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Society

27th Society Meeting

"Adhesive Interactions in Health and Disease"

Birmingham 24th 2015

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UK CELL ADHESION Society

We are delighted to welcome you to Birmingham for the 27th UK Cell Adhesion Society meeting.

The UK Cell Adhesion Society (formerly known as UK Adhesion Society) is a forum created in 1996 by leukocyte and endothelial cell biologists with the aim of promoting discussion and exchange of ideas on the role of adhesion molecules and intercellular interactions in all the aspects of adhesive responses relevant to physiology and pathophysiology including cell trafficking, immunology, cancer cell biology and angiogenesis. To join the Society or for more information on the forthcoming annual meeting please visit www.ukcelladhesion.org

We have representatives from across the UK and abroad.

The meeting has three themes:

- Platelet Regulation of Inflammation
- Specialised Vasculature
- New Imaging Modalities in Health and Disease

Each theme features at least one of our keynote speakers, accompanied by talks from students and early career researchers.

The Birmingham UK CAS organising Committee

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Lewis Clarke
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Sahithi Kuravi
Helen McGettrick
Ed Rainger*

UK CAS Web Manager

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Welcome to Birmingham



UK CELL ADHESION Society

SESSION 1: Platelet Regulation of Inflammation

Chair: Sahithi Kuravi

- 09:20 – 09:25 Welcome and short introduction: Helen McGettrick
- 09:25 – 10:00 **Oliver Borst (University Clinic of Tuebingen):**
Platelet-derived chemokines in inflammation.
- 10:00 – 10:20 **Abhishek Chauhan (University of Birmingham)**
Platelet activation via CLEC2 drives liver damage in acute inflammatory hepatitis.
- 10:20 - 10:40 **Silvia Rosini (University of Bristol)**
Understanding the role of thrombospondin-1 in collagen fibril organisation.
- 10:40 – 11:00 **Aigli Evryviadou (University of Birmingham)**
Characterisation of a novel interaction between monocytes and platelet-derived microvesicles.
- 11:00 – 11:25 Coffee/Tea. Poster Viewing.**

SESSION 2: Specialized Vasculature

Chair: Myriam Chimen

- 11:25 – 12:00 **John Greenwood (University College London):**
Specialised vascular barriers of the CNS and their influence on leukocyte migration.
- 12:00 – 12:20 **Ben Ward (University of Sheffield)**
Disturbed flow promotes adhesion of neutrophil-derived microvesicles to endothelial cells.
- 12:20 – 12:40 **Scott Davies (University of Birmingham)**
T-lymphocytes wanted - dead or alive; unexpected consequences of CD4+ T cell adhesion to hepatocytes.
- 12:40 – 13:45 Lunch. Poster Viewing.**

SESSION 3: Novel Imaging Modalities in Health and Disease

Chair: Helen McGettrick and Lewis Clarke

- | | |
|----------------------|---|
| 13:45 – 14:20 | Steve Thomas (University of Birmingham):
Platelet adhesion and the actin cytoskeleton: Peeking beyond the diffraction limit. |
| 14:20 – 14:40 | Samantha Arokiasamy (Queen Mary's University London)
TNFα drives CCR7-dependent migration of neutrophils into afferent lymphatic vessels <i>in vivo</i>. |
| 14:40 – 15:00 | Rebar N. Mohammed (Cardiff University)
L-Selectin/CD62L dependent homing of influenza-specific CD8⁺ T cells is essential to control pulmonary infection. |
| 15:00 – 15:30 | Coffee/Tea. Poster Viewing. |
| 15:30 – 15:50 | Leila Thuma (University of Bristol)
Modelling of immune cell diapedesis from vessels to tissue damage in the <i>Drosophila</i> pupal wing veins. |
| 15:50 – 16:10 | Tamara Girbl (Queen Mary's University London)
The role of chemokines in the regulation of neutrophil-pericyte interactions <i>in vivo</i>. |
| 16:10 – 16:45 | Michael Sandholzer (HelmholtzZentrum Munich):
Micro-CT in preclinical applications: From embryogenesis to cardiovascular diseases. |
| 16:45 – 17:00 | Closing Remarks and Prizes: Ed Rainger and Myriam Chimen |
| 17:00 | Departure |



Oliver Borst

University Clinic of Tuebingen, Germany

Oliver Borst is a Consultant/Registrar in Cardiology at Eberhard Karls University Tuebingen, Germany, whose research focuses on the role of platelets in inflammation and their contribution to the development and progression of cardiovascular diseases. He has a particular interest in the intracellular signalling pathways responsible for platelet activation and secretion, as well as interactions with leukocytes.



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John Greenwood

Hugh Davson Professor of Biomedical Research and Head of Department of Cell Biology, University College London, London, UK.

The specialised vascular endothelial cells that line the vessels of the brain and retina form an impermeable but selective barrier between the blood and the neural parenchyma. Under normal physiological conditions this critical interface, termed the blood-brain/retinal barrier, strictly limits the passage of solutes and cells between these two compartments. During disease, however, the endothelial cells become activated resulting in a change of phenotype and an alteration in their regulatory function. Thus, in neuroinflammatory diseases such as multiple sclerosis and posterior uveitis the function of these vascular barriers changes resulting in an enhanced influx of leukocytes. Accordingly, the endothelial cells of the CNS are recognised as playing a pro-active role in the propagation, maintenance and possibly resolution of CNS inflammatory lesions. Over the last decade increasing evidence has emerged showing that the endothelial cell responds to adherent leukocytes in variety of ways resulting in immediate facilitation of diapedesis to the longer-term regulation of gene expression. Many of these outside-in signalling cascades are generated through the engagement of endothelial immunoglobulin superfamily adhesion molecules such as ICAM-1, which act as signal transducers leading to the activation of the small GTPase rho, eNOS, phospholipase C, protein kinase C, src kinase and release of intracellular calcium. In addition, downstream activation of MAP kinases, re-arrangements of the actin cytoskeleton and tyrosine phosphorylation of various cytoskeletal associated proteins has also been reported. Deciphering the end-points of these signaling networks, and indentifying potential pharmacological targets, has been a major focus of our laboratory. In this seminar I will present our current understanding of the role the CNS endothelial cell plays in facilitating leukocyte migration, including the role of various immunoglobulin superfamily adhesion molecule and chemokine signaling pathways, and how the endothelial cell junction, the vascular basement membrane and the pericyte influence this event.





Steve Thomas

Lecturer in Cardiovascular Sciences, Institute of Cardiovascular Science, University of Birmingham, UK

Steve Thomas is a lecturer in Cardiovascular Sciences whose research focuses on the role and regulation of the actin cytoskeleton in platelets and their progenitor cell, the megakaryocyte. Originally coming from a plant biology background, he has developed an interest in the cytoskeleton, firstly as a key regulator of programmed cell death in pollen and more recently in understanding how the cytoskeleton is involved in both platelet production from megakaryocytes and in regulating platelet activation, adhesion and thrombus formation. More recently he has been using super-resolution microscopy to provide deeper insights into the organisation of the cytoskeleton in these cells and how podosomes and platelet actin nodules are important for platelet function.



**UNIVERSITY OF
BIRMINGHAM**

Michael Sandholzer

Head of the Preclinical Imaging Unit, Institute of Experimental Genetics, HelmholtzZentrum Munich, Germany

Michael graduated from the University of Vienna in 2010 with a M.Sc. in Human Biology, with a focus on biomedical imaging, osteology and radiology. Between 2011 and 2014 he then went on to do a Ph.D. at the University of Birmingham, during which he worked intensively on the application of micro-CT imaging in biomedical research. In early 2014 he took up a Bioimaging postdoctoral position at MRC Harwell, researching the 3-D visualisation of gene expression in mouse embryos using tomographic and microscopic methods. Since April 2015 he heads the Preclinical Imaging Unit at the Institute of Experimental Genetics at the HelmholtzZentrum Munich. This current position focuses on in-vivo micro-CT, preclinical MRI and other imaging modalities to phenotype mice as part of an international multi-centre collaboration.

HelmholtzZentrum münchen
German Research Center for Environmental Health

SESSION 1: Platelet Regulation of Inflammation

Keynote Speaker:

Platelet-derived chemokines in inflammation

Oliver Borst

University Clinic of Tuebingen, Germany

Platelets are essential for primary hemostasis at sites of vascular injury, but are also critically important for the development of acute thrombotic occlusion, the major pathophysiological mechanism underlying myocardial infarction and ischemic stroke. Over the last decade there is growing evidence that platelets further act as multifunctional cells in many inflammatory processes involved in immune defense, wound healing, tissue repair and regeneration. Platelets are a rich source of different cytokines and chemokines, stored in their granules, such as stromal-derived factor 1 (SDF-1; CXCL12), CXCL16, interleukin 1 β (IL1 β), macrophage migration inhibitory factor (MIF), high mobility group box 1 (HMGB1) or cyclophilin A (CyPA) which could regulate vascular injury, inflammation as well as tissue repair and are crucial to survival of different cell types. Moreover, several platelet functions are modulated by inflammatory chemokines via different intracellular platelet signaling pathways linking inflammation to arterial thrombosis.

Short talks:

PLATELET ACTIVATION VIA CLEC2 DRIVES LIVER DAMAGE IN ACUTE INFLAMMATORY HEPATITIS

Abhishek Chauhan David H Adams, Steve P Watson and Patricia F Lalor

Center for Liver Research, Institute of Biomedical Research, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TH, UK.

Platelets are fundamental players in liver pathobiology; driving inflammation, fibrosis, cancer and even aiding regeneration. Platelet interaction with the sinusoidal endothelium drives leukocyte recruitment, thus initiating and perpetuating cycles of iterative inflammation or acute hepatitis. The specific molecular basis of platelet activation in the context of liver inflammation and thus damage remains elusive. We have been investigating the platelet ITAM receptor CLEC-2 in this regard. Platelet based CLEC-2 mediates powerful platelet activation on meeting its ligand Podoplanin. Interestingly homozygous loss of CLEC-2 does not give rise to the bleeding diathesis seen with traditional platelet inhibitors. We have studied mice deficient in platelet CLEC-2 (via PF4 cre) in the carbon tetrachloride model of acute murine hepatitis. Our data shows that hepatic necroinflammation post CCL4 injection is markedly less in PF4 cre mice (mean serum ALT 105 \pm 14 U/ml compared to WT mice 230 \pm 10 U/ml). We next demonstrate that in the inflamed liver, resident and non-resident macrophages (F480+CD11b+) up-regulate the only known naturally occurring CLEC-2 ligand-Podoplanin. We demonstrate that abrogating the platelet based CLEC-2 signal (PF4 Cre mice) or using a function- blocking podoplanin antibody results in reduced hepatic accumulation of F480+CD11b+ cells (11 X10⁴ Cells/gm liver tissue compared to 16 X10⁴ cells/gm) and results in less liver damage. In addition to mediating macrophage entry to the liver we demonstrate that PF4-cre mice exhibit reduced TNF- α production thus providing an explanation for the observed hepatoprotective effect seen in these animals. We next wished to investigate a role for this axis in human liver disease. In keeping with our murine data we demonstrate a dramatic upregulation of Podoplanin on Kupffer cells and vascular endothelium in diseased human livers. These findings together indicate that platelets and specifically the CLEC-2:podoplanin axis plays an important role in acute inflammatory liver disease and thus presents an exciting avenue for further research into potential therapies for acute hepatitis.

UNDERSTANDING THE ROLE OF THROMBOSPONDIN-1 IN COLLAGEN FIBRIL ORGANISATION

[Silvia Rosini¹](#), [Dominique Bihan²](#), [Rachael Stone²](#), [Richard W. Farndale²](#), [Josephine C. Adams¹](#)

¹School of Biochemistry, Biomedical Sciences Building, University of Bristol, Bristol BS8 1TD, UK ²Department of Biochemistry, Downing Site, University of Cambridge, Cambridge, CB2 1QW, UK. Supported by MRC K018043.

Introduction : Fibrillar collagens are the major structural proteins responsible for the organisation and 3D architecture of the extracellular matrix (ECM). In chronic pathological conditions such as fibrosis or scleroderma, tissues are characterized by a high deposition of collagens that also correlates with an increase of Thrombospondin-1 (TSP1) within the ECM. TSP1 is a calcium-binding glycoprotein secreted and retained as *puncta* in the ECM. In common with other TSPs, TSP1 affects the 3D assembly and the packing of collagen fibrils. Biochemical studies have indicated that TSPs may interact with collagens, but the binding site on fibrillar collagen is unknown for any TSP. The aim of this project is to map the binding sites between fibrillar collagens and TSP1. **Materials and Methods**: A solid phase, ELISA-type binding assay (SPBA) was used to screen fibrillar collagens I, II, III and the libraries of Collagen II and III Toolkit peptides, that span the triple helical regions of these collagens, for binding of native human platelet TSP1. After optimization of conditions, screens were performed in presence of 15 mM ZnSO₄ and 2 mM CaCl₂. **Results** : SBPA showed that platelet-TSP1 binds to collagens type I, II, III under physiological cation concentrations, with greatest binding to fibrillar collagen type I and weakest to monomeric collagen type I. The screens with Toolkit triple-helical peptides identified the 6-triplet region of interest GøOGøOGøKGHRGøOGøO (where ø is a hydrophobic residue) responsible for binding to TSP1. Using specific peptide mutants we could identify that this region contains, as key binding motif, the collagen cross-linking site KGHR. This motif is present in the alpha 1 chains of human fibrillar collagens type I, II, III, V, XI and in the alpha 2 chains of human fibrillar collagens type V and XI and is central to the role of Lysyl Oxidase (LOX) in crosslinking collagens in order to form fibres. **Discussion**: Possible models are proposed for a mechanism of direct interaction between TSP1 and collagen cross-linking sites responsible for regulating collagen fibril organisation in the ECM.

CHARACTERISATION OF A NOVEL INTERACTION BETWEEN MONOCYTES AND PLATELET-DERIVED MICROVESICLES

[Aigli Evryviadou](#), [Clare I Box](#), [Myriam Chimen](#), [Matthew J Harrison](#), [Stephen P Watson](#), [Gerard Nash](#), [G Ed Rainger](#)

Leukocyte Trafficking Group, NG15 IBR Link, College of Medical and Dental Sciences, University of Birmingham, Birmingham, B15 2TT

We have recently described a novel paradigm where platelets support monocyte recruitment to the vessel wall in a model of inflammation by acting as adhesive bridges between monocytes and endothelial cells. Given this observation, the aim of this study was to investigate the dynamics of platelet-monocyte aggregates formation in whole blood. We assessed binding of platelets to monocytes upon platelet stimulation in whole blood by measuring platelet-specific GPIb on CD14- and CD16-labelled monocytes using flow cytometry. Adhesion of monocytes was followed in flow conditions using phase-contrast videomicroscopy on recombinant vWF and TGF-β-stimulated endothelial cells. Platelet activation in blood resulted in monocytes acquiring GPIb in significantly smaller amounts compared to the amount of GPIb present on a single platelet, surprisingly suggesting that monocytes formed aggregates with platelet-derived microvesicles rather than whole platelets. The formation of these heterotypic aggregates was found to be time-dependent, monocyte-specific but not exclusive to any particular monocyte subset. This trend was also observed when platelet microvesicles were externally provided to whole blood but also when incubated with isolated monocytes. Incubation of monocytes with platelet microvesicles resulted in significantly increased numbers of monocytes adhering to both recombinant vWF and TGF-β-stimulated endothelial cells. The binding of monocytes was found to be GPIb-dependent. We speculate that by delivering adhesion receptors to monocytes, platelet-derived microvesicles can regulate the recruitment and potentially the differentiation and inflammatory function of monocytes.

SESSION 2: Specialised Vasculature

Keynote Speaker:**The specialised vascular barriers of the CNS and their influence on leukocyte migration****John Greenwood**

University College London, London, UK.

The specialised vascular endothelial cells that line the vessels of the brain and retina form an impermeable but selective barrier between the blood and the neural parenchyma. Under normal physiological conditions this critical interface, termed the blood-brain/retinal barrier, strictly limits the passage of solutes and cells between these two compartments. During disease, however, the endothelial cells become activated resulting in a change of phenotype and an alteration in their regulatory function. Thus, in neuroinflammatory diseases such as multiple sclerosis and posterior uveitis the function of these vascular barriers changes resulting in an enhanced influx of leukocytes. Accordingly, the endothelial cells of the CNS are recognised as playing a pro-active role in the propagation, maintenance and possibly resolution of CNS inflammatory lesions. Over the last decade increasing evidence has emerged showing that the endothelial cell responds to adherent leukocytes in variety of ways resulting in immediate facilitation of diapedesis to the longer-term regulation of gene expression. Many of these outside-in signalling cascades are generated through the engagement of endothelial immunoglobulin superfamily adhesion molecules such as ICAM-1, which act as signal transducers leading to the activation of the small GTPase rho, eNOS, phospholipase C, protein kinase C, src kinase and release of intracellular calcium. In addition, downstream activation of MAP kinases, re-arrangements of the actin cytoskeleton and tyrosine phosphorylation of various cytoskeletal associated proteins has also been reported. Deciphering the end-points of these signaling networks, and indentifying potential pharmacological targets, has been a major focus of our laboratory. In this seminar I will present our current understanding of the role the CNS endothelial cell plays in facilitating leukocyte migration, including the role of various immunoglobulin superfamily adhesion molecule and chemokine signaling pathways, and how the endothelial cell junction, the vascular basement membrane and the pericyte influence this event.

Short talks:**DISTURBED FLOW PROMOTES ADHESION OF NEUTROPHIL-DERIVED MICROVESICLES TO ENDOTHELIAL CELLS****Ben Ward¹, Ingrid Gomez¹, Paul Evans¹, Victoria Ridger¹**¹ Department of Cardiovascular Science, Faculty of Medicine, Dentistry and Health, University of Sheffield, Sheffield, UK.

Rationale & hypothesis: Atherosclerosis is an inflammatory disease of the arteries that preferentially develops at areas exposed to disturbed blood flow patterns. At these sites adhesion molecule expression is elevated on the surface of the endothelial cells (EC), facilitating transendothelial migration of inflammatory cells. Although neutrophil depletion is associated with reduced lesion progression, neutrophils are seldom found co-localised within developing plaque and their role in atherosclerosis remains uncertain. Upon activation, neutrophils release small, membrane microvesicles (MV) expressing cell surface proteins and pro-inflammatory cytoplasmic contents. MVs are able to interact with the endothelium, via binding ICAM-1 expressed on the EC surface. Previous unpublished data from our group has shown that neutrophil-derived MVs exclusively adhere to the inner curvature of the aorta arch (disturbed flow region) APO^{-/-} mice. Thus we hypothesise that disturbed flow regulates the interactions between MVs and the endothelium, contributing to the focal nature of atherogenesis. **Objectives:** To determine whether pre-conditioning under disturbed flow influences the adhesion of neutrophil-derived MVs to ECs. **Methodology:** Human umbilical cord vein endothelial cells (HUVEC) were exposed to flow using an in vitro parallel plate system (Ibidi). The cells were exposed to physiologically relevant levels of shear stress, either low oscillatory flow (4 dynes/cm² oscillating at 1 Hz) or high unidirectional flow

(13dynes/cm²). To measure the effect of disturbed flow on adhesion molecule expression, the cell surface expression of ICAM-1 and VCAM-1 was determined at 0, 4, 24, 48 and 72 hours following the onset of flow using flow cytometry. To investigate the influence of preconditioning on MV adhesion, HUVECS were under these flow conditions, with and without TNF- α (10 ng/ml) for 72 hours, or cultured statically and treated with TNF- α for 4 hours. Preconditioned cells were then incubated with fluorescently labelled MVs (1×10^{-6}), isolated from human peripheral blood neutrophils using fMLP (10^{-3} M for 1h), for 2 hours. Adhesion was determined using fluorescence microscopy. **Findings:** The expression of ICAM-1 in the disturbed flow condition was increased ($P < 0.01$), suggesting that 72 hours is a suitable time point for determining the effects of preconditioning ECs on MV adhesion ($P < 0.01$). Both disturbed flow ($P < 0.05$) and inflammation ($P < 0.05$) increase the level of adhesion compared to high unidirectional control. The addition of TNF- α to the high unidirectional shear stress condition did not increase MV adhesion ($P > 0.05$).

Conclusions: In conclusion, disturbed flow preferentially enhances the adherence of MVs to ECs. We propose that the mechanism involves the increase in the expression of adhesion molecule ICAM-1 on ECs exposed to disturbed flow. These findings implicate endothelial cell preconditioning in the exclusive adhesion of MVs to the inner curvature of the aortic arch in atherogenic APO^{-/-} animal models. These mechanisms may therefore contribute to our understanding of the focal nature of atherosusceptability.

T-LYMPHOCYTES WANTED - DEAD OR ALIVE; UNEXPECTED CONSEQUENCES OF CD4+ T CELL ADHESION TO HEPATOCYTES

S. Davies, N. Weight, S. Nakum, K. Wang, E. Humphries, G. Reynolds, P. Lalor, Y. Huang, D. Adams, Z. Stamataki

Immunity and infection, University of Birmingham, Birmingham, B15 2TT UK

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The department of infectious disease, the third affiliated hospital, Sun Yat-sen University, Guangzhou City, 510630, China

T lymphocytes frequently infiltrate into the liver parenchyma where, they come into contact with hepatocytes. We have recently found that, following their adherence to hepatocytes, CD4+ T cells were preferentially internalised whilst still alive in a process similar to entosis. Hepatocytes are also competent phagocytes, clearing dead cells in the liver parenchyma. Although phagocytosis and entosis have been previously characterised in other systems, details regarding the molecular mechanisms of each process, as well as the fate of internalised live cells, have not been unearthed. We set out to characterise the kinetic and molecular differences between the endocytosis of live and dead T cells in hepatocytes. We performed scanning electron microscopy to analyse the adhesion and capture mechanisms for live and dead T-cells by hepatocytes. Blebbing of the hepatocyte membrane was observed during the adhesion and capture of live T cells. Conversely, the engulfment of dead T cells ubiquitously involved membrane ruffling characteristic of phagocytosis. In order to assess how the kinetics of these processes may differ and be modulated, we co-cultured fluorescently labelled hepatocyte cell lines with live or dead CD4+ T-cells in the presence or absence of different cytokines or glucocorticoids. The frequency of entosis and phagocytosis was determined by counting internalised T cells per hepatocyte using confocal microscopy. We show that dead T cells were more readily internalised than live CD4+ T-cells. Furthermore, that IFN gamma and dexamethasone were able to increase the rate of phagocytosis within hepatocytes, whilst failing to alter the rate of entosis. Finally, we performed immunostaining for Rab proteins and used pH indicators to analyse intracellular vacuoles containing live or dead T cells. Vacuoles containing live cells were less likely to acidify than those containing dead cells. Additionally, entotic and phagocytic vacuoles were both Rab 5 and Rab 11 negative. Of note, vacuoles containing live cells did not associate with Rab 9, unlike those containing dead cells. These results suggest that the processes of entosis and phagocytosis, as conducted by hepatocytes, are distinct, and they can be distinguished by variations in adhesion and engulfment, differences in endosomal trafficking and in response to environmental cues.

SESSION 3: Part I: Novel Imaging Modalities in Health and Disease

Keynote Speaker:

Platelet adhesion and the actin cytoskeleton: Peeking beyond the diffraction limit.

Steven Thomas

University of Birmingham, Birmingham, UK.

Fluorescence microscopy is commonly used in cell biology to dissect out the structure and function of many cellular processes and is widely used in the study of platelets and megakaryocytes. However, there is a physical limit to the resolving power light microscopes can achieve which limits the extent of the spatial information we can obtain. Several techniques have been developed which are able to circumvent this limit and are known collectively as super-resolution microscopy. In this talk, I will introduce the platelet actin nodule, a structure which we identified in platelets and is associated with early spreading, and describe how we have used super-resolution microscopy to dissect out the possible role of this structure. I will also present data showing how we believe that the actin nodule is important for platelet adhesion both to the ECM and to other platelets in the growing thrombus.

Short talks:

TNF α DRIVES CCR7-DEPENDENT MIGRATION OF NEUTROPHILS INTO AFFERENT LYMPHATIC VESSELS *IN VIVO*

S. Arokiasamy^{1, 2}, W. Migo¹, J.A. Dilliway¹, W. Wang², S. Nourshargh¹, M.-B. Voisin¹

¹ Centre for Microvascular Research William Harvey Research Institute, Barts and The London SMD, QMUL, UK;

² Institute of Bioengineering, QMUL, UK.

Key effectors of innate immunity, neutrophils, are capable of promoting and/or regulating adaptive immune responses following migration into the draining lymph nodes of infected tissues or following immunisation. However, the mechanisms of this migration are still poorly understood and a matter of controversy in the literature. In the present study, we developed a murine model of cremasteric inflammation to directly visualise *in vivo* the migration of neutrophils into tissue-associated afferent lymphatic vessels. Upon stimulation with Complete Freund's Adjuvant plus antigen neutrophils rapidly migrated into both the inflamed tissue and the cremasteric lymphatic vasculature in a time-dependent manner. Interestingly, this intravasation response was partially inhibited in mice genetically deficient in both TNF α receptors (p55 and p75) as well as in CCR7KO animals, as compared to control mice. Furthermore, whilst TNF α alone induced the migration of neutrophils into afferent lymphatics in WT animals, this response was completely suppressed in CCR7KO mice (more than 95% inhibition). Taken together, our results demonstrate for the first time a critical role for TNF α in priming the neutrophils to migrate into the lymphatic vessels *in vivo* in a CCR7-dependent manner.

L-SELECTIN/CD62L DEPENDENT HOMING OF INFLUENZA-SPECIFIC CD8⁺ T CELLS IS ESSENTIAL TO CONTROL PULMONARY INFECTION

[Rebar N. Mohammed](#), [Ian R Humphreys](#) and [Ann Ager](#)

Institute of Infection and Immunity, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN

Cytotoxic CD8⁺ T lymphocytes (CTLs) play a critical role in host defence against viruses. The ability to secrete cytotoxic chemicals and cytokines is considered pivotal for eliminating virus from infected tissues. Of equal importance is how CTLs home to virus-infected tissues. L-selectin is a type I transmembrane adhesion molecule that is well characterized as a lymph node homing receptor on the surface of naïve CD8⁺ T cells. Upon activation, CD8⁺ T cells downregulate L-selectin expression using two independent mechanisms of ectodomain proteolytic shedding and gene silencing. The role of regulated expression of L-selectin in the homing of activated CD8⁺ T cell to sites of virus infection has not been determined.

We have found that L-selectin is downregulated on virus-specific CD8⁺ T cells in lymph nodes which drain the site of virus inoculation within 24-48 hours. However, L-selectin is re-expressed before activated CD8⁺ T cells leave lymph nodes. Activated CD8⁺ T cells released into the bloodstream express high levels of L-selectin and recruitment from the bloodstream into virus-infected tissues is L-selectin dependent. To determine the role of L-selectin dependent homing in protective immunity to virus infection, CD8⁺ T cells co-expressing the F5 T cell receptor and different levels of L-selectin were intravenously transferred to immunodeficient RAG^{-/-} mice. Mice were infected intranasally with H17 influenza A virus which expresses the cognate nucleoprotein peptide for F5 T cell receptor and virus titres measured in the lungs at day 8 post-infection. F5 CD8⁺ T cells expressing wildtype L-selectin controlled pulmonary influenza infection whereas F5 CD8⁺ T cells deficient in L-selectin (L-sel^{-/-}) had no effect of virus levels in the lungs, despite being activated normally in the lung draining, mediastinal lymph node. F5 CD8⁺ T cells expressing a mutant L-selectin (LΔP), which is not downregulated in activated T cells, provided enhanced protection against pulmonary influenza infection. In contrast to intravenously transferred F5 CD8⁺ T cells, L-selectin expression had no effect on the ability of F5 CTLs to control virus replication following their installation directly into the airways of influenza-infected mice.

Collectively, our data show that re-expression of L-selectin on the surface of activated CD8⁺ T cells after initial priming and activation in lymph nodes is required to enable recruitment of these cells from the bloodstream into virus-infected lungs and limit the replication of influenza virus. These results connect homing and a function of virus-specific CD8⁺ T cells for the first time to a single molecule, L-selectin.

SESSION 3: Part II: Novel Imaging Modalities in Health and Disease

Short talks:

MODELLING OF IMMUNE CELL DIAPEDESIS FROM VESSELS TO TISSUE DAMAGE IN THE *DROSOPHILA* PUPAL WING VEINS

[Leila Thuma](#)¹, [Helen Weavers](#)² & [Paul Martin](#)^{1,2}

¹School of Physiology & Pharmacology, ²School of Biochemistry, Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK

Diapedesis, the migration of immune cells through intact blood vessel walls towards damaged tissue, plays an essential role in host defence. Exactly how this rate-limiting component of the wound inflammatory response is regulated is still not completely understood. Inflammatory disorders, such as leukocyte adhesion deficiency, resulting from abnormal diapedesis lead to recurrent infections and impaired wound healing; research into this process therefore has important therapeutic application for the treatment of these diseases. To gain further insight into the molecular mechanisms underlying diapedesis, we propose a novel diapedesis model making use of the developing pupal and adult *Drosophila* wing. Importantly, *Drosophila*, as a simple model system with powerful genetic capabilities, offers a fast-track approach for screening and the potential for high resolution *in vivo* imaging to dissect out the underlying mechanisms of diapedesis. Although largely speaking the cardiovascular system of *Drosophila* consists of an open circulatory system, vein-like vessels are found in the developing pupal and mature adult wings that carry the insect blood (hemolymph) and the macrophage like - immune cells called hemocytes. In undamaged flies, hemocytes flow within the hemolymph through these vein-like channels facilitated by so-called wing hearts, which contract in a pulsatile fashion. High-resolution live imaging reveals how, upon wounding, hemocytes stall and migrate from the vein lumen towards the damaged tissue, where they clear up necrotic wound debris. After emigration from the veins, hemocytes migrate through the extracellular matrix (ECM) to reach the damaged tissue and we are currently analyzing this at the ultrastructural level to reveal more information about the mechanisms of migration. Exploiting the sophisticated genetic tools available in *Drosophila*, we can live image remodeling of Collagen IV after injury, using a fluorescent Collagen IV reporter, and we will extend that analysis to other ECM components such as Perlecan. Using this new model, we hope to provide further insight into the molecular mechanisms of diapedesis and by performing a large scale *in vivo* screen, uncover new players in this process.

THE ROLE OF CHEMOKINES IN THE REGULATION OF NEUTROPHIL-PERICYTE INTERACTIONS *IN VIVO*

[Tamara Gírl](#), [Mathieu-Benoit Voisin](#), [Sussan Nourshargh](#)

Centre for Microvascular Research, William Harvey Research Institute, Barts & The London School of Medicine, Queen Mary University of London, UK

The passage of blood leukocytes through venular walls is a key component of an effective immune response. Whilst the function of chemokines in leukocyte-endothelial cell (EC) interactions is well defined, little is known about their role in leukocyte-pericyte interactions. To address this issue, we have developed a confocal intravital microscopy (IVM) platform for tracking neutrophils within the pericyte layer of cremaster muscle venules of α SMA-RFPcherry;Lys-EGFP-ki mice (express RFP⁺ pericytes & EGFP⁺ neutrophils; *Proebstl et al.*, *JEM*, 2012). With this approach we observed that neutrophils extensively crawl on pericytes within venular walls before entering interstitial tissues via gaps between adjacent pericytes. The aim of the present study was to investigate the potential role of chemokines in this novel phase of neutrophil migration. Using the IVM method detailed above, we found that the administration of a blocking anti-CXCL1 mAb decreased the crawling speed and directional migration of neutrophils on pericytes as compared to a control mAb, collectively inhibiting the displacement of neutrophils on the pericyte layer (69.6% inhibition, $p=0.007$) during TNF-induced inflammation.

This was associated with a substantial retention of neutrophils within venular walls, with only a small fraction of the neutrophils breaching the pericyte layer in anti-CXCL1 mAb-treated tissues as compared to control tissues (6.3% vs 83.1%, respectively, $p=0.005$). Interestingly, blockade of CXCL1 led to a significant number of neutrophils in the sub-EC space exhibiting reverse trans-EC migration back into the circulation (16.7%), suggesting that CXCL1 is critical in guiding neutrophils into the extravascular tissue. Furthermore, our results indicate that cultured TNF-stimulated cremaster muscle pericytes are a rich source of CXCL1. Collectively, our results demonstrate that CXCL1 is essential both for supporting neutrophil crawling on pericytes and providing the directional cues necessary for full breaching of venular walls.

This work was funded by the British Heart Foundation (FS/14/3/30518) & the People Programme (Marie Curie Actions) of the EU (FP7/2007-2013, REA grant agreement 608765).

Keynote Speaker:

Micro-CT in preclinical applications: From embryogenesis to cardiovascular diseases

Michael Sandholzer

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For over two decades, X-ray micro-computed tomography (micro-CT) has been almost exclusively used to produce ex-vivo 3-D data sets of mineralised tissue. The nature of X-rays leads to a higher attenuation by mineralised tissue compared to soft tissue. The rediscovery of histological stains and development of preclinical contrast agents in the past five years enabled micro-CT technology to also be applied for soft-tissue applications. This provided further understanding of animal morphology and developmental, genetic and physiological processes. Additionally, novel in-vivo micro-CT scanners for rodents now also allow high-resolution imaging for longitudinal studies of developmental defects, disease progression and pharmaceutical kinetics at a low X-ray dose. This lecture is going to give an overview of micro-CT imaging in preclinical applications, with a focus on experimental genetics, cardiovascular and immunological research. Moreover, complementary imaging methods for these research areas such as optical projection tomography (OPT) and high-resolution episcopic microscopy (HREM) will be highlighted.

P1: CHARACTERISING THE THERAPEUTIC POTENTIAL OF A PEPTIDE INHIBITOR OF T CELL TRANSENDOTHELIAL MIGRATION

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Peptide inhibitor of transendothelial migration (PEPITEM) is a novel 14-aa peptide secreted by B cells, which inhibits T cell trafficking across inflamed vascular endothelium. In autoimmune and chronic inflammatory diseases, e.g. type-1 diabetes and rheumatoid arthritis, this homeostatic mechanism is lost leading to inappropriate T cell migration with pathological consequences. We aim to develop PEPITEM as a therapeutic agent. Here, we characterise the pharmacokinetics of PEPITEM or peptides subject to single alanine substitutions, and establish their functional efficacy. PEPITEM spiked into plasma *in vitro* showed no significant degradation after 1h. However *in vivo* studies with radiolabelled PEPITEM demonstrate a circulating half-life of <2min. Thus, PEPITEM was probably not degraded by circulating peptidases but was likely cleared rapidly from the circulation by renal filtration. We confirmed this using a fluorescent version and whole animal *in vivo* imaging, which demonstrated rapid localisation in the kidneys after intravenous injection. These data imply that a modified version of PEPITEM with a longer half-life is required for therapeutic applications. Initially we investigated whether some amino acids were functionally essential. Alanine substitutions suggested that no individual amino acid was vital for function. This is consistent with NMR spectroscopy data, indicating a simple flexible structure with no secondary interactions. Blocking the N & C termini reduced functional efficacy, suggesting functional importance here. In order to develop a therapeutic agent to T cell-mediated chronic inflammatory diseases, the circulating half-life of PEPITEM must be extended whilst maintaining function. New variations of the peptide must now be formulated and tested *in vitro* and in disease models for efficacy.

P2: CHARACTERISING INTRACELLULAR TRAFFICKING OF JUNCTIONAL ADHESION MOLECULE C (JAM-C)

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The vascular endothelium has a key role in the response to infection or tissue injury. Dysfunction of the endothelium can predispose the vessel wall to leukocyte adhesion, platelet activation, oxidative stress, thrombosis, and chronic inflammation such as that involved in cardiovascular disease. Of particular interest are endothelial cell junctions, which govern permeability and are remodelled to allow leukocyte transmigration. One junctional molecule, Jam-C, has a role in polarised transendothelial cell migration, angiogenesis, and vascular permeability, yet little is understood about the molecular mechanisms which regulate Jam-C junctional formation and remodelling. We show here that in endothelial cells, as well as being present at the cell surface Jam-C is present in vesicles inside the cell, and that the number of these vesicles increase under certain conditions including TNF α stimulation and calcium-mediated junctional disassembly. We have also begun to define specific residues of Jam-C which affect intracellular trafficking and recycling. Removal of the C-terminal PDZ interaction motif results in accumulation of protein at the Golgi, whilst an ubiquitination site in the intracellular domain controls the rate of Jam-C degradation. As Jam-C function has been implicated in multiple inflammatory diseases including arthritis, peritonitis, pancreatitis, ischaemia reperfusion injury, pulmonary inflammation and atherosclerosis, gaining a mechanistic insight into Jam-C trafficking is an essential first step in characterising, and eventually modifying, its physiological role.

P3: PHOSPHOINOSITIDE 3-KINASES (PI3K) ACTIVATE EXTRACELLULAR SIGNAL-REGULATED KINASES (Erk) THROUGH MTORC2 AND PAK IN ADHESION DEPENDENT SIGNALLING IN NEUTROPHILS

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Background. Neutrophils are important components of innate immunity. Their activity is tightly controlled to ensure adequate protection from infections whilst avoiding excessive inflammation. The PI3K-Akt-mTOR and Ras-Raf-Mek-Erk signalling pathways are important regulators of cellular responses of neutrophils. These two signalling pathways regulate separate downstream effects, but a number of studies indicate some cross talk takes place. Here, we analyse the interrelationship between both pathways in human and mouse neutrophils with a focus on adhesion dependent stimulation. **Methods.** All experiments were performed with freshly prepared primary human or mouse neutrophils that had or had not been preincubated with specific small molecule inhibitors prior to stimulations. To analyse signalling events, Western blots were performed with phospho-specific antibodies (for kinases) or by G-LISA assay (for small GTPases). **Results.** On stimulation of integrins or FcγR, Erk activation was completely dependent on class IA PI3K in both mouse and human neutrophils. The contribution of individual PI3K isoforms differed between species. Erk activation was further downstream of mTORC2, but not of mTORC1 or PKB/Akt. Interestingly, Erk was not regulated in the canonical pathway (Ras-Raf-Mek-Erk), and indeed Ras does not lie downstream of PI3K. We assessed the involvement of a number of alternative MAP3Ks, and identified Pak acts as a MAP3K in this context. Current experiments address the Rho family small GTPase that lies upstream of Pak. Moreover, we are analysing whether this novel pathway provides the mechanism by which Mac1/CD11b/αMβ₂ activity is being regulated downstream of FcγR activation.

P4: MESENCHYMAL STEM CELLS INFLUENCE THE ABILITY OF INFLAMED ENDOTHELIAL CELLS TO RECRUIT NEUTROPHILS: A COMPARATIVE STUDY.

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Mesenchymal stem cells (MSC) are multi-potent stromal precursor cells capable of self-renewal and differentiation that reside within the perivascular niche of almost all tissues. Recently we have shown that bone marrow MSC (BMMSC) modify the response of vascular human umbilical vein endothelial cells (HUVEC) to cytokines and suppress neutrophil recruitment from flow. Here we compare the ability of primary human BMMSC to modulate the responses of endothelial cells (EC) from different tissues and influence their ability to support the recruitment and migration of circulating neutrophils.

Two forms of co-cultures were developed: (1) To assess effects on recruitment, EC and MSC were cultured on opposite sides of porous filters and incorporated into a novel flow chamber. Neutrophil were perfused and observed as they bind to the EC surface. (2) To examine effects on migration, EC monolayers were formed on the apical surface of a collagen gel in which MSC were incorporated. Neutrophil migration through the construct and their location within the gel were assessed.

MSC significantly suppressed neutrophil adhesion to stimulated HUVEC and dermal blood vascular endothelial cells (BEC). In the multi-cellular gel model, MSC co-cultured with BEC were also capable of suppressing neutrophil adhesion and thus further migration into the gel. The presence of MSC did not appear to affect the depth to which recruited neutrophils migrated.

Our results suggest MSC are able to modify the response of EC from different tissues, suppressing the recruitment of circulating neutrophils and their onward migration. Thus MSC are endogenous tissue-resident regulators of inflammation.

P5: THE ROLE OF MAPKS IN ICAM-1 SIGNALING IN HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS.

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Endothelial ICAM-1 is known to play a crucial role in the transendothelial migration (TEM) of leukocytes across the endothelial barrier. Our previous data demonstrated the role of MAP kinases ERK, p38 and JNK during ICAM-1-mediated lymphocyte migration across brain microvascular endothelial cells (BMVECs). All three MAPKs were activated by LFA-1-ICAM-1 interaction or antibody mediated clustering. Moreover, all of them controlled ICAM-1 dependent transcription of many inflammatory genes, but only JNK played a role in facilitating diapedesis. Some of the diapedesis-related function in brain endothelial cells have been confirmed in non-brain endothelial cells, thus some of the ICAM-1 induced signalling appear to be similar across the entire vascular bed. Therefore, here we investigate the potential brain specificity of the ICAM-1-induced MAPK response. To do that we studied the role of ERK, p38 and JNK in response to ICAM-1 engagement in human dermal microvascular endothelial cells (hDMEC) to compare the results to those obtained in brain cells. Furthermore we assayed TEM and MAPK involvement using hDMEC and human CD4⁺ harvested from peripheral blood. These results shed novel light on our understanding of vascular inflammation.

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P6: PLATELET DERIVED EXTRACELLULAR VESICLES IN NEUTROPHIL RECRUITMENT TO ENDOTHELIUM DURING INFLAMMATION.

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Introduction: Platelet extracellular vesicles (PEV) account for a large proportion of circulating extracellular vesicles and have been suggested to promote leukocyte recruitment to the vascular endothelium. The rate of PEV binding to endothelial cells (EC), their influence on neutrophil recruitment and mechanisms involved are not well understood. We aimed to determine the binding kinetics of PEV to EC and the resultant effects on neutrophil recruitment from flow.

Methods: PEV were generated from CD41-labeled platelets, stimulated with collagen related peptide (CRP-XL, 1µg/ml) and were incubated with EC. PEV-mediated stimulation of EC was assessed by flow cytometry for adhesion receptors. Flow-based adhesion assay assessed neutrophil recruitment on PEV-coated on glass capillaries or on EC grown in flow chambers and treated with combinations of PEV and different concentrations of TNF-α. Blocking studies were performed to assess the role for chemokine receptors. Chemokines in cell supernatants were measured using multiplex chemokine array.

Results: PEV binding to EC was detected within 1hr and maximal by 4h with >60% dual positivity for CD41 and VE-cadherin on EC. The PEV uptake resulted in upregulation of endothelial activation markers (E-selectin and VCAM-1). Neutrophils bound directly to PEV enabling frequent inflow capture and low levels of stable adhesion to a PEV-coated surface. Similar effects of PEV were observed on unstimulated or minimally stimulated (1U/ml TNF-α) EC. Blocking studies revealed roles for P-selectin, platelet activating factor and chemokine receptors in PEV mediated neutrophil capture and adhesion. Furthermore, PEV supernatants contained platelet chemokines such as platelet factor 4 and RANTES along with Interleukin-8, GRO-α, ENA-78 and MCP-1.

Conclusions: Surface-bound PEV can directly capture flowing neutrophils and also activate endothelial cells. Thus PEV may promote neutrophil recruitment in inflammation, by potentiating effects of low levels of cytokines acting on EC.

P7: ADAM15 AFFECTS TIGHT JUNCTION FORMATION IN BREAST CANCER

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ADAM15 is a multidomain multifunction transmembrane protein. It participates in protein ectodomain shedding via the metalloproteinase domain. ADAM15 also interacts with integrins via the disintegrin domain¹. ADAM15 mRNA is subject to complex processing, generating different isoforms as a result of alternative splicing. The splicing affects the intracellular domain (ICD) of the protein, generating distinct SH3 domain binding ligand regions. The expression of specific splice forms correlate with breast cancer prognosis².

We generated an isogenic panel expressing each ADAM15 isoform in MDA-MB-231 cells. Comparative characterisation of the panel demonstrated distinct differences between isoforms, such as catalytic function dependant or independent effects on the growth rate. We discovered ADAM15 isoform-dependant upregulation of tight junction protein Claudin1. Immunofluorescence analysis demonstrated co-localisation of ADAM15 with Claudin1 and another tight junction protein ZO1 at cell-cell junctions. Further immunoprecipitation analysis showed potential complex formation with ADAM15 and ZO1. ADAM15-mediated Claudin1 upregulation did not result in enhanced trans-epithelial resistance as measured by TEER. However, claudin1 upregulation might play a role in protecting cancer cells from anoikis, as well as facilitate collective cell migration.

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P8: THE REGULATION OF L-SELECTIN ACTIVITY BY PROTEOLYSIS

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L-selectin is a type I transmembrane protein found in the plasma membrane of lymphocytes. The lectin domain of L-selectin binds heavily glycosylated ligands that express 6-sulphated Sialyl Lewis X (sLex) tetrasaccharide, such as the glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1). This interaction allows tethering of lymphocytes to high endothelial venules in peripheral lymph nodes.

L-selectin is cleaved in the ectodomain by ADAM metalloproteinase 17 (ADAM17) generating a membrane retained fragment (MRF). After ectodomain cleavage, many type I transmembrane proteins are ripped in the intramembrane region by γ -secretase. Presenilin is the catalytic component of gamma-secretase responsible for this proteolysis. There are two isoforms of presenilin, presenilin 1 (PS1) and presenilin 2 (PS2). This study hypothesises that following ectodomain proteolysis, the MRF of L-selectin is cleaved in the transmembrane region by either PS1 or PS2.

Using western blot analysis, our results show ADAM17 ectodomain proteolysis cleaves full length L-selectin to generate a membrane retained fragment. The amount of MRF detectable in presenilin double knockout (PSdKO) mouse embryonic fibroblast (MEF) cells was significantly higher than in wild type MEF cells. Complementing PSdKO MEF cells with PS1 reduced the level of MRF, whereas expression was unaltered after introducing PS2. These results show that L-selectin undergoes two proteolytic events. First L-selectin is shed at its ectodomain by ADAM17. The resulting MRF is then cleaved in the transmembrane region by PS1 releasing the cytoplasmic tail into the intracellular compartment.

P9: HUMAN LIVER B CELLS ARE ENRICHED IN CD24 NEGATIVE POPULATIONS

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B cells are found in the liver in proportions similar to blood, yet little is known about their subset composition in healthy and diseased liver (autoimmunity, viral infection, dietary injury). We defined nine subpopulations of B cells isolated from human liver tissue explants by flow cytometry: *Naïve Mature* (CD19+IgM+IgD+CD27-CD38-), *Natural Effector* (CD19+IgD+CD27+CD38-), *Memory* (CD19+IgD-CD27+CD38-), *Plasmablasts* (CD19+IgD- CD27+CD38hi), *Plasma cells* (CD19+IgD-CD27+CD38hiCD138+), *B10-like B cells* (CD19+CD5+CD1dhi), *Human B1-like cells* (CD19+CD70-CD43+CD27+), *centroblasts* (CD19+CD77+) and *Transitional/Regulatory B cells* (CD19+CD38hiCD24hi). We compared healthy and diseased liver and blood to spleen and mesenteric lymph nodes. We discovered that CD24 negative B cells, which were low/absent in patient blood, were enriched in matched liver specimens. CD24 is a glycosyl phosphatidylinositol (GPI)- anchored glycoprotein found in most B cells associated with lipid rafts. In cancer cells, it operates as an adhesion molecule that binds to P-selectin and can facilitate tumour evasion. It's role in healthy B cells is unclear, yet recent experiments in mice have shown that CD24 expression reduced SDF-1-mediated cell migration and signalling via CXCR4. CD24 is highly expressed in early stages of B cell development and can be lost as B cells experience antigen recognition, potentially leading to increased susceptibility to apoptosis. Our experiments detected CD24 negative cells in multiple B cell subsets including naïve B cells, and did not show an increase of CD24 negative cells in activated or memory B cells in the liver. Liver and tonsil immunohistochemistry indicated that CD24 negative B cells localized in B cell follicles and near P-selectin expressing blood vessels. Further staining indicated that CD24 negative B cells were most broadly distributed in patients with viral or autoimmune hepatitis. Our current experiments investigate the mechanism and impact of CD24 loss in B cell biology. We provide a thorough characterisation of B cell subsets in the human liver, in health and in chronic inflammation. Our results reveal differences in the B cell compartment amongst patients with different liver diseases, and highlight a role for the adhesion molecule CD24 in human liver B cell biology.

P10: CORTISOL REGULATES THE EXPRESSION OF ADIPONECTIN RECEPTORS ON B LYMPHOCYTES IN PATIENTS WITH TYPE 1 DIABETES.

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We recently discovered a novel pathway regulating the trafficking of T lymphocytes during inflammation through the action of adiponectin on B lymphocytes. Adiponectin binding to the adiponectin receptors (AdipoR1 and AdipoR2) on B lymphocytes induces release of a novel peptide, called PEPITEM that binds to the endothelium and subsequently inhibits T lymphocyte transmigration. The expression of adiponectin receptors on B lymphocytes from patients with Type 1 Diabetes (T1D) and rheumatoid arthritis is lower compared to matched healthy controls. This results in a loss of control on T cell trafficking by adiponectin, which facilitates chronic inflammation. However, the determinants regulating expression of adiponectin receptors remain unknown. Here, we aimed to determine the effect of cortisol on adiponectin receptor expression on B lymphocytes over time.

We quantified the expression of both adiponectin receptors in the presence or absence of cortisol and in a solvent (methanol) control using flow cytometry. Cell viability was measured using the Zombie Aqua dye. Mass spectrometry was used to quantify serum steroid concentrations for patients with T1D and healthy controls, which was then correlated with expression of AdipoRs on B lymphocytes.

B lymphocyte viability remained above 80% over the 72 hour time-course. The expression of AdipoRs declined on B lymphocytes after 48 and 72 hours compared to day 0 pre-cortisol treatment. This could be rescued by culture of B lymphocytes in the presence of cortisol but not methanol. In addition, preliminary data indicates that circulating concentrations of cortisol and 11-deoxycortisol tend to be lower in patients with T1D compared to matched healthy controls. Additionally, the expression of AdipoR1 positively correlated with 11-deoxycortisol concentrations. Altogether, this data indicates that there may be a role for glucocorticoids, such as cortisol, in regulating the expression of AdipoRs on circulating B lymphocytes.

P11: THE TSPANC8 TETRASPANINS TSPAN5 AND TSPAN17 REGULATE THE TRANSMIGRATION OF LYMPHOCYTES BY MODULATING ADAM10-DEPENDENT SHEDDING OF VASCULAR ENDOTHELIAL (VE)-CADHERIN

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ADAM10 is a ubiquitously expressed transmembrane metalloprotease responsible for the proteolytic shedding of key cell surface proteins. On endothelial cells these include the cellular adhesion molecule vascular endothelial (VE)-cadherin and transmembrane chemokines. Their shedding by ADAM10 has been implicated in leukocyte transmigration in cell line models. ADAM10 is regulated by the TspanC8 subgroup of six tetraspanin proteins, and emerging evidence suggests that different TspanC8s might target ADAM10 to different substrates. The aim of the present study was to determine the mechanism by which ADAM10 promotes leukocyte transmigration in primary human cells, and to determine whether one or more TspanC8s facilitate this process.

Utilising an *in vitro* flow-based adhesion assay and video microscopy, siRNA knockdown or pharmacological inhibition of ADAM10 on human umbilical vein endothelial cells (HUVECs) was found to impair transmigration of primary human lymphocytes, but not neutrophils. This effect was associated with enhanced endothelial electrical resistance, a reduction in VE-cadherin shedding and a subsequent increase in VE-cadherin surface expression. Partial knockdown of VE-cadherin, in the presence of ADAM10 knockdown or inhibition, reduced VE-cadherin levels to normal and restored basal lymphocyte transmigration. Systematic knockdown of TspanC8s in HUVECs revealed that the presence of either Tspan5 or Tspan17 was sufficient to maintain basal lymphocyte transmigration and normal VE-cadherin surface expression levels. Tspan5 and Tspan17 are functionally uncharacterised tetraspanins, but they are the most highly related TspanC8s by sequence (78% amino acid identity) and may share a common role in the lymphocyte transmigration by regulation of ADAM10 and VE-cadherin.

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P12: LAR PROTEIN TYROSINE PHOSPHATASE REGULATES FOCAL ADHESION VIA CDK1

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Focal adhesions are large, complex multi-molecular structures that link the actin cytoskeleton to the extracellular matrix via integrin adhesion receptors. Adhesion complexes play a key role in regulation of many cellular functions and their assembly is regulated by growth factor signalling via receptors such as the PDGF receptor. LAR is a member of the receptor-like subfamily of protein tyrosine phosphatases and a known regulator of PDGF signalling. Using mouse embryo fibroblasts expressing a truncated form of LAR we observed that loss of LAR phosphatase activity results in reduced numbers of focal adhesions, and significantly decreased cell adhesion to fibronectin. To understand how LAR regulates cell adhesion we used SILAC-based mass spectrometry to analyse global phosphorylation events in wild type and LAR phosphatase-deficient cells. We found that loss of LAR activity resulted in reduced phosphorylation of a number of cyclin-dependent kinases including CDK1, which has recently been identified as a regulator of cell adhesion. Subsequent experiments established that the c-Abl tyrosine kinase acts downstream of LAR to regulate CDK1 activity via the serine/threonine kinase PKB/Akt. This study identifies a novel role for LAR phosphatase in regulating CDK1 activity and hence cell adhesion to the extracellular matrix.

P13: CAFFEINE DAMPENS AMINE OXIDASE-DEPENDENT IMMUNE REGULATION

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Introduction: Vascular adhesion protein (VAP)-1 is expressed within the liver where it has been shown to modulate inflammation and fibrosis, and the catalytic activity of VAP-1 has been associated with immune trafficking. We sought to define the role of the enzymatic activity of this protein in the context of inflammatory liver disease, and investigate the potential of caffeine as an inhibitor of VAP-1. **Materials and Methods:** VAP-1 expression in patient tissue was assessed by immunohistochemistry and catalytic activity was quantified by a novel Amplex UltraRed assay. Leukocyte adhesion to primary human hepatic sinusoidal endothelial cells (HSEC) was assayed by flow-based adhesion and confocal microscopy. **Results:** The expression of VAP-1 was associated with inflammatory liver disease and increased enzyme activity in alcoholic livers. Immunopurified VAP-1 prepared from tissue lysates of normal and inflamed human livers was sensitive to inhibition by caffeine in a dose-dependent manner. Pre-treatment of HSEC with caffeine reduced the proportion of cells that transmigrated across the monolayer without affecting the total number of adherent leukocytes, however caffeine had no effect on adhesion to recombinant protein. Confocal microscopy of HSEC transfected with GFP-tagged VAP-1 protein revealed an intimate association between endothelial VAP-1 and inflammatory cells. **Conclusions:** Human VAP-1 is expressed in inflammatory liver disease and is sensitive to inhibition by caffeine, associated with a reduction in leukocyte recruitment. These data are consistent with previous studies demonstrating the protective effect of caffeine in hepatic inflammation and forms the basis of a healthy volunteer study (NOCTUA, <https://clinicaltrials.gov/ct2/show/NCT02098785>).

P14: THE TRANSMIGRATION OF CD4⁺ T CELLS THROUGH HEPATIC EPITHELIA REVEALS FUNCTIONAL AND PHENOTYPIC DIFFERENCES NOT EVIDENT IN STATIC CO-CULTURE

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The ability of a T cell to migrate to its target organ is intrinsically linked to efficient functionality. In the liver, the mechanisms and stimuli involved in the adhesion and migration of T cells across the sinusoidal endothelium are now starting to become understood. However, T cell- hepatocyte interactions remain uninvestigated. This is important as T cell function may be modulated by hepatocytes upon entry into the tissue. Therefore we asked the question: does the adhesion and migration of T cells through hepatic epithelia affect their phenotype and/or function? To investigate this we used transwell systems whereby hepatocyte cell lines were cultured to confluency on transwell inserts before human blood or liver derived CD4⁺ T cells were migrated through the transwells overnight. Subsequently, markers of phenotype and function were assayed by flow cytometry. We show that a higher percentage of the migrated T cells produced IFN- γ , IL-2 and TNF- α than their non-migrated counterparts and T cells alone. The migrated cells also showed a more activated, effector memory phenotype. Critically, these differences were not evident when comparing to T cells in static co-culture with hepatocytes, where T cells adhered to hepatocytes but did not transmigrate. Assaying CD4⁺ T cells from the blood of patients with different chronic liver diseases showed differences in expression of these cytokines depending on disease origin: autoimmune liver disease, viral hepatitis or healthy donor T cells. These differences in T cell responses between patients with liver disease were only apparent following hepatocyte transmigration. In summary, CD4⁺ T cell transmigration across hepatocyte monolayers revealed a preference for activated, effector memory T_H1 cells with relatively high proportions of effector cytokine-expressing cells, which was not observable in static co-culture. Continuing these studies will allow us to determine if the effect takes place in vivo and would provide clues as to how hepatocytes modulate T cell function in health and disease.

Notes

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28th Society Meeting

“Focus:
Integrins and beyond”

University College London
Thursday 22th September 2016

Keynote Speakers:

Jim Norman – CRUK Beaston Institute, Glasgow, UK

Martin Humphries – University of Manchester, UK

Susanna Fagerholm – University of Helsinki, Finland