords: muscle, dystrophy, glucocorticoid, biomarker, translational, titin

PP-184

ANTILEISHMANIAL ACTIVITY EVALUATION OF GALLIC ACID

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Leishmaniasis is a complex disease caused by a protozoa parasite from over 20 Leishmania species. It is transmitted to humans by the bite of infected female phlebotomine sandflies. An estimated 900 000–1.3 million new cases and 20 000 to 30 000 deaths occur annually. There are 3 main forms of the disease: cutaneous, visceral and mucocutaneous. The main treatment available is the pentavalent antimony. However, the application of these drugs is limited due to low efficacy, life-threatening side effects, high toxicity, induction of parasite resistance, length of treatment and high cost. Overall the development of antileishmanials has been generally slow. Thus, the bioactive phytocompounds present in the plant derivatives including the crude extracts, essential oils, and other useful compounds can be a good source for discovering and producing new antileishmanial drugs. In the present study, we evaluated the effect of gallic acid against Leishmania major, L. infantum and L. killicki promastigotes, THP-1 cells and L. infantum amastigotes. The obtained results showed that gallic acid exhibited a very good leishmanicidal activity on both amastigote and promastigote forms. Furthermore, gallic acid showed a low cytotoxicity on THP-1 cell line.

The present study shows a plant extract, gallic acid, exhibiting interesting antileishmanial properties in vitro experimental study.

Keywords: gallic acid, leishmaniasis, leishmanicidal activity

Vascular Processes

PP-177

INTEREST OF DEXTRAN SULFATE ON ANTI-REDNESS TREATMENT

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Rosacea is a chronic skin disorder characterized by inflammation and vascular abnormalities of the facial skin. The vascular endothelial growth factor (VEGF) pathway contribute to vascular changes and immune infiltration observed in rosacea. The cathelicidin LL-37 and the metalloproteinase MMP-9 are also increased and activated in rosacea skin. Moreover, MMPs have a role in LL-37 activation by activating kallikreins (KLKs).

The aim of this study was to evaluate the activity of dextran sulfate on the inflammatory and vascular responses implicated in rosacea. The anti-inflammatory activity of dextran sulfate was evaluated on the prostaglandin PGE2 production after PMA stimulation on the keratinocyte cell line NCTC-2544. The inflammatory and vascular responses were evaluated on normal human epidermal keratinocytes (NHEK) in a rosacea environment. Finally, the anti-angiogenic activity of dextran sulfate was assessed by analysing the formation of pseudotubes on human microvascular endothelial cells (HMVEC) and normal human dermal fibroblasts (NHDF) co-culture.

These experiments showed that dextran sulfate strongly and significantly inhibited PMA-induced PGE2 production at 0.2 and 2 mg/ml. Dextran sulfate, at 10 μg/ml, inhibited the mRNA expression of proteases KLK5 and MMP9 and the production of IL1α and VEGF in NHEK rosacea environment. Finally, dextran sulfate, at 10, 30 and 100 μg/ml, showed a highly significant inhibitory effect on VEGF-induced pseudotube formation in the HMVEC/NHDF co-culture.

Altogether these results showed that dextran sulfate had very interesting soothing and anti-redness properties and could be suitable for topical adjunctive treatment in rosacea.

Keywords: rosacea, dextran sulfate, VEGF, keratinocytes

PP-178

INTEREST OF TRP-REGULIN®, PONGAMIA OIL AND HESPERIDIN METHYL CHALCONE ON ANTI-REDNESS TREATMENT

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Rosacea is a chronic skin disorder characterized by inflammation and vascular abnormalities of the facial skin. Skin biopsies of rosacea are also characterized by an increase on the activation of the capsaicin receptor, the transient receptor potential vanilloid 1 (TRPV1).

The aim of this study was to evaluate the activity of different compounds on the inflammatory and vascular responses implicated in rosacea. In one hand, we evaluated the effect of TRP-regulin® (pentylene glycol 4-t-butylcyclohexanol) on TRPV1 activation, and the effect of the association of TRP-regulin® and pongamia oil on the inflammatory response of normal human epidermal keratinocytes in a rosacea environment. On the other hand, the activity of Hesperidine Methyl Chalcone (HMC) compound was assessed to modulate vascular responses and IL-8 cytokine production after a Substance P (SP) stimulation on human skin explants.

In this study, we showed that TRP-regulin® significantly inhibited TRPV1 activation by capsaicin. These experiments also showed that TRP-regulin® and pongamia oil inhibited the mRNA expression of the chemokines CXCL1 and CXCL6 and the IL8 protein production. In addition, we demonstrated a synergy of these two compounds association on the inhibition of IL8 production. Finally, in the SP stimulated-skin model, HMC significantly decreased the proportion of dilated vessels, the total vessel area and the IL8 production.

Altogether these results showed that TRP-regulin®, pongamia oil and HMC compounds have complementary and very interesting soothing and anti-redness properties and could be suitable for topical adjunctive treatment in rosacea.

Keywords: rosacea, TRPV1, keratinocyte, TRP-regulin, pongamia oil, HMC

PP-179

ASSOCIATIONS BETWEEN AN ANTI-ANGIOGENIC VASCULAR ENDOTHELIAL GROWTH FACTOR-A ISOFORM AND ACUTE MYOCARDIAL INFARCTION IN HUMANS

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Background: A growing body of evidence suggests the potential interplay between angiogenesis and inflammation. New microvessel formation is an essential process to restore microvascular perfusion in the jeopardized myocardium in the weeks following reperfused ST-segment-elevation myocardial infarction (STEMI) and thus to prevent the transition to heart failure. VEGF-A165b was recently identified as the main anti-angiogenic VEGF-A splice variant, however its role in acute myocardial infarction (AMI) remains unknown.

Objective and Methods: In the present study, we aimed to investigate the dynamics of circulating VEGF-A165b levels and their association with Cardiac Magnetic Resonance (CMR)-derived infarct size and left ventricular ejection fraction (LV) ejection fraction (LVEF) in 50 STEMI patients undergoing primary percutaneous coronary intervention (PCI).

Results: Serum VEGF-A165b levels were significantly increased before primary PCI, reaching the highest levels at 24h after PCI. Additionally, extensive infarct size and depressed left ventricular ejection fraction were associated with higher VEGF-A165b levels. Immunohistochemistry analysis revealed a marked up-regulation of VEGF-A165b expression and leucocyte inflammatory infiltration in myocardial infarct areas from patients with a previous history of acute infarction. Finally, in an *ex vivo* morphogenesis assay using human coronary artery endothelial cells, neutralization of serum VEGF-A165b from STEMI patients significantly increased tubulogenesis.

Conclusion: VEGF-A165b might play a deleterious role in AMI as an endogenous inhibitor of angiogenesis in the human myocardium. Neutralization of VEGF-A165b could represent a novel pro-angiogenic therapy for completing the treatment of jeopardized myocardium in STEMI patients. This study was supported by grants PIE15/00013, CPII13/00025, PI15/00082, CB16/11/00486 from the Carlos III Health Institute, the Spanish Ministry of Economy and Competiveness, and the European Regional Development Fund (FEDER).

Keywords: Acute myocardial infarction, Angiogenesis, Vascular Endothelial Growth Factor

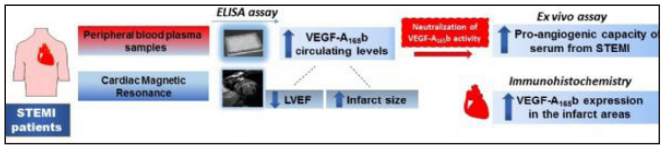

Figure 1. Graphic Results

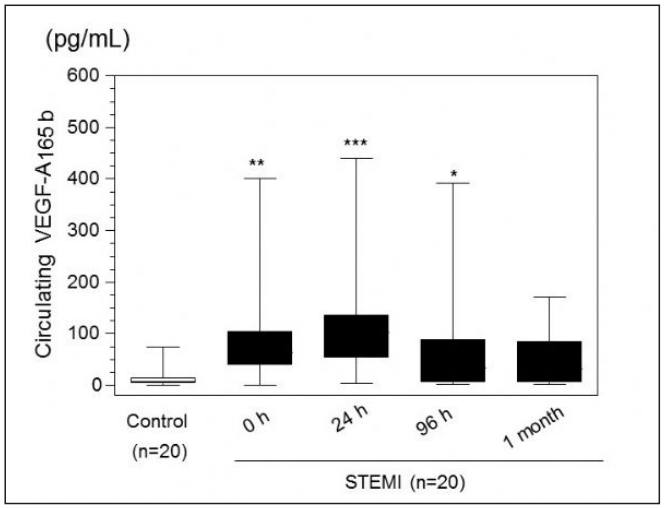

Figure 2. Temporal changes in levels of serum VEGF-A165b in STEMI patients and control subjects. Data are from controls (n=20) and STEMI patients (n=20) before reperfusion (time=0 h) and at 24 h, 96 h and 1 month after primary percutaneous coronary intervention. Box plots shows median values and interquartile range. ***P<0.001, **P<0.01 *P<0.05 vs. control subjects.

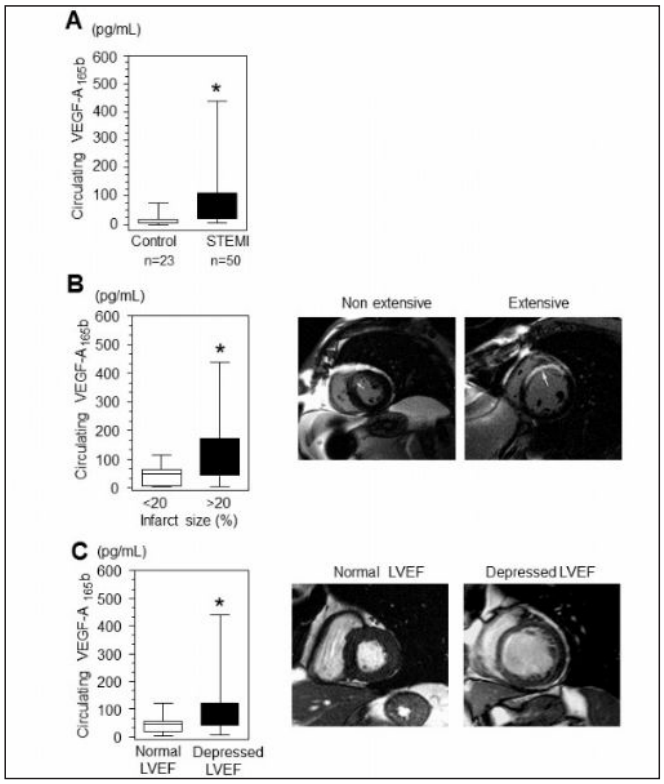

Figure 3. High VEGF-A165b levels in STEMI patients are associated with an extensive infarct size and depressed left ventricular ejection fraction (LVEF). A, Circulating VEGF-A165b levels from STEMI patients (n=50) at 24 h after reperfusion and control subjects (n=23). B, STEMI patients with extensive infarct size (infarct size >20% of LV mass) have higher VEGF-A165b levels than patients with non-extensive infarct size. Right panels show representative images of extensive infarct and non-extensive infarct. C, STEMI patients with depressed LVEF have higher VEGF-A165b levels than patients with preserved LVEF. Right panels show representative images of preserved LVEF and depressed LVEF. Box plots show median values and interquartile range. *P<0.05 vs control subjects.

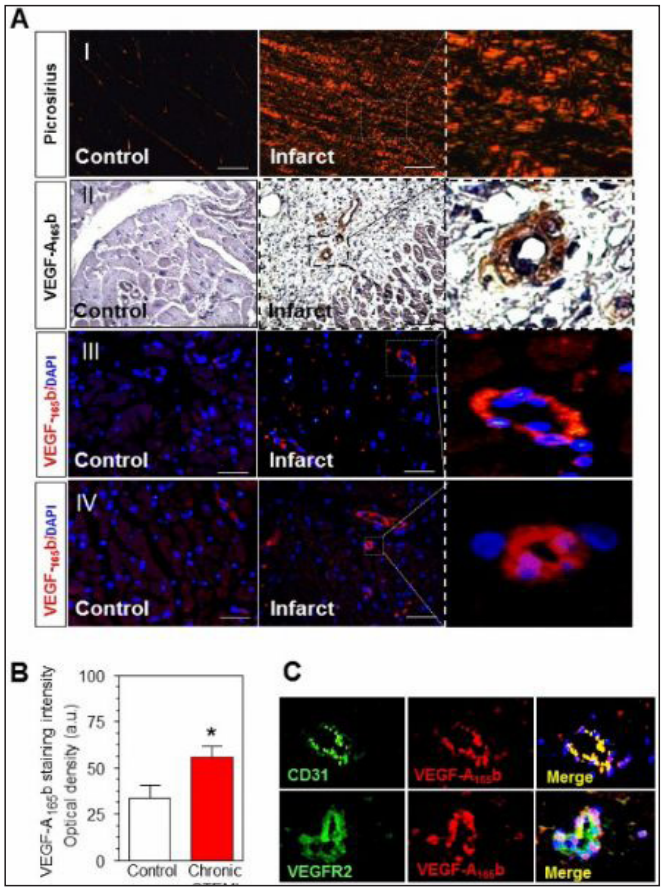

Figure 4. Immunohistochemistry analysis of VEGF-A165b expression in heart tissue from autopsies of patients with previous history of myocardial infarction. **A, I)** representative images of picrosirius staining. **II)** Myocardial sections were incubated with a mouse anti-human VEGF-A165b antibody (5 μg/ml) and specific labelling was detected with a biotin-conjugated goat anti-mouse secondary antibody. **III)** and **IV)** Myocardial sections were incubated with a mouse anti-human VEGF-A165b antibody (5 μg/ml) and immunoreactivity was visualized using Alexa Fluor 594 *(VEGF-A165b, red)* secondary antibodies. Nuclei were stained with DAPI *(blue)*. Bars=500 μm. B, Densitometric analysis of VEGF-A165b immunofluorescent staining. Images from infarct and control sections were captured and digitized *(Axio Observer A1, Carl Zeiss)* and then analyzed with Image-Pro Plus analysis software (Media Cybernetics). Scoring was performed blinded on coded slides. Data represent mean±SD of optical density in arbitrary units (a.u.). *P<0.05 vs control. C, Representative images showing colocalization of CD31/VEGF-A165b or VEGFR-2/VEGF-A165b in infarct myocardial tissue. Immunoreactivity was visualized using Alexa Fluor 488 (CD31 or VEGFR2, green) and Alexa Fluor 594 (VEGF-A165b, red) secondary antibodies. Nuclei were stained with DAPI (blue).

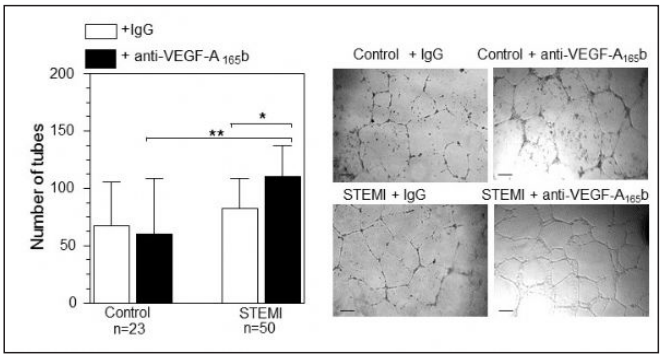

Figure 5. Blocking serum VEGF-A165b from STEMI patients with a neutralizing antibody induces angiogenesis. HCAEC were incubated with diluted serum (10%) from STEMI patients (n=50) or controls (n=23). Some cells were incubated in the presence of a mouse monoclonal anti-human VEGF-A165b blocking antibody (10 μg/mL) or irrelevant isotype- and concentration-matched IgG. Phase contrast images were taken after 24 h and the number of tube-like structures was counted. Data represent mean±SD of the number of tube-like structures in 5 low-magnification (×100) fields. Bars=300 μm. *P<0.05 vs irrelevant isotype-matched immunoglobulin. Right panels show representative images of endothelial cell differentiation on Matrigel. *P<0.05 **P<0.01

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