Frontiers in comparative immununology: dendritic cells and macrophages

22 & 23 March 2023

Radisson Blu Hotel, Edinburgh City Centre

WEDNESDAY 22 MARCH

13:00	Registration for ECR event
13:30	ECR event begins
14:15	Registration for main meeting
14:45	ECR event ends
15:00	**Opening remarks** Wilhelm Gerner, Chair of CVIG, Pirbright Institute, UK Rebecca McLean, Veterinary Representative at BSI Membership Forum Pirbright Institute, UK
	SESSION 1
15:15	**Of Mice and Men: The Mononuclear Phagocyte System, past and present** Siamon Gordon, University of Oxford, UK– *invited speaker*
15:45	**Functional specialization of human tonsil macrophages is controlled by both cell-intrinsic and tissue-derived factors** Elodie Segura, Centre for Immunotherapy of Institut Curie, France– *invited speaker*
16:15	Panel discussion
17:00	Posters and networking reception
19:00	Meeting close and evening Event – Ceilidh and buffet at Surgeons Quarter (guest list only)

THURSDAY 23 MARCH

SESSION 1 – Role of DC's and macrophages in disease

Chair(s): Amanda Gibson, Aberystwyth University, UK

09:00	**Porcine Dendritic cells and Macrophages populations**

	Nicolas Bertho, ONIRIS, France– *invited speaker*
09:35	**Altering Future Immune Challenges, Priming and Metastasis Through Long-Term Changes to Acute Influenza Infection** Ryan Devlin, Beatson Institute for Cancer Research, UK– *selected short talk*
09:50	**Depletion of Cx3cr1+ mononuclear phagocytes in the gut delays oral prion disease pathogenesis** Rachel Young, The Roslin Institute, UK – *selected short talk*
10:05	**Co-transfer of antigen and contextual information harmonises peripheral and lymph node cDC activation** Edward Roberts, Beatson Instititute for Cancer Research– *selected short talk*
10:20	Refreshment break and meet the exhibitors
	SESSION 2 – Interplay of DC's and macrophages with non-classical immune cells Chair(s): Ambre Chapius, Moredun Institute, UK Rebecca McLean, Pirbright Institute, UK
11:00	**Placenta macrophage ontogeny and function** Naomi McGovern, University of Cambridge, UK – *invited speaker*
11:35	**Multi-organ functions of yolk sac during human early development** Issac Emmanuel Goh Kaien, Newcastle University, UK – s*elected short talk*
11:50	**A 3D in-vitro model of macrophages in the subcutaneous tissue for drug development and disease modelling** Asme Boussahel, The University of Bristol, UK – s*elected short talk*
12:05	**Ankyrons™ – the future of target-binding reagents beyond antibodies** Jeremy Fry, ProImmune, UK – *sponsored talk*
12:25	Lunch and meet the exhibitors
	SESSION 3 – Bridging immunity – the innate immunity interface Chair(s): Wilhelm Gerner, Pirbright Institute, UK
13:30	**Comparative transcriptomics to unravel DC and monocyte biology** Stephanie Talker, University of Bern, Switzerland – *invited speaker*
14:05	**Comparing the total proteomes of mouse and human macrophages treated with LPS and IL4 –** Christa Baker, University of Dundee, UK **-** s*elected short talk*

14:20	**Single-cell RNA-Seq Reveals Multiple Sub-Populations of Bovine Afferent Lymphatic Dendritic Cells Draining the Skin under Steady-State Conditions** Heather Mathie, The Roslin Institute, University of Edinburgh, UK – *selected short talk*
14:35	**A new taxonomy for human monocytes, dendritic cells and macrophages relevant for rheumatoid arthritis (RA)** Sebastien Viatte, University of Manchester, UK – *selected short talk*
14:50	**Closing remarks** Amanda Gibson, Aberystwyth University, UK
15:00	Meeting close

Poster presentations

P.01 Using an in-vitro cell-culture model of inflammation to examine the interaction of dendritic cells and the innate immune system in allergic asthma.

Georgina Brown, University College London, UK

P.02 MHC-II expression by glia cells in the CNS after lysolecithin-induced demyelination

Kristina Ulicna[1], Newcastle University, UK, Wellcome-Wolfson Institute for Experimental Medicine, Queen´s University Belfast, UK

P.03 Universal platforms to study the primary cellular and recall responses to candidate vaccines.

William C. Davis, Washington State University, USA

P.04 Chicken CSF2 and IL-4-, and CSF2-dependent bone marrow cultures differentiate into macrophages over time.

Dominika Borowska, The Division of Immunology, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK

P.05 Atypical chemokine receptor ACKR2 controls macrophage dynamics

Gillian J Wilson, Chemokine Research Group, School of Infection and Immunity, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK.

P.06 The effect of LPS on the Mouse Bone Marrow Derived Macrophage Proteome and Metabolism

Jordan Warner, Department of Cell Signalling and Immunology, School of Life Sciences, University of Dundee, UK

P.07 M. bovis PPD Enhances Respiratory Bioenergetics of Human vs. Bovine Macrophages

Amanda J. Gibson, Pathobiology and Population Science, Royal Veterinary College, UK, Department of Life Science, Aberystwyth University, UK*

P.08 Characterisation of macrophages and dendritic cells in ovine pulmonary adenocarcinoma

Helen Todd, Moredun Research Institute, Edinburgh, UK

The British Society for Immunology (www.immunology.org) and the BSI London Immunology Group would like to thank the following sponsors for their support:

Selected talks abstracts

Altering Future Immune Challenges, Priming and Metastasis Through Long-Term Changes to Acute Influenza Infection

Ryan Devlin, Chiara Pirillo, Amy Shergold, Alberto Bravo-Blas, Sarwah Al-Khalidi, Leo Carlin, Ed Roberts

Beatson Institute for Cancer Research, UK

Trained immunity has been shown to impact the course of subsequent infections. We therefore hypothesised that prior infections could alter immune responses to tumours. Characterisation of the lung and lymph node of specific pathogen free mice 28 days post PR8 influenza A virus (IAV) infection showed significant changes in both the stromal and immune compartments, despite the virus being cleared from the lungs and bone marrow changes returning to baseline. Gross alterations in the organisation of immune cells within the lung were also evident at this late timepoint post-infection, with an increase in conventional dendritic cells (cDCs) within the lung which were organised in clusters in the peribronchiolar space. We subsequently investigated whether these changes could impact pulmonary primary tumour development or metastasis to the lung. Following IAV infection resolution, mice developed more lung tumours either in response to a carcinogen, urethane, or intravenous injection of metastatic cells. However, despite this increased tumour burden, the raised levels of cDC correlated with improved anti-metastasis CD8+ T cell priming even when tumour abundance was controlled for. This raised the possibility that increasing levels of cDC may improve anti-metastatic immune responses. Experimentally increasing cDC levels through administration of FLT3L, thereby investigating cDC in isolation of the other factors driven by infection, not only increased cDC numbers but led to a highly significant decrease in metastatic seeding. We now aim to investigate how cDC numbers remain elevated, the role of flu in increasing tumour burden, and the potential of FLT3L as an anti-metastatic therapeutic.

Depletion of Cx3cr1+ mononuclear phagocytes in the gut delays oral prion disease pathogenesis

Rachel Young, David S. Donaldson, Barry M. Bradford, Neil A. Mabbott

The Roslin Institute & Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK

Infections with prions cause chronic neurodegenerative diseases that affect humans and animals. Prion infections can be acquired orally through the consumption of contaminated food or pasture. Replication upon stromal follicular dendritic cells (FDC) in the gut-associated lymphoid tissues (GALT) is essential to establish infection and for prion spread to the brain. We have previously shown that replication on FDC is dependent on prions transiting the gut epithelium by M cells in GALT before being shuttled towards FDCs by CXCR5+CD11c+ mononuclear phagocytes (MNP). The role of other gut MNP populations in this process is unclear, particularly CX3CR1+ MNPs that acquire particulate antigens after M cell uptake. To determine if CX3CR1+ MNPs also play a role in the delivery of prions to the GALT FDC, CX3CR1-expressing MNPs were transiently depleted immediately prior to oral prion exposure in MafB-cre Cx3cr1-iDTR transgenic mice by diphtheria toxin treatment. The specific ablation of CX3CR1+ MNPs before oral prion infection significantly increased survival time compared to controls, suggesting that CX3CR1+ MNPs play an important role in establishing infection on FDC following oral prion exposure. Accordingly, the early accumulation of prions upon FDC in the GALT was reduced in CX3CR1+ MNP ablated mice. Therefore, our data suggest that CX3CR1+ MNPs are important for the efficient delivery of orally acquired prions onto GALT FDC. A further understanding of this pathway and its impact on prion susceptibility may help identify novel intervention strategies and will also give insights into the role of different GALT MNP populations in generating mucosal immune responses to M cell sampled antigens.

Co-transfer of antigen and contextual information harmonises peripheral and lymph node cDC activation

Pirillo C[1], Al Khalidi S[1], Sims A[2], Pinto R[2], Jasim S[2], Shearer P[3], Devlin R[1], Bravo-Blas A[1], Loney C[2], Perona-Wright G[3], Hutchinson E[2,3], Roberts EW[1,4]

[1]CRUK Beatson Institute, Glasgow, UK, [2]MRC-University of Glasgow Centre for Virus Research, UK, [3]School of Infection and Immunity, University of Glasgow, UK, [4]School of Cancer Sciences, University of Glasgow, UK

T cell responses against infections and cancer are directed by dendritic cells (cDC) in lymph nodes distant from the site of challenge. Migratory cDC which travel from the tissue to the lymph node drive initial T cell activation but also transfer antigen to lymph node resident cDC. These resident cells have essential roles defining the character of the resulting T cell response, however, it is unknown how they can appropriately process and present antigens to suitably direct responses given their spatial separation. Here using a novel strain of influenza A and a modified melanoma model we show that tissue and lymph node cDC activation is harmonized and that this is driven by co-transfer of contextual cues. In the tumour, incomplete cDC activation in the tumour microenvironment is mirrored by lymph node resident cDC: whilst during influenza infection, pathogen associated molecular patterns co-transferred with antigen drive TLR signalling in the resident cDC and their subsequent robust activation. This co-transfer mechanism explains how individual antigens can be handled distinctly by resident cDC and how signals driving poor tumoral cDC activation further impact the lymph node. Our findings clarify how tissue context dictates antigenic and, consequently, T cell fate in the lymph node.

Multi-organ functions of yolk sac during human early development

Issac Goh[1,2†,] Rachel A Botting[1†,] Antony Rose[1,2‡,] Simone Webb[1,2‡,] Justin Engelbert[1], Yorick Gitton[3], Emily Stephenson[1,2], Mariana Quiroga Londoño[4], Michael Mather[1], Nicole Mende[4], Ivan Imaz-Rosshandler[4,5], Lu Yang[2], Dave Horsfall[1], Daniela Basurto-Lozada[1], Nana-Jane Chipampe[2], Victoria Rook[2], Jimmy Tsz Hang Lee[2], Mai-Linh Ton[4], Daniel Keitley[2], Pavel l Mazin[2], MS Vijayabaskar[4], Rebecca Hannah[4], Laure Gambardella[2], Kile Green[6], Stephane Ballereau[2], Megumi Inoue[3], Liz Tuck[2], Valentina Lorenzi[2], Kwasi Kwakwa[2], Clara Alsinet[2], Bayanne Olabi[1], Mohi Miah[1], Chloe Admane[1,2], Dorin-Mirel Popescu[1], Meghan Acres[1], David Dixon[1], Thomas Ness[7], Rowen Coulthard[7], Steven Lisgo[1], Deborah J Henderson[1], Emma Dann[2], Chenqu Suo[2], Sarah J Kinston[4], Jong- eun Park[8], Krzysztof Polanski[2], John Marioni[2], Stijn Van Dongen[2], Kerstin B Meyer[2], Marella de Bruijn[9], James Palis[10], Sam Behjati[2,11], Elisa Laurenti[4], Nicola K Wilson[4], Roser Vento- Tormo[2], Alain Chédotal[3], Omer Bayraktar[2], Irene Roberts[1,2], Laura Jardine[1*], Berthold Göttgens[4*], Sarah A Teichmann[2*], Muzlifah Haniffa[2,1*]

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[1]Biosciences Institute, Newcastle University, UK, [2]Wellcome Sanger Institute, Wellcome Genome Campus, UK, [3]Sorbonne Université, INSERM, CNRS, Institut de la Vision, France, [4]Department of Haematology, Wellcome-MRC Cambridge Stem Cell Institute, UK, [5]MRC Laboratory of Molecular Biology, UK, [6]Translational and Clinical Research Institute, Newcastle University, UK, [7]NovoPath, Department of Pathology, Newcastle Hospitals NHS Foundation Trust, UK, [8]Korea Advanced Institute of Science and Technology, South Korea, [9]MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK, [10]Department of Pediatrics, University of Rochester Medical Center, USA, [11]Department of Paediatrics, University of Cambridge, UK, [12]Department of Paediatrics, University of Oxford, UK

The yolk sac (YS) represents an evolutionarily-conserved extraembryonic structure that ensures timely delivery of nutritional support and oxygen to the developing embryo. However, the YS remains ill-

defined in humans. We therefore assemble a comprehensive single cell 3D map of human YS from 3-8 post conception weeks by integrating multiomic protein and gene expression data. Beyond its known role as a site of haematopoiesis, we reveal a YS-specific accelerated route to macrophage production that seeds developing organs. We further provide evidence that the YS synthesises proteins required for metabolism, coagulation, vascular development and haematopoiesis regulation. We reconstruct the emergence of YS haematopoietic stem and progenitor cells from hemogenic endothelium and their decline upon stromal support modulation. The multi-organ functions of YS are superseded as intraembryonic organs develop, effecting a multifaceted relay of vital functions as pregnancy proceeds.

A 3D in-vitro model of macrophages in the subcutaneous tissue for drug development and disease modelling

Asme Boussahel, Tiah Oates, Louise Dolan, Adam Perriman

University of Bristol, UK

Macrophages in the subcutaneous tissue have an array of homeostatic, immune and pathophysiological functions. Following injection of biopharmaceuticals subcutaneously, macrophages are a site of first pass catabolism, responsible for controlling the degradation and absorption of drugs. This function is likely to be impacted by their phenotype, which is continuously changing in response to stimuli. Macrophages are also thought to have a significant role in subcutaneous adipose tissue related pathophysiologies such as metabolic dysfunction. They mediate chronic inflammation in the adipose tissue in obesity leading to the recruitment of more M1 polarised macrophages. This sustained adipose tissue pro-inflammatory state leads to insulin resistance and diabetes. In-vitro models of macrophages in the subcutaneous tissue are needed to develop a better understanding of their role in drug absorption and how it can be assessed and modulated in-vitro; and to provide more insight into the mechanisms underlying their role in metabolic dysfunction and their potential as therapeutic targets. However, the dynamic nature of macrophages, their niche specific behaviour and changing phenotypes make them difficult to model in-vitro. We have developed a 3D biomimetic hydrogel for the co-culture of adipocytes and macrophages to produce a biomimetic subcutaneous tissue microenvironment niche to model macrophage phenotype and function in-vitro. We have characterised the macrophages' identity, viability, polarisation, and motility in the 3D hydrogel and we are currently scrutinising their interaction with adipocytes. Further work will look at testing the degradation/absorption of a range of biopharmaceuticals and at modelling metabolic dysfunction in the developed subcutaneous tissue model.

Comparing the total proteomes of mouse and human macrophages treated with LPS and IL4

Christa P Baker[1], Thomas Helps[2], Jordan Warner[1], Iain Phair[3], Rena, G.[3], McLean, M.H.,[2], Arthur JSC[1]

[1]Cell Signalling and Immunology, School of Life Sciences, University of Dundee, UK, [2]Molecular and Clinical Medicine, School of Medicine, University of Dundee, UK, [3]Cellular Medicine, School of Medicine, University of Dundee, UK

Mouse bone marrow derived macrophages are an established model in studying the innate immune response. However, how closely these macrophages model the human macrophage response is less known. Conventionally, macrophages stimulated with LPS or IL4 model either an inflammatory macrophage or a tissue resolving macrophage response, respectively. We use DIA-MS total proteomics to compare the response of LPS and IL4 on bone marrow derived macrophage response and human monocyte derived macrophages. Although side by side comparisons between mouse and human proteins can be difficult to unfold, there appears to be reasonable correlation for highly expressed

proteins between mouse and human. Additionally, we found the LPS response to both mouse and human macrophages have a distinct interferon response. Understanding which pathways are conserved or not cons.

Single-cell RNA-Seq Reveals Multiple Sub-Populations of Bovine Afferent Lymphatic Dendritic Cells Draining the Skin under Steady-State Conditions

Heather Mathie [1, 2], Barbara Shih [1], Anirudh Patir [1], Mark Gray[1], Charlotte Bell[1], Irene McGuinnes[1], Lindsey Waddell[1], Anna Raper[1], Prakash Ramachandran[3], Marianna Beltran-Sierra[3], Neil Henderson[3], Tom Freeman[1], Ivan Morrison[1], Jayne Hope[1]

[1]The Roslin Institute, University of Edinburgh, UK, [2]The University of Glasgow, UK, [3]The Queen's Medical Research Institute, University of Edinburgh, UK.

Vaccination is a key component in the control of animal pathogens that impact the livestock industry. However, deployable, efficacious vaccines remain unavailable for many prevalent diseases, particularly those requiring cell-mediated immunity. Dendritic cells trafficking from the site of vaccination are key to determining the downstream adaptive immune response to vaccine antigens. We have utilised a bovine afferent lymphatic cannulation model to collect skin-draining afferent lymphatic dendritic cells (ALDC) in order to perform analysis directly ex vivo in both naïve and vaccinated cattle. We have performed an in-depth characterisation of ALDC in naïve cattle using single cell RNA-Seq and identified multiple subpopulations and trajectories using pseudotime analysis. In addition, we have identified a novel cell population that has a similar gene expression profile to monocytes and has functions related to antigen presentation.

A new taxonomy for human monocytes, dendritic cells and macrophages relevant for rheumatoid arthritis (RA)

Emily Amies[1,2], Ben Mulhearn[1,2,3], Maria Christofi[1,2], Megan Sutcliffe[2], Anne Barton[2,4], Tracy Hussell[5], Soumya Raychaudhuri[2,6,7,8], Paul Martin[2,5,9], Sebastien Viatte[2,4,5,9]

[1]Equal contribution, [2]Versus Arthritis Centre for Genetics and Genomics, Centre for Musculoskeletal Research, Manchester Academic Health Science Centre, The University of Manchester, Manchester, UK, [3]Department of Life Sciences, University of Bath, UK, [4]NIHR Manchester Musculoskeletal Biomedical Research Centre, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, UK, [5]Lydia Becker Institute of Immunology and Inflammation, Faculty of Biology, Medicine and Health, The University of Manchester, UK, [6]Center for Data Sciences, Brigham and Women's Hospital and Harvard Medical School, USA, [7]Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, USA, [8]Division of Rheumatology, Inflammation, and Immunity, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, USA, [9]Co-senior authors.

Background: Deep characterisation of monocytes, dendritic cells and macrophages (myeloid cell subsets) by single cell RNA sequencing (scRNA-seq) across healthy and inflamed tissues in RA has led to the identification of new subsets in 5 recent large scale studies. Subset overlap across studies has not been exhaustively investigated. Methods: First, peripheral blood mononuclear cells from 3 healthy volunteers and 3 RA patients were enriched for monocytes by negative selection and subjected to scRNA-seq. Clustering of 20,746 cells was performed in Seurat (generation of a template). Second, all published myeloid cell subsets were mapped onto this template using the following approach: a

module expression score from each published subset was calculated for each cluster in the template. Hierarchical clustering was applied to merge similar clusters to create a consensus map. Third, random forests were used to identify novel myeloid cell subsets. Results: We generated an exhaustive cartography of recently published myeloid cell subsets across anatomical compartments relevant for RA. For example, we show that the highly inflammatory synovial macrophage subsets Alivernini cluster 2, AMP cluster M1 and AMP2 cluster M8 all share a similar transcriptional signature and map to an EGR1+ HLAlow+ monocyte subset equally present in the peripheral blood of RA patients and healthy volunteers. We also identified 3 new subsets, including an HLA-DRhigh+ monocyte subset. Discussion: We define a new myeloid cell atlas and taxonomy relevant for RA comprising a total number of 18 continuous cell states dynamically transitioning into each other across conditions and compartments.

Poster Abstracts

Using an in-vitro cell-culture model of inflammation to examine the interaction of dendritic cells and the innate immune system in allergic asthma.

Georgina Brown, Howard Clark, Jens Madsen

University College London, UK

Allergic asthma is an inflammatory disease that features a T2-type lymphocyte response. This is due to sensitisation to allergens such as dust mites, pollen, and fungi. Dendritic cells induce a Th2 response when presenting allergens to T cells, leading to the release of inflammatory cytokines. Re-exposure to the allergen can cause mast cell degranulation via IgE binding. Mast cells have been found to regulate dendritic cell behaviour, feeding the inflammatory cycle.

Surfactant proteins A and D (SP-A and SP-D) are innate immune molecules that bind to non-self glycoproteins. They have multiple functions in the immune systems, notably the ability to bind allergens and modulate the subsequent immune response. A recombinant form of SP-D has been developed that preserves most functions of the full-length protein.

This project aims to integrate mast and dendritic cell interaction with the regulation of surfactant proteins to model the immune interaction occurring in allergic asthma.

Validation of immortalised mast cells has been completed, and a protocol for differentiating dendritic cells from monocytes has been developed. This was through the investigation of cell viability, cell surface marker expression, and immunofluorescence. A co-culture of these cell types has been established. The interaction of these cells with each other and surfactant proteins will be investigated by mass spectrometry and RT-qPCR.

This project presents the opportunity to research the anti-inflammatory roles of surfactant proteins, providing the groundwork for their possible use treating allergic asthma. This could potentially alleviate the chronic inflammation characteristic of the disease.

Universal platforms to study the primary cellular and recall responses to candidate vaccines.

William C. Davis, Asmaa M. Mahmoud, Gaber S. Abdellrazeq, Lindsay M.

Washington State University, USA

Progress in developing vaccines has been impeded by a lack of methods to study the cellular immune response to candidate vaccines, especially peptide-based vaccines. We developed methods to use dendritic cells as antigen primed APC to study the primary and recall responses to mycobacterial pathogens and a mycobacterial peptide ex vivo. We developed a flow cytometric assay to characterize the proliferative response of responding cells. We developed a quantitative PCR assay to study the killing of intracellular bacteria by CD8 cytotoxic T cells. Through the use of these assays, we were able to obtain data showing tri-directional signaling at the interface of the APC is essential for eliciting development of MHC restricted CD8 cytotoxic lymphocytes. As described in this summary, signals delivered to either CD4 or CD8 by DC, primed with Mycobacterium a. paratuberculosis or a membrane peptide (MMP), are blocked in the presence of antibody against MHC I or MHC II. The methods developed in these studies can be used with any species to study the immune response to candidate vaccines.

Chicken CSF2 and IL-4-, and CSF2-dependent bone marrow cultures differentiate into macrophages over time.

Dominika Borowska, Samantha Sives, Lonneke Vervelde, Kate M Sutton

The Division of Immunology, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK

Chicken bone marrow-derived macrophages (BMMΦ) and dendritic cells (BMDC) are utilized as models to study the mononuclear phagocytic system (MPS). A widely used method to generate macrophages and DC in vitro is to culture bone marrow cells in the presence of colony-stimulating factor-1 (CSF1) to differentiate BMMΦ and granulocyte-macrophage-CSF (GM-CSF, CSF2) and interleukin-4 (IL-4) to differentiate BMDC, while CSF2 alone can lead to the development of granulocyte-macrophage-CSF-derived DC (GMDC). However, in chickens, the MPS cell lineages and their functions represented by these cultures are poorly understood. Here, we decipher the phenotypical, functional and transcriptional differences between chicken BMMΦ and BMDC along with examining differences in DC cultures grown in the absence of IL-4 on days 2, 4, 6 and 8 of culture. BMMΦ cultures develop into a morphologically homogenous cell population in contrast to the BMDC and GMDC cultures, which produce morphologically heterogeneous cell cultures. Temporal transcriptomic analysis indicated that all cultures expressed high levels of CSF3R, MERTK, SEPP1, SPI1 and TLR4, genes associated with macrophages in mammals. In contrast, low levels of FLT3, XCR1 and CAMD1, genes associated with DC, were expressed at day 2 in BMDC and GMDC after which expression levels decreased. Collectively, chicken CSF2 + IL-4- and CSF2-dependent BM cultures represent cells of the macrophage lineage rather than inducing conventional DC.

Atypical chemokine receptor ACKR2 controls macrophage dynamics

Gillian J Wilson, Ayumi Suzuki, Samantha Love, Jiwon Kim, Marieke Pingen, Alan Hayes, Gerard J Graham.

Chemokine Research Group, School of Infection and Immunity, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK.

Chemokines and their receptors play fundamental roles in controlling cell migration within the body. The atypical chemokine receptor, ACKR2 has been shown to be an important regulator of macrophages, during embryonic development of the lymphatic system and in the mammary gland during postnatal development. ACKR2−/− mice display precocious mammary gland development, increased macrophage recruitment and a denser ductal epithelial network. We observed an inverse phenotype, with decreased density of the ductal epithelial network, in the mammary glands of CCR1-/- mice. Here we reveal that ACKR2 also controls macrophages within the peritoneum of naïve mice. There are 2 main macrophage populations in the peritoneal cavity, large peritoneal macrophages (LPM, F4/80 high) and small peritoneal macrophages (SPM, F4/80 low). Specifically the number of SPMs are reduced in the peritoneum of ACKR2-/- mice. We confirm expression of ACKR2 by a population of innate-like B cells (B1d) in the peritoneal cavity and identify a population of ACKR2 fibroblasts in the peritoneal wall. We also show that SPMs expand in WT mice but not in ACKR2-/- mice, 48 h after intraperitoneal challenge with Escherichia coli. Therefore, ACKR2 is an important regulator of macrophages in diverse biological contexts; at rest, in development and during infection.

The effect of LPS on the Mouse Bone Marrow Derived Macrophage Proteome and Metabolism

Jordan Warner, Iain Phair, Linda Sinclair, Simon Arthur

Department of Cell Signalling and Immunology, School of Life Sciences, University of Dundee, UK

Macrophages are innate immune cells responding to a variety of stimuli. Macrophage activation by inflammatory stimuli such as LPS not only alters the biological molecules that macrophages produce, but also the methods through which they derive energy. Insight into the mouse macrophage proteome has been obtained through experiments with mouse bone marrow derived macrophages differentiated in vitro. The observed changes were then correlated with the results of assays looking into cytokine production, glucose uptake and glucose utilisation. While glucose uptake does increase in response to LPS, the largest increase in transporter expression is significantly delayed, and it appears that the initial increase in glucose consumption can be partially fuelled by the macrophage's glycogen stores.

M. bovis PPD Enhances Respiratory Bioenergetics of Human vs. Bovine Macrophages

Marie-Christine Bartens[1, 2], Sam Willcocks [2, 4], Dirk Werling [1], Amanda J. Gibson [1, 3]

[1]Pathobiology and Population Science, Royal Veterinary College, UK, [2]Department of Infection Biology, London School of Hygiene and Tropical Medicine, UK; [3]Department of Life Science, Aberystwyth University, UK*; [4]Department of Life Sciences, Brunel University, UK*

The role of macrophage cellular metabolism and reprogramming during TB infection is of great interest, due to the influence of Mycobacterium spp. on macrophage bioenergetics. Recent studies have shown that M. tuberculosis induces a TLR2-dependent shift towards aerobic glycolysis and metabolic reprogramming, comparable to the established LPS induced M1 MQ polarisation. Distinct differences in the metabolic profile of murine and human macrophages, suggest evidence for species-specific differences in bioenergetics. So far, studies examining the metabolic potential of cattle are lacking, thus

the basic bioenergetics of bovine and human macrophages were explored in response to a variety of stimuli. Cellular energy metabolism kinetics were measured concurrently for both species (Seahorse XFe96 platform) to generate a profile for known TLR2 and TLR4 ligands, FSL-1 and LPS respectively. Despite previous reports of species-specific differences in TLR signalling and cytokine production between human and bovine macrophages, we observed similar respiratory profiles for both species. Basal respiration remained constant between stimulated macrophages and controls, whereas TLR ligands induced increased glycolysis. In contrast to macrophage stimulation with M. tuberculosis PPD, M. bovis PPD treatment significantly enhanced basal respiration rates and glycolysis observed for human macrophages only. Respiratory profiling further revealed significant elevation of ATP-linked OCR and maximal respiration suggesting a strong OXPHOS activation upon M. bovis PPD stimulation in human macrophages. Our results provide an exploratory set of data elucidating the basic respiratory profile of bovine vs. human macrophages which lay the foundation for future studies to investigate host-tropism of the M. tuberculosis complex.

Characterisation of macrophages and dendritic cells in ovine pulmonary adenocarcinoma

Helen Todd, Sophia Z Thanasi, Hannah Elliot, S Jo Moore, Chris Cousens

Moredun Research Institute, Edinburgh

Ovine pulmonary adenocarcinoma (OPA) is a naturally occurring disease of sheep of importance to the sheep industry and as a potential model of human lung cancer. OPA results from transformation of alveolar type II cells by Jaagsiekte sheep retrovirus (JSRV). The role of tumour associated macrophages and other immune cells in the control vs development and metastasis of solid tumours has been widely investigated in recent years in mice and humans. However, there have been no reports of such studies in sheep tumours. Here we are using immunofluorescence, initially with markers CD68, CD11b, CD163 and DC-LAMP to differentiate M1, M2 macrophages and dendritic cells and an anti-JSRV marker to label OPA cells to describe the presence and location of these cells in and around OPA tumour nodules in early and advanced cases of OPA. To date we have shown that macrophages in the alveolar spaces around the tumours express CD163, whilst cells expressing CD68 can be found in the tumour stroma and may also express DC-LAMP or CD11b, which is indicative of plasmacytoid dendritic cells or of the M1 macrophage phenotype. Access to samples from differing stages of disease progression has given us the opportunity to study early tumours where the immune response is actively engaging through to advanced disease where immune cells may perform various roles including pro-tumour activities. Whilst the outbred status of the sheep may increase variability compared to inbred rodent tumour models, this is likely to be more reflective of what is happening in humans.