9th Annual Meeting

of the

Irish Cytometry Society

25th – 26th February 2014

Astra Hall

University College Dublin

Welcome Message

Dear Registrant

You are very welcome to the Irish Cytometry Society (ICyS) Annual Meeting at the Astra Hall in University College Dublin. Now in its tenth year, we are delighted to have such an excellent programme, with world-renowned speakers coming from Ireland, UK, and mainland Europe. Further we have a terrific array of posters in the main exhibition area, so be sure to check them out.

We are indebted to our trade exhibitors for their generous support which allows us to host this meeting free of charge. Please be sure to visit the exhibition stands, as not only can you discuss your requirements with them, but they tend to be very knowledgeable on all things cytometry. We are running a vendor raffle with some fantastic prizes as further coercion. And don't forget to join us for Happy Hour in the College Bar, sponsored by eBioscience!

Cytometry continues to be one of the most important techniques available to life scientists, both in research, industrial, and clinical settings. As applications rapidly expand it is essential for users to be aware of these developments, and how they can use such methods to maximize their work output. We feel that this meeting, and indeed the Irish Cytometry Society as a whole, serves as an important resource in ensuring that scientists have the knowledge and resources in cytometry they need to excel in their particular fields. If you would like to become more involved, please let us know.

For more information and notifications for courses and conferences please visit our website at:

http://irishcytometry.wix.com/icys

We hope you enjoy the meeting!

Barry Moran

on behalf of the ICyS 2014 Organising Committee.

Tuesday, February 25th

8:30 – 9:30 **Registration, Tea & Coffee.**

9:30 – 9:40 **Welcome**

Alfonso Blanco University College Dublin, Ireland

Tuesday, February 25th

Session 1: Advanced Applications

Joint Chair: Alfonso Blanco and Andy Riddell

9:40 – 10:15 **Invited Speaker**

Antonio Cosma CEA: Alternative Energies and Atomic Energy Commission, France

Managing the Data Flow of Mass Cytometry *Sponsored by* DVS Sciences

10:15 – 10:30 **Selected Speaker**

Simon McCallum University of Cambridge, UK

Cambridge Biomedical Research Centre Cell Phenotyping Hub - A Shared Laboratory Resource for Cellular Analysis at the University of Cambridge Clinical School

10:30 – 11:05 **Invited Speaker**

Andy Filby Cancer Research UK, UK

A Tri-Partite Cytometric Approach to Measuring Proliferation in Transformed Cells by Dye Dilution

11:05 – 11:35 **Poster Session & Break**

11:30 – 13:00 ***Parallel Workshop*** *for Infinicyt™ Flow Cytometry software* Conway Institute, UCD

11:35 – 12:10 **Invited Speaker**

Peter O'Toole University of York, UK

2 in 1: Novel Quantitative, Label-Free to Super-Resolution and Correlative Imaging

12:10 – 12:25 **Selected Speaker**

Filippo Bertoli University College Dublin

Unveiling the Dark Side of the Protein Corona: a Study on its Cellular Uptake and Evolution

12:25 – 13:00 **Invited Speaker** *Sponsored by* EXCYTE EXPERT CYTOMETRY

Rui Gardner Gulbenkian Instituto de Ciência, Portugal

Purity yields to Recovery: Getting Priorities Right when Evaluating Sorter Performance

13:00 – 14:00 **Poster Session & Lunch**

Session 2: Multicolour Analysis & Clinical Cytometry

Joint Chair: Sean Rooney and Ian Dimmick

14:00 – 14:35 Invited Speaker

Ian Dimmick University of Newcastle, UK

Instrument and Cell Population Monitoring in Clinical Flow Cytometry based Applications

14:35 – 14:55 Industrial Presentation

Roberto Juanes Juanes Cytognos *Sponsored by* cytognos

Infinicyt™ Flow Cytometry Software: The EuroFlow Approach

14:55 – 15:10 Selected Speaker

Melissa Conroy Trinity College Dublin

Investigating the Role of Chemokines in the Recruitment of pro-Inflammatory T cells to the Liver and Visceral Adipose Tissue of Cancer Patients

15:10 – 15:40 **Poster Session & Break**

15:40 – 16:00 Invited Speaker

Fiona Murray University College Hospital, Galway

Refractory Celiac Disease Diagnosis by Flow

16:00 – 16:15 Selected Speaker

Nahidul Islam National University of Ireland, Galway

Flow Cytometry based Novel Observations to Characterize Immunosuppression after Major Surgical Trauma

16:15 – 16:30 Selected Speaker

James Bojdo Queen's University Belfast

Outgrowth Endothelial Cells as a Potential Cell-based Therapy for Ischaemic Retinopathies

16:30 – 17:00 Invited Speaker

Andy Riddell University of Cambridge, UK *Sponsored by* Fannin Scientific

Sorting and Maintaining Haploid Stem Cells using a Beckman Coulter MoFlo Legacy and a Bio-Rad S3 by Light Scatter

17:15 – 18:15 **Happy Hour!** College bar, UCD, sponsored by affymetrix eBioscience

Wednesday, February 26th

Session 3: Cancer & Stem Cell Biology

Joint Chair: Shirley Hanley and Peter O'Toole

9:30 – 10:10 **Invited Speaker**

Michael O'Dwyer National University of Ireland, Galway

An Emerging Role for Glycosylation in Multiple Myeloma

10:10 – 10:25 **Selected Speaker**

Christian Hellwig *Royal College of Surgeons of Ireland*

Employing a Novel Activation Platform for the Initiator Caspase-8 to Induce Apoptosis in Chemo-resistant Cancer Cells

10:25 – 10:40 **Selected Speaker**

Paul Lohan National University of Ireland, Galway

Potential of Primary Chondrocytes for Allogeneic Cell Therapy

10:40 – 11:15 **Invited Speaker**

Breandan Kennedy University College Dublin

Applying Cytometry in Zebrafish to Cancer and Vision Research

11:15 – 11:45 **Poster Session & Break**

11:45 – 12:10 **Invited Speaker**

Rhodri Ceredig National University of Ireland, Galway

Do Cytokines Instruct Hematopoietic Lineage Choice?

12:10 – 12:25 ***Selected Speaker***

Lisa O'Flynn Orbsen Therapeutics Ltd, National University of Ireland, Galway

CD362: a Functional Marker enabling MSC Isolation & Clinical Development

12:25 – 12:50 **Invited Speaker**

Pat Casey University College Cork

Reduce, Refine, Replace: Use of Bioluminescence and Biofluorescence Technology in Life Science Research

12:50 – 13:10 **Industrial Presentation**

Lisa Tang Medical Supply Company *Sponsored by* MSC

Dynamic Methods for Biomarker Discovery and Multiplexed ELISAs

13:10 – 14:10 **Poster Session & Lunch**

Session 4: Immunology

Joint Chair: Barry Moran and Jean Fletcher

14:10 – 14:50 **Invited Speaker**

Kingston Mills Trinity College Dublin

Immunotherapeutics for Cancer and Autoimmune Diseases

14:50 – 15:05 **Selected Speaker**

Sarah Chambers Queens University Belfast

Myeloid Angiogenic Cells (MACs) Attain a more M1 Pro-inflammatory Phenotype when exposed to High Glucose

15:05 – 15:20 **Selected Speaker**

Yasmeen Ghnewa Trinity College Dublin

Human Invariant Natural Killer T cells Control Multiple B cell Functions in vitro

15:20 – 15:40 **Poster Session & Break**

15:40 – 16:15 **Invited Speaker**

Cormac Taylor University College Dublin

Regulation of IL-1β-induced NF-κB Activity by Hydroxylases Links key Hypoxic and Inflammatory Signaling Pathways

16:15 – 16:30 ***Selected Speaker***

Fionnuala Hickey Trinity College Dublin

Flow Cytometry as a Tool for the Analysis of Mitochondrial Mass and Energy Metabolism in Immune Cells

16:30 – 17:00 **Invited Speaker**

Jean Fletcher Trinity College Dublin

Regulation of Human Th17 Cells

17:00 **Raffle, Oral, & Poster Presentation Prizes**

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Oral Presentation Abstracts

in order of presentation:

Managing the Data Flow of Mass Cytometry

Antonio Cosma

CEA: Alternative Energies and Atomic Energy Commission, France

Mass cytometry, also known as Cytometry by Time-of-Flight (CyTOF), addresses most of the limitations of conventional fluorescence-based flow cytometry. Notably the number of parameters and the spectral overlap normally observed with fluorescent probes. Mass cytometry can detect up to 40 different markers at the single cell level with minimal spectral overlap, thanks to antibody probes tagged with rare heavy metals and a detection system based on time of flight mass spectrometry. Currently, the main challenge of this innovative technology is data analysis. Several tools are now available, but the main issues are the choice of the tool and the training of personnel for analysing complex datasets. Moreover, data need to be organized, stored and compared with data generated by other assays. Thus, following a first "sample analysis approach", data need to be integrated to the whole information generated by the laboratory. I will discuss some of the available solutions and try to give some indications / suggestions on how to start analysing multi-parameters datasets.

Cambridge Biomedical Research Centre Cell Phenotyping Hub - A Shared Laboratory Resource for Cellular Analysis at the University of Cambridge Clinical School

Simon McCallum

Anna M. Petrunkina, Reiner Schulte, Simon A. McCallum, Chris Bowman, Natalia Savinykh, Chiara Cossetti, Michal Maj

The Cambridge Biomedical Research Centre Cell Phenotyping Hub

The Cambridge Biomedical Research Centre Cell Phenotyping Hub is a central shared laboratory resource within the University of Cambridge Clinical School. It was established in 2012 to offer campus-wide access to researchers across all themes, to enable the collaborative operation of two interdependent facilities and to liaise with other core facilities involved in translational research at the University of Cambridge.
The principal vision behind the establishing of a central shared laboratory resource was to allow the rapid processing of unscreened human samples, and to promote patient-based research. The Hub is an integral element of the Cambridge BRC infrastructure; it provides vital technical and educational services that result in major publications. A further function of the Hub is to collect and disseminate technological and scientific expertise for patient-based research and clinical translation.
Given the Hub's mission, choice and integration within it of the latest technology is of paramount importance. Thus far the Hub is equipped with 30 instruments across two sites, including major equipment such as multiple cell sorters, analysers and microscopes, and ancillary equipment such as auto-samplers, high-throughput systems and cell counters. It also houses three microbiological safety cabinets for processing unscreened human samples.
However, in addition to technical capacity, there are major issues and challenges in establishing and developing a shared laboratory resource; these involve a variety of topics such as expansion of operations, equipment resources, recruitment and retention of staff, education of users, and professional development of Hub staff, all within the constraint of a cost recovery and limited external funding. Further important organisational factors defining our directions and strategies for further development and optimization of the Hub services include biosafety aspects, instrument reliability and inter-compatibility, ease of training of operators and flexibility of adapting the instruments to researchers' demands.

A Tri-Partite Cytometric Approach to Measuring Proliferation in Transformed Cells by Dye Dilution.

Andy Filby

FACS Laboratory, London Research Institute, Cancer Research UK, London, UK

Cell proliferation is a key measure of biological consequence from immunology to cancer biology. Several methods exist that are able to provide some measure of the proliferative history of a cell population. These include radioactive assays such as measuring tritium incorporation as well as more favourable fluorescent-based approaches. One of the most powerful methods utilise a family of fluorescent dyes that include Carboxy-Fluorescein Succinimidyl Ester (CFSE) that bind to intracellular proteins in live cells. As the labelled input population divides, the fluorescence is apportioned between daughter cells producing generational peaks of decreasing intensity. As such, the proliferative history of any single cell can be determined and powerful metrics calculated. While this method is easily applied to uniform cell types such as resting primary T cells, successful peak resolution of heterogeneous transformed cells lines is often impossible making the dye dilution approach ineffectual. We have developed an approach that utilises Conventional Flow Cytometry (CFC) sorting and analysis in combination with Imaging Flow Cytometry (IFC) to determine what factors influence the ability to resolve division peaks in the context of transformed cell lines. These data have significance to the study of cancerous proliferation and the effects of various chemotherapeutic compounds, especially when dealing with asynchronous populations.

2 in 1: Novel quantitative, label-free to super-resolution and correlative imaging

Peter O'Toole

University of York, UK

We are currently involved with development of multiple new imaging technologies. There is an increasing realisation that we need to vastly improve and exploit label free techniques. Ptychography is a quantitative label-free, high-contrast, live-cell imaging technique. We have been exploring the capabilities of this technique and have demonstrated its ability to be used in the studies of cell cycle (1), apoptosis, differentiation and is now being applied by groups focussed cancer research , immunology, stem cell and neurobiology.
We are also developing novel correlative/integrated light and electron microscopy techniques. Recent advances in biological imaging have seen new techniques developed in an attempt to improve resolution using light microscopy (PALM/STORM/STED etc) and using Correlative Light and Electron Microscopy (CLEM). Despite these intricate and exciting developments, they are still not ideal for many biological studies and the methods are far from simple to apply by non-specialists. To achieve a full understanding of complex biological systems that underlie disease/infections and fundamental biology, it is imperative to really understand all aspects of the mechanisms involved. Recent years have seen genomic and proteomic studies further our understandings, but we are still unable to visualise any of these processes directly in an intact living cell. For example, we can find out the atomic structure of a protein, and yet are unable to directly study its assemblies in vivo. Whilst the new techniques are bringing us closer to this goal, there still remains a critical resolution gap between electron and light microscopy. Our approach in collaboration with Lucy Collinson at the CRUK London Research Institute, is to use a novel electron Super Resolution Microscopy (eSRM) technique, based on the novel ClairScope (2), that will seamlessly couple the technologies of electron and light microscopy together to achieve address key functional questions.
(1) J Marrison, L Räty, P Marriott and P O'Toole; Ptychography – a label free, high-contrast imaging technique for live cells using quantitative phase information. Sci. Rep. 3 (2013), 2369; DOI:10.1038/srep02369.
(2) IEG Morrison, CL Dennison, H Nishiyama, M Suga, C Sato, A Yarwood and PJ O'Toole; Atmospheric Scanning Electron Microscope for Correlative Microscopy. Invited Chapter for Methods in Cell Biology: Correlative Light and Electron Microscopy, edited by Thomas Müller-Reichert and Paul Verkade. Methods in Cell Biology (2012) 11:307-324

Unveiling the Dark Side of the Protein Corona: a Study on its Cellular Uptake and Evolution

Filippo Bertoli

Filippo Bertoli, Marco P. Monopoli, Anna Salvati, Kenneth A. Dawson

Centre for Bionano Interactions (CBNI), University College Dublin

Nanomaterials (and specifically nanoparticles), as a result of their unique properties (like small size and their large surface area), offer great promise for drug delivery systems and medicine terapies[1, 2] ; therefore, in last ten years, a great scientific effort has been employed in determining how nanoparticles interact with biological systems and, specifically, with cells. Different studies have showed how nanoparticle - cell interactions are governed by different parameters, one of the main being the layer of proteins and other biomolecules adsorbed on the nanoparticle surface from the surrounding biological media (protein corona)[1]. Although the composition and extracellular stability of the protein corona of various nanoparticles have been characterized and studied[3] little is still understood about the intracellular uptake and evolution of this layer and how nanoparticle characteristics (like surface properties) affect these processes. Here we fluorescently labeled the protein corona formed on polystyrene nanoparticles with different surface properties (amino and carboxyl modified) and we follow its intracellular uptake and evolution with different fluorescence based techniques and we emphasize the fundamental role the surface plays in the corona final destiny; specifically we show how different surfaces leads to different kinetics in the persistence and processing of the corona proteins by the cells. To achieve a better understanding of the final fate of the proteins of the corona subcellular fractionation techniques have been employed to isolate the organelles in which the corona localized and different techniques (like fluorescent microscopy, flow cytometry, 1 D SDS PAGE and mass spectrometry) have been used to understand the processing of these proteins from the cells. The findings show that, when corona proteins reach the lysosomes, they are there degraded by the lysosomal proteases. We then compare the amount of proteins the two nanoparticle types bring into the lysosomes with the amount of serum internalized in normal conditions, and we have found that nanoparticles may bring on their surface an unsuspected amount of proteins and traffic them to specific organelles.

1. Nel, A.E. et al. Understanding biophysicochemical interactions at the nano-bio interface. *Nat Mater* **8**, 543-57 (2009).
2. Webster, T.J. Nanomedicine: what's in a definition? *Int J Nanomedicine* **1**, 115-6 (2006).
3. Monopoli, M.P., Aberg, C., Salvati, A. & Dawson, K.A. Biomolecular coronas provide the biological identity of nanosized materials. *Nat Nanotechnol* **7**, 779-86 (2012).

Purity yields to Recovery: Getting priorities right when evaluating sorter performance

Rui Gardner

Instituto Gulbenkian Ciencia, Portugal

For many years Purity has been the gold standard metric to evaluate sort performance. Though Purity is objectively defined as the ratio between the number of sorted cells of interest and the number of total cells in the sorted tube, its interpretation has always been a subjective art, mastered by the most experienced operators. Still, even in very clear situations when there are no doubts about the measured Purity, its actual value may tell us very little of how well the sorter is performing. This can be demonstrated by sorting an extremely diluted sample where the drop delay is set one drop away from the correct one. In this simple case, the instrument will sort mostly empty drops without compromising Purity. Recovery, on the other hand – defined here as the fraction of sorted cells of interest relative to the original number in the sample – will always be compromised as each wrong sort decision will fail to place the cell of interest in the sorted tube. Increasing sample concentration will start to affect Purity, but Recovery will still be equally affected. What this simple example demonstrates is that Purity fails as a measurement of instrument performance, whereas anything affecting the sorter's ability to deflect the correct drop will affect Recovery. For the researcher who is sorting in Purity mode, measuring Purity still remains a priority to assess experimental performance. Low Recovery, on the other hand, can in most cases be circumvented by bringing more cells to the sorter and therefore of less importance. But for the sorter operator to evaluate whether the cell sorter is performing well, Purity has little meaning in contrast to Recovery, which is sensitive to any loss of instrument performance.

In this talk, I would like to present a few examples on how Purity fails to give us a clear indication on how the instrument is performing, and show arguments in favor of using Recovery to evaluate sort performance. Given that measuring absolute Recovery is usually associated with large errors, a method has been developed to calculate the instrument's maximum Recovery – Rmax – that provides a quantitative metric of how well the instrument is sorting. Rmax can be used to setup the instrument, monitor performance, and Quality Control.

Instrument and Cell Population Monitoring in Clinical Flow Cytometry based Applications

Ian Dimmick

University of Newcastle, UK

Investigating the role of chemokines in the recruitment of pro-inflammatory T cells to the liver and visceral adipose tissue of cancer patients

Melissa Conroy

Melissa J. Conroy, Karen C. Galvin, Anne-Marie Mongan, Aoife Cannon, Katie O'Sullivan, Maria Kavanagh, Gillian Moore, John V Reynolds and Joanne Lysaght.

Department of Surgery, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland.

Introduction: Oesophageal adenocarcinoma (OAC) is increasing rapidly in Ireland with a 5-year survival rate of approximately 15%. OAC has the strongest association with obesity providing a clinically relevant model for the study of T cells in adipose tissue and liver inflammation. We have previously shown that the omentum, part of visceral adipose tissue (VAT), is a rich source of activated pro-inflammatory T cells. We propose that such cells are trafficked to and sequestered in VAT and contribute to obesity-associated inflammation.
Methods: The effector memory phenotypes, cytokine profiles and chemokine receptor expression profiles of T cells were examined in the blood, liver and omentum of obese and non-obese OAC patients, along with the levels of a panel of secreted chemokines and cytokines using flow cytometry and MSD multiplex ELISAs.
Results: Significantly higher proportions of effector/memory T cells were observed in the omentum when compared to blood ($p<0.05$). The proportions of IFN-γ-producing $CD4^+$ and $CD8^+$ T cells in omentum and liver were also significantly elevated when compared to blood while frequencies of IL-17-producing $CD4^+$ T cells were higher in only the omentum ($p<0.05$). Levels of secreted GM-CSF, IL-12, IL-1β, IL-2 and IL-6 were also higher in the omentum and/or liver ($p<0.05$). Significantly higher IL-8, Fractalkine, IP-10, MIP-3α and MIP-1α levels were observed in the omentum and/or liver compared to blood serum ($p<0.05$), while levels of TARC, ITAC and RANTES were markedly lower ($p<0.0001$). The differential expression levels of the chemokines in blood serum, liver and omentum were reflected in the expression profiles of their known receptors on T cells at these sites indicating that these chemokines are actively recruiting T cells to omentum and liver in these OAC patients.
Conclusion: Chemokine-mediated recruitment of inflammatory T cells into omentum and liver may contribute to obesity-associated inflammation in OAC.

Refractory Celiac Disease Diagnosis by Flow

Fiona Murray

University College Hospital, Galway.

Flow Cytometry based Novel Observations to Characterize Immunosuppression after Major Surgical Trauma

Nahidul Islam

Nahidul Islam[1,2,3], Michael Whitehouse[2], Sanchit Mehandale[2], Michael Hall[3], Ashley Blom[2], Gordon Bannister[2], John Hinde[4], Shirley Hanley[1], Rhodri Ceredig[1], Benjamin Bradley[1,2,3]

[1] Immunology & Transplant Biology Group, Regenerative Medicine Institute, NCBES, Nursing and Health Sciences, NUI Galway, Ireland. [2] Orthopaedic Research Unit, Avon Orthopaedic Centre, Southmead Hospital, University of Bristol, United Kingdom. [3] Shannon Applied Biotechnology Centre, Institute of Technology Tralee, Tralee, Co.Kerry, Ireland. [4] School of Mathematics, Statistics and Applied Mathematics, NUI Galway, Ireland.

Introduction: Immunity consists of multi-layered defences against innumerable lethal infections experienced throughout our billion years of evolution from simple prokaryote creatures to modern day human. Major trauma

from whatever cause leads to suppression of immunity, thereby increasing vulnerability to infections after major surgery. In this context, our previous investigations showed that post traumatic immunosuppression (PTI) following joint replacement surgery was worsened by allogeneic blood transfusions, but, by contrast autologous filtered blood re-infusion reversed PTI resulting in systemic immuno-stimulation*. In this project we have confirmed and extended these observations.

Methods: Two cohorts of knee arthroplasty patients were compared; one received no blood transfusion (n=18) and the other was re-infused with autologous salvaged blood (n=25) retrieved from the wound site between 0 and 6 hours post-operatively. Venous blood samples were taken from each patient pre-operatively and within 2-5 days post-operatively. Samples of salvaged blood were taken from the collection bag. Thirteen cytokines were simultaneously measured by Flow Cytometric Bead Array using Accuri C6 Flowcytometer. Other cytokines, chemokines and danger factors (DAMPS) were measured by ELISA. For each individual patient, *post-operative blood, wound site blood*, and *re-transfused salvaged blood* were compared with *pre-operative* levels using the T-test and only significant changes are listed below.

Results: Surgical trauma suppressed pro-inflammatory (IL-1β/2/17A,IFN-γ,TNF-α) and increased anti-inflammatory cytokines (IL-5/10). In contrast re-infusion of autologous salvaged blood increased pro-inflammatory (IL-1β/2/17A,IFN-γ,TNF-α) and decreased anti-inflammatory cytokines (IL-4/5/9/10/13). This was attributed to a cocktail of immune-stimulants in wound blood that included chemokines (IL-8, MCP-1, MIP-1α) and DAMPS (HMGB-1, S100-A8/A9, α-Defensin, HSP-27/70/60, Annexin-A2) as well as cytokines (IL-6/1ra/2/12p70,IFN-γ,TNF-α etc).

Conclusions: Whilst trauma causes depletion of systemic immune capacity rendering patients vulnerable to pneumonia and bacteraemia, it enhances immune resources in peripheral wound sites. Furthermore, natural substances in the form of chemokines, DAMPS, cytokines, and as yet unidentified substances that accumulate in these sites are of potential therapeutic benefit when given intravenously for reversing post-traumatic immunosuppression.

* A. Gharehbaghian, K.M.G. Haque, C. Truman, R. Evans, R. Morse, J. Newman, G. Bannister, C. Rogers, B.A. Bradley. Effect of autologous salvaged blood on postoperative natural killer cell precursor frequency. Lancet, 2004; 363: 1025–30.

Outgrowth Endothelial Cells as a Potential Cell-based Therapy for Ischaemic Retinopathies

James Bojdo

James Bojdo, Christina L. O'Neill, Sarah EJ. Chambers, Jasenka Guduric-Fuchs, Emma Reid, and Alan W. Stitt, Reinhold J. Medina.

Centre for Experimental Medicine, Queen's University Belfast

Ischaemic retinopathies, often regarded as the most feared of ophthalmic diseases, ultimately result in vision impairment and blindness in affected patients. This pathology can be found at all ages: from neonates (retinopathy of prematurity), through to the working population (diabetic retinopathy), and to the elderly (retinal vein occlusions).

Ischaemic retinopathies share similar pathogenesis, i.e. critical loss of blood vessels. Therefore, a novel and logical strategy would be to adopt a cell-based therapy aiming to revascularise the ischaemic sites. There are, however, significant discrepancies defining the widely used term endothelial progenitor cell (EPC). This has led to ongoing debates regarding the ideal cell of choice for these therapies and terminology for cell populations.

Here, we describe the isolation methodology, the molecular characterisation, and the pre-clinical use of a well-defined EPC subset called Outgrowth Endothelial Cells (OECs), also known as late EPCs and Endothelial Colony Forming Cells.

OECs are isolated from human blood. They possess high proliferative potential and belong to the endothelial lineage as demonstrated by flow cytometry-immunophenotyping, microarray-based transcriptomes, and electron microscopy ultrastructure. We now have empirical evidence to demonstrate the vascular reparative properties OECs exhibit using *in vitro* and *in vivo* experimental models. In further detail, we have shown that OECs significantly contribute to revascularisation of murine ischaemic retinas.

There are still various challenges before translating OEC cytotherapy into the clinics, this is despite the promising results that have been attained. For example, *ex-vivo* long-term expansion of OECs is coupled to replicative senescence; OEC isolation from diabetic patients might be challenging; and optimisation of cell culture conditions using animal-free components and under strict GMP regulations are some fundamental concerns that will need to be addressed in the near future.

To summarise, OECs have the potential to be used as the cell therapy of choice for vascular repair. In particular, the ischaemic retina represents an ultimate test bed for first-in-human trials.
Acknowledgements: This study is supported by the Fight for Sight UK, the Sir Jules Thorn Trust, and the Juvenile Diabetes Research Foundation.

Sorting and maintaining haploid stem cells stems cells using a Beckman Coulter MoFlo Legacy and a Bio-Rad S3 by light scatter.

Andy Riddell

FCCF, Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute (CSCI), University of Cambridge

Maintenance of viable mammalian haploid stems cell lines used in the CSCI constitute a difficult challenge. Sorts using traditional DNA dyes result in heavy losses in post sort cell cultures. This is possibly due to the cytotoxic effects of standard supravital DNA dyes, in particular the vital stain Hoechst 33342 and others (1,2,3). The time taken in preparing the cells for sorting and the sorting itself may also induce cell damage leading to poor viability. We assumed that if we can minimise the time the cells are out of culture, we could increase the survival of these cells post sorting. We noticed that by carefully adjusting the Forward Scatter (FSC) and Side Scatter (SSC) collection optics on the MoFlo, we could enrich for G0/G1 cells without staining (4,5,6). We applied this technique to the haploid sorts which significantly reduced the total processing time and gave us better survival of the cells in culture post sort.
Recently we added a Bio-Rad S3 into the facility and began testing the system to see if we could perform the FSC-SSC haploid sort. This talk will cover the basic elements of the assay and include a discussion of the S3, particular its ability to sort haploid G0/G1 cells without DNA dyes.
1) Kotecki M., Sanjeeva Reddy P. and Cochran B.H., "Isolation and Characterization of a Near-Haploid Human Cell Line". Experimental Cell Research 252, 273–280 (1999)
2) Crissman H. A., Hofland M. H., Stevenson, A. P., Wilder M. E. and Tobey, R. A., "Use of DiO-C5-3 to improve Hoechst 33342 uptake, resolution of DNA content, and survival of CHO cells". Exp. Cell Res. 174, 388–396 (1988)
3) Wlodkowic D. and Darzynkiewicz Z., "Destruction of chromatin structure by supravital nucleic acid probes", Cytometry A. 73(10), 877–879 (2008)
4) Zucker R. M., Daniel K. M., Massaro E. J., Karafas S. J., Degn L. L. and Boyes W. K., "Detection of Silver Nanoparticles in Cells by Flow Cytometry Using Light Scatter and Far-Red Fluorescence", Cytometry A. 83AA 962-972, (2013)
5) Ormerod M. G., Paul F., Cheetham M. and Sun X. M., "Scatter detection Discrimination of Apoptotic Thymocytes by Forward Light Scatter" Cytometry 21 300-304 (1995)
6) The European Cytometry Network: http://www.eurocyt.net/page/sorting-out-g0g1-cells-without - http://www.eurocyt.net/page/sorting-out-g0g1-cells-without

An Emerging Role for Glycosylation in Multiple Myeloma

Michael O'Dwyer

National University of Ireland, Galway

Glycosylation is a stepwise procedure of covalent attachment of oligosaccharide chains to proteins or lipids, and alterations in this process, especially increased sialylation, have been associated with malignant transformation and metastasis. Multiple myeloma (MM) is characterized by the presence of multiple lesions throughout the skeleton, indicating continuous trafficking of tumor cells to multiple bone marrow niches. This trafficking is potentially influenced by glycosylation as MM cells express a variety of adhesion molecules, many of which are dependent on glycosylation for their function. Our studies implicate glycosylation as having a critical role in the growth, survival and dissemination of multiple myeloma with the potential to identify new biomarkers and therapeutic targets.

Employing a novel activation platform for the initiator caspase-8 to induce apoptosis in chemo-resistant cancer cells

Christian Hellwig

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We recently identified that cancer cells which are resistant to genotoxic chemotherapeutics can be eliminated by apoptosis upon forming autophagy-dependent non-canonical caspase-8 activation platforms. However, the required platform for caspase-8 activation has not yet been isolated, and its components are still unknown.
We identified caspase-8 as the apical caspase activated after proteasome inhibition and its ability to induce apoptosis was independent of necroptosis or ROS generation. We collected data on the dose-response relationships and the time required for non-canonical caspase-8 activation. We showed by cell fractionation and size exclusion chromatography that in contrast to canonical caspase-8 activation, proteasome inhibition-induced caspase-8 activation occurs in high molecular weight complexes in the cytosol and is associated with unique ultrastructural changes, as seen by electron microscopy. The activation complexes are not ripoptosomes, but instead represent a novel, unknown caspase-8 activation platform. We determined by FRET flow cytometry that the majority of cells respond with caspase-8 activation, which is sufficient to induce apoptosis execution in human colon cancer HCT-116 $(Bax/Bak)^{-/-}$ cells, which lack the mitochondrial (intrinsic) apoptosis pathway and are therefore resistant to genotoxic chemotherapeutics.
This study provides evidence for a mechanism that promotes caspase-8 activation upon proteasome inhibition, able to kill chemotherapy-resistant cancer cells.

Potential of Primary Chondrocytes for Allogeneic Cell Therapy

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Damaged or injured articular cartilage is a debilitating condition caused by injury or osteoarthritis. Articular cartilage has a low intrinsic regenerative capacity and therefore autologous chondrocyte implantation (ACI) is often required to restore function to the joint. However ACI requires two procedures to be carried out on the patient. Here we investigate the immunological characteristics of allogeneic articular cartilage derived primary chondrocytes.
We use a fully allogeneic rat strain combination of Dark Agouti(DA) to Lewis(LEW). Primary chondrocytes were isolated from the articular cartilage on the condyles of the knee of 6-12 week old male Dark Agouti rats. The cartilage was digested in protease and collagenase and isolated cells cultured in either 2% or 19% oxygen. 2% oxygen was used as the knee is an avascular and hypoxic environment.
Chondrocytes were treated with cytokines interferon-γ, interleukin-1β and tumour necrosis factor-α alone or in combination for 48 hours to mimic inflammatory environments which the cells may encounter *in vivo*.
The cell surface expression of immunologically relevant molecules MHCI, MHCII, CD80 and CD86 were measured by flow cytometry in all conditions and results showed that chondrocytes expressed low basal levels of these before cytokine treatment. Interferon-γ treatment resulted in a significant upregulation of MHCI in 19% O_2 cultured chondrocytes while interleukin-1β treatment resulted in a significant MHCI upregulation in 2% O_2 treated chondrocytes. However in functional assays where primary chondrocytes were co-cultured with allogeneic lymphocytes to measure the immunogenicity of these cells we observed that chondrocytes were weakly immunogenic, as measured by low levels of T cell proliferation and activation. We found that chondrocytes were capable of providing an effective MHC signal to allogeneic T cells but were unable to provide co-stimulation.
Primary Chondrocytes were co-cultured with CD3/CD28 stimulated allogeneic lymphocytes to determine their ability to suppress ongoing T cell proliferation. We found that primary chondrocytes could suppress ongoing T cell proliferation when they were in contact with the T cells and also when there was no contact. We found that nitric oxide was secreted by primary chondrocytes in these cultures which may be part of the mechanism of primary chondrocyte mediated immunosuppression.
This study reveals that primary chondrocytes may have the potential to be used in an allogeneic cartilage repair model due to their weak *in vitro* immunogenicity and their immunosuppressive activity.

Applying Cytometry in Zebrafish to Cancer and Vision Research

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Do Cytokines Instruct Hematopoietic Lineage Choice?

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Flow cytometry has been instrumental in developing our understanding of the process of blood cell formation, or haematopoiesis, in the bone marrow. Using this technology, the heterogeneity of cell subpopulations within this haematopoietic organ has been dissected down to the single cell level. These studies have confirmed that in both mouse and man, haematopoietic stem cells (HSC) truly exist at the single cell level. Despite these studies, how haematopoiesis actually works is still a matter of debate. It is known that the process of haematopoiesis is dependent upon contact with mesenchymal-derived stromal cells as well as signals emanating from soluble mediators or cytokines. However, whether cytokines play permissive or instructive roles in lineage choice is a hotly debated issue. Thus cytokines either support, or are permissive for, the differentiation of cells that have already made lineage decision choices or alternatively by their signaling they directly instruct lineage choice decisions. Recent evidence using animals over-expressing cytokines critical for early haematopoiesis tends to favor an instructional role for cytokines [1, 2]. In this regard, in collaboration with Prof Ton Rolink's laboratory at the University of Basel, we have generated a transgenic mouse line over-expressing Flt3L, one of these key cytokines involved in myelopoiesis [3]. As well as containing a considerably expanded number of functional dendritic cells, a finding that may be of interest to immunologists, careful analysis of the bone marrow reveals significant lineage skewing away from erythrocyte, megakaryocyte and B lymphocyte lineages. Co-administration of another key cytokine, Interleukin-7, to such mice partially corrects the B lineage deficiency. Taken together, these results are most easily interpreted as indicating an instructive role for Flt3L in myeloid lineage commitment.

1. Grover, A., et al., *Erythropoietin guides multipotent hematopoietic progenitor cells toward an erythroid fate.* J Exp Med, 2014. **211**(2): p. 181-8.
2. Mossadegh-Keller, N., et al., *M-CSF instructs myeloid lineage fate in single haematopoietic stem cells.* Nature, 2013. **497**(7448): p. 239-43.
3. Tsapogas, P., et al., *In vivo evidence for an instructive role of fms-like tyrosine kinase-3 (FLT3) ligand in hematopoietic development (in press).* Haematologica, 2014.

CD362: a functional marker enabling MSC isolation & clinical development.

Lisa O'Flynn

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Human tissue-derived Mesenchymal Stromal Cells (MSC) comprise a mixed population of fibroblastic cells that can be isolated from bone marrow, umbilical cord and adipose tissue by adherence to tissue culture plastic and formation of colony forming unit-fibroblasts (CFU-F). MSC secrete potent immunomodulatory and angiogenic factors and so represent a valid therapeutic option for complicated inflammatory and ischemic diseases. Questions remain over the endogenous identity and function of MSC *in vivo*, as well as the heterogeneity and function of therapeutic MSC preparations *in vitro.*

In this study, CD362, a heparan sulfate proteoglycan, was identified as a stromal cell protein that labels MSC isolated from human, murine, rat, rabbit and equine marrow. Antibodies to CD362 enabled prospective isolation of defined MSC from human, mouse and equine tissues. In human marrow, CFU-F were highly and exclusively enriched in the

$CD271^{+}CD45^{-}$ mononuclear cell (MNC) fraction expressing either $CD362^{lo}$ or $CD362^{+}$. FACS-based purification of $CD362^{+}CD271^{+}$MNC from human marrow yielded CFU-F:MNC ratios in the range of 1:3 to 1:10, representing a >3,000-fold enrichment of CFU-F compared to current plastic adherence methods. MSC derived from $CD362^{+}CD271^{+}$ MNC gave rise to stromal cell cultures with similar flow cytometric profiles and tri-lineage capacity. Moreover, $CD362^{+}CD271^{+}$ demonstrate immunosuppressive capacity *in vitro* by the reduction of allogeneic human $CD4^{+}$ T lymphocyte proliferation in response to αCD3/αCD28 stimulation. CD362 surface protein was maintained through several passages with a pattern of slowly diminishing expression. In the C57BL/6 mouse bone marrow, $CD362^{+}CD45^{-}$ MNC also express Sca1, Nestin, PDGFRα and gp38 (Podoplanin). Murine MSC derived from $CD362^{+}gp38^{+/-}$ MNC exhibited similar growth kinetics and reduced syngeneic murine $CD4^{+}$ T lymphocyte proliferation in response to anti-CD3/APC stimulation. $CD362^{+}CD45^{-}$ MNC were identified in multiple murine tissues. Finally, antibodies to CD362 were used to successfully isolate all MSC from equine marrow by FACs and also from human umbilical cord by magnetic column separation (MACS).
In conclusion based on these results, CD362 represents the first MSC marker that enables prospective isolation of a defined and therapeutic stromal cell from human, equine and murine tissues. Moreover, CD362 protein represents a potential potency assay of MSC efficacy and work is on-going to elucidate the role of CD362 in MSC biology. Grant Nos. EU FP7/2012/305736 and SFI 09/SRC/B1794

Reduce, Refine, Replace: Use of Bioluminescence and Biofluorescence Technology in Life Science Research

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Immunotherapeutics for Cancer and Autoimmune Diseases

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The immune system has evolved to protect us against infection and tumours and when dysregulated can respond to self antigens and tissues leading to autoimmunity. CD4+ T cells that secrete IL-17 (Th17 cells) or IFN-γ (Th1 cells) play a pathogenic role in many autoimmune diseases, but also function with Th1 cells to mediate protective immunity to infection and tumours. Regulatory T (Treg) cells can suppress Th17 responses and thereby prevent autoimmunity, but can also suppress protective immunity to pathogens and tumours. Stimulation of dendritic cells by pathogen recognition receptor, including Toll-like receptors (TLR) and NOD-like receptors (NLR) promotes maturation and T-cell promoting cytokines. We have shown that TLR and NLR agonists induce IL-1α and IL-1β which synergize with IL-23 to promote activation of memory Th17 cells and γδ T cells. The induction of function of Th17 cells is regulated by cytokines secreted by the other major subtypes of T cells, especially IL-10 and TGF-β production by Treg cells. The induction of natural and adaptive Treg cells is stimulated by TGF-β, IL-10 and IL-27 in response to certain virulence factors from pathogens that have evolved to subvert protective immunity, but also in response to TLR and NLR agonists and this constrains their potential as adjuvants and therapeutics against infection and cancers. We have shown that inhibitors of MAP kinase and PI3K signalling pathways can block IL-10 and TGF-β, while sparing or enhancing IL-12 production, thereby promote Th1, but not Treg cells. This has been successfully applied to a therapeutic intervention against cancer in pre-clinical models. Finally, we have identified approaches for activation of regulatory cytokine production by innate immune cells for selective induction of Treg cells, without Th1 or Th17 cells. These approaches been effective in pre-clinical models of autoimmunity and cancer and are also being translated to clinical studies in humans.

Myeloid Angiogenic Cells (MACs) Attain a more M1 Pro-inflammatory Phenotype when Exposed to High Glucose

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Endothelial progenitor cells (EPCs) promote angiogenesis, maintain and regenerate the vasculature. An EPC subtype called myeloid angiogenic cells (MACs) are molecularly and functionally similar to alternatively activated M2-macrophages. MACs therapy significantly enhances vascular repair in murine ischaemic retina and is mediated by paracrine release of angiogenic factors, such as IL8. This pro-angiogenic potential of MACs could be harnessed as a novel cellular therapy for treatment of diabetic retinopathy. However, since MACs are like M2-macrophages which are plastic in phenotype, switching between pro-inflammatory M1 and pro-angiogenic M2 depending on their environment, the plasticity and pro-angiogenic function of MACs needs to be assessed in the diabetic milieu.
In vitro diabetic milieu was achieved by 4days exposure to high D-glucose (DG) 25mM. An *in vitro* 3D-angiogenesis assay demonstrated that high DG significantly reduced the pro-angiogenic potential of MACs conditioned medium on BREC tube formation ($P<0.0001$), compared to the untreated control (5mM DG) and the osmotic control (5mM DG & 20mM L-Glucose LG). Analysis of conditioned media by an angiogenesis protein array highlighted that DG treated MACs secreted reduced levels of pro-angiogenic cytokine IL8. Gene expression analysis from high DG treated cells were compared to M1 and M2-macrophages, cultured from CD14+ve sorted cells (98% pure by flow cytometry). DG exposure induced a more M1 pro-inflammatory profile, with higher levels of IL1α, IL6, ICAM-1 and lower levels of M2 markers CD209, CD163 and angiogenic promoter IL8. Before observing MACs *in vivo,* a model of STZ-induced diabetes was observed for pathological changes over a period of 4-9months diabetes using Fluorescence Angiography and Optical Coherence Tomography.
This study shows that although MACs are capable of inducing angiogenesis and act as M2 macrophages, they have a reduced capacity to promote angiogenesis when exposed to high DG. This suggests that their phenotype changes towards a more M1 profile, and alters their secretome profile to release fewer angiogenic cytokines. This switch in phenotype is an important consideration when delivering MACs as a cell therapy into a diabetic environment, and highlights that more research is required to understand how MACs behave *in vivo*, and how we maintain their M2 phenotype before these cells can be used successfully as a cellular therapy.

Human Invariant Natural Killer T cells Control Multiple B cell Functions in vitro

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Human B cells express CD1d and therefore are likely to present glycolipid antigens to invariant natural killer T (iNKT) cells. We investigated if B cells could present the iNKT cell agonist glycolipid, α-galactosylceramide (α-GC) to expanded autologous iNKT cells in vitro and if α-GC-stimulated iNKT cells could reciprocally activate the B cells. Since iNKT cells comprise functionally-distinct subsets based on CD4 and CD8α expression, we analysed the effects of co-culturing sorted CD4+, CD8α+ and CD4-CD8α- double negative (DN) iNKT cells with B cells. B cells were capable of presenting α-GC to CD4+ and DN (and to a lesser degree CD8α+) iNKT cells resulting in IFN-γ, TNF-α, IL-4, IL-5 and IL-13 secretion by iNKT cells. However B cells were 10-200-fold less efficient than DC at stimulating the production of these cytokines by iNKT cells. Reciprocally, all subsets of iNKT cells could induce antibody production by autologous B cells in vitro, but interestingly, α-GC was not required. Culture of B cells with CD4+ or DN iNKT cells resulted in the expansions of cells with regulatory B (BREG; CD1dhiCD5+, CD24hiCD38hi) phenotypes and CD4+ iNKT cells promoted IL-10 production by some B cells. These BREG phenotypes were induced without the need for α-GC. These results suggest that human iNKT cells can differentially control adaptive immune responses, depending on whether or not stimulatory glycolipid ligands are present. In the presence of α-GC, human B cells can weakly activate iNKT cells, resulting in Th1 and Th2 cytokine secretion. In the absence of α-GC, B cells do not stimulate cytokine secretion by iNKT cells, but iNKT cell subsets can induce antibody production and promote differentiation of B cells into IL-10-producing BREG cells.

Regulation of IL-1β-induced NF-κB activity by hydroxylases links key hypoxic and inflammatory signaling pathways

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Hypoxia is a prominent feature of chronically inflamed tissues. However, this is rarely taken into account in studies of inflammatory signaling pathways. Oxygen-sensing hydroxylases control transcriptional adaptation to hypoxia through regulation of Hypoxia-Inducible Factor (HIF). Furthermore, pharmacologic hydroxylase inhibition reduces inflammation in animal models of colitis and sepsis. However, the underlying mechanisms that link hydroxylase activity to inflammatory signaling remain unclear. IL-1β, a major pro-inflammatory cytokine, activates NF-κB and is associated with numerous inflammatory pathologies. We demonstrate that a combination of PHD1 and FIH hydroxylase isoforms regulate IL-1β–induced NF-κB at the level or downstream of the activation of the TRAF6 complex. Several proteins of the IL-1β-signaling pathway are subject to hydroxylation and form complexes with PHD1 or FIH. We propose that hydroxylation is a key post-translational modification regulating IL-1β signaling and subsequent inflammatory gene expression.
Hydroxylase inhibition represents a new approach to the inhibition of IL-1β-dependent inflammatory signaling.

Flow cytometry as a tool for the analysis of mitochondrial mass and energy metabolism in immune cells

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Glucose metabolism not only provides energy for physical activity, but also mediates a variety of physiological processes. Under normal physiological conditions glucose is used via oxidative phosphorylation. This process involves the mitochondrial electron transport chain and is dependent on oxygen availability. In the absence of oxygen cells switch to an alternative pathway of energy production termed glycolysis, although this pathway is far less efficient in the production of energy.
Otto Warburg first noted in 1924 that cancer cells predominantly generate energy through the process of glycolysis despite the availability of normal levels of oxygen. This phenomenon of aerobic glycolysis, now termed the Warburg effect; has been extensively studied in cancer cells. More recently, a similar phenomenon has been reported during inflammation i.e. a switch from respiration to aerobic glycolysis occurs in pro-inflammatory but not in anti-inflammatory cells. As such, the measurement of bioenergetics in primary immune cells is a rapidly growing field.
We are using a combination of flow cytometry and other methods to analyse mitochondrial mass and glucose metabolism in peripheral blood mononuclear cells (PBMCs) from patients with autoimmunity. Using mitotracker green uptake in combination with markers for T cells, B cells and monocytes allows us to analyse mitochondrial mass in each of these populations in a single tube from 10^5 PBMCs. This data could also be obtained using real-time PCR of mitochondrial and nuclear DNA. However, sorting of cells would be required to study individual populations by this method and therefore would require much greater numbers of cells..
We are also measuring metabolism by a combination of flow cytometry and extracellular flux analysis. In particular, glycolysis is being measured using a fluorescently labelled 2-deoxyglucose analogue. This compound can be taken up by glucose transporters but cannot be broken down by glycolysis and as such accumulates in cells acting as an indicator of glucose uptake and glycolysis. Again, using markers for specific cell population allows glucose uptake in numerous cell types to be analysed simultaneously in a single tube from very few cells. Further experiments are being carried out using extracellular flux analysis (Seahorse Biosciences) which allows the simultaneous measurement of respiration and glycolysis. However, 1.5×10^6 cells are required per sample meaning that the analysis of specific cell populations requires very large numbers of donor cells.
In conclusion, the use of flow cytometry allows the measurement of parameters of mitochondrial mass and cellular metabolism in multiple specific cell types from minimal numbers of cells.

Regulation of Human Th17 Cells

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Poster Presentation Abstracts

in alphabetical order (first author):

Common Variable Immunodeficiency: a role for innate T cells in antibody deficiency?

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BACKGROUND - Common Variable ImmunoDeficiency (CVID) is the most commonly diagnosed primary immunodeficiency, clinically heterogeneous but always characterized by abnormally low immunoglobulin titres and recurrent infections.
AIM - Since T cell help is required for B cell differentiation and immunoglobulin production, we investigated if circulating T and innate subpopulations are numerically or phenotypically altered in CVID.
METHODS - Blood was obtained from 28 healthy controls and 21 CVID patients attending St. James's Hospital, Dublin. Using a whole blood protocol, we used flow cytometry to determine the frequencies and absolute numbers of plasmablasts, naive and switched memory B cells, $CD4^+$ and $CD8^+$ T cells, Natural Killer (NK) cells, γδ T cell subsets, invariant Natural Killer T (iNKT) cells and Mucosal-Associated Invariant T (MAIT) cells. Clinical data were also collected from patient records.
RESULTS - B cell maturation was skewed in patients, with significantly increased frequencies of naive B cells and decreased switched memory B cells and plasmablasts. NK cells and γδ T cell subsets were normal, however iNKT and MAIT cells were significantly depleted in patients ($p<0.0001$ and $p<0.01$, respectively). MAIT cells also exhibited an atypical CD4/CD8 distribution, and their frequencies were particularly low in patients with CVID complicated by autoimmunity ($p<0.005$).
CONCLUSIONS - Circulating MAIT and iNKT cells were found to be depleted in CVID patients. iNKT cells can influence B cell differentiation and antibody production. MAIT cells require B cells to expand and could, like iNKT cells, affect B cell function. Their reduction in patients with autoimmune complications moreover suggests they have regulatory functions in peripheral tissues.

USING FLOW CYTOMETRY TO DIRECTLY CLONE MESENCHYMAL STROMAL CELLS FROM MOUSE BONE MARROW

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Since first described by Friedenstein in the mid-70s [1], mesenchymal stromal cells (MSC) have raised a huge interest in the research community for their differentiation capacity and their immune-modulatory properties. These cells have been isolated from several tissues; including liver, muscle, and placenta with bone marrow being still the most common source for clinical studies [2]. Human MSC are already widely used in clinical trials, e.g in Myocardial infarction or critical limb ischemia [3]. Despite the routine isolation of human MSC, mouse MSC (mMSC) are far more difficult to isolate due to their contamination with hematopoietic cells, and their characterisation is hindered by inbred mouse strain differences in numbers, proliferation, surface markers and other properties [4][5]. This restricts the use of rodent genetic models, which are needed to understand the basic biology, engraftment and therapeutic potential of MSCs [6]. One of the major obstacles in the isolation of mMSC is the heterogeneity of mouse MSC cultures obtained by routine plastic adherence isolation used for human MSC. These bulk cultures may contain individual clonal populations which differ in their differentiation profile and immune-modulatory potency.
Single cells FACS offers a promising approach in clonally isolate progenitor/stem cell population. We initially isolated cells based on Sca-1 expression by FACS following collagenase digestion of either flushed bone marrow plugs or crushed bones. Even though we were able to expand them *in vitro*, the CFU-F plating efficiency was too low to allow cloning by single cell. However, by combining hypoxic (< 5% oxygen) culture conditions, gelatine coating of 96-well plates and the use of additional cell surface markers we were able to significantly improve CFU-F plating efficiency and therefore were able to clonally isolate single cells from mouse bone marrow. Thus $CD45/Ter119^-/Sca\text{-}1^+/CD90^+/PDGFRa^+$ cells had a plating efficiency of about 1 in 2. Further investigation will be carried out to address the lineage potential and transcriptomic profiles of these cells.

[1] A. J. Friedenstein et al., *Experimental hematology*, vol. 4, no. 5, pp. 267–74, Sep. 1976.

[2] H. K. Salem and C. Thiemermann, *Stem cells (Dayton, Ohio)*, vol. 28, no. 3, pp. 585–96, Mar. 2010.
[3] "http://clinicaltrials.gov/." [Online]. Available: http://clinicaltrials.gov/.
[4] D. G. Phinney et al., *Journal of cellular biochemistry*, vol. 72, no. 4, pp. 570–85, Mar. 1999.
[5] A. Peister et al., *Blood*, vol. 103, no. 5, pp. 1662–8, Mar. 2004.
[6] S. Morikawa et al., *The Journal of experimental medicine*, vol. 206, no. 11, pp. 2483–96, Oct. 2009.

REGULATORY T-CELLS OF MOUSE DISPLAY DISTINCTIVE SURFACE GLYCOSYLATION PATTERNS THAT VARY ACROSS DIFFERENT ANATOMICAL SITES AND ACTIVATION STATES

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Background: Regulatory T-cells (T-reg) are a subset of $CD4^+$ T lymphocytes whose specialized functions act to suppress the activities of different effector immune cells. T-reg differ phenotypically from other T-cell populations by the higher expression levels of some molecules such as CD25, CTLA-4 and GITR, but mostly by the intracellular expression of the transcription factor FoxP3. Increasing evidence supports an important role for carbohydrate modification (glycosylation) of surface molecules in the determination of lymphocyte biological function.
Aims: The main goals of this work were to identify surface glycosylation characteristics that distinguish T-reg from conventional $CD4^+$ T-cell populations (T-conv) in mouse, to evaluate differences in the glycosylation pattern between different T-reg subsets according to their activation state (based on CD62L/CD25 expression) and to correlate T-reg surface glycosylation with expression of specific proteins known to mediate T-reg suppressive functions.
Methods: Cell suspensions of lymphoid organs and peripheral blood were obtained from C57BL/6-FoxP3-EGFP mice in which GFP expression is exclusive to T-reg. Surface glycosylation and marker expression was then evaluated by multi-colour flow cytometric analysis using a panel of 17 biotinylated lectins (carbohydrate binding proteins) and 9 fluorochrome-labeled antibodies with gating on T-reg (GFP^+) and T-conv (GFP^-).
Results: Freshly-isolated splenic T-reg demonstrated significantly higher surface binding of 5/17 lectins (GSL-I, DSL, PHA-E, PHA-L and SNA-I) compared to T-conv. In the resting state, T-reg from subcutaneous lymph nodes showed similar lectin binding profiles to splenic T-reg. However, T-reg from thymus and bone marrow had additional variances in lectin binding levels compared to T-conv from the same sites. T-reg of differing activation state displayed variable surface glycomes. For example, $CD4^+FoxP3^+CD62L^+CD25^+$ "naïve" T-reg had higher global glycosylation compared to non-naïve T-reg subpopulations ($CD62L^-/CD25^+$ and $CD62L^-/CD25^-$). Using 6-colour flow cytometric analysis, it was also demonstrated that higher glycan expression at T-reg surface correlated with higher levels of expression multiple known mediators of T-reg suppressive function including GITR, ICOS, CD73, PD-1, PDL-1 and CTLA-4. **Conclusions:** The surface glycosylation of mouse T-reg is distinct from that of T-conv in the resting state. $CD4^+$ T-cell glycosylation is variable across different anatomical sites in healthy animals and changes according to activation state. On-going experiments are investigating the hypothesis that enhanced T-reg glycosylation pathways contribute to T-reg expansion and suppressive function in vivo.

Development of a Quantitative, Flow Cytometry-based Assay for Human Blood Monocyte Adhesion to and Transmigration through an Endothelial Layer

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Monocytes are blood cells of myeloid lineage, which originate in the bone marrow and migrate to circulation as short lived, non-dividing cells with diverse roles in tissue homeostasis, tissue repair and immune defense. They are also implicated in many diseases, particularly atherosclerosis. Based on CD14 and CD16 expression, human blood monocytes can be divided into 3 distinct subpopulations, namely $CD14^{++}CD16^-$ "Classical", $CD14^{++}CD16^+$ "Intermediate" and $CD14^+CD16^{++}$ "Non-Classical" subsets. Recently, we have shown that the Intermediate monocyte subset can be further subdivided based on HLA-DR (MHC II) and CD16 surface expression, into 2 additional subsets which we refer to as DR^{mid} and DR^{hi} intermediate monocytes.
A key functional characteristic for which individual monocyte subsets may differ is their propensity to adhere to and transmigrate across activated endothelium. In this study, we have developed an in vitro monocyte transendothelial

migration assay in which primary human aortic cells (HAECs) are seeded onto fibronectin-coated polycarbonate membranes (3.0□m pore size) and cultured in a Transwell® system. The HAEC monolayer is stimulated with the pro-inflammatory cytokine TNF-□, resulting in up-regulation of vascular adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1). Unfractionated human peripheral blood mononuclear cells (PBMCs) are introduced to the upper chamber of the Transwell® system then cultured for 15 minutes to 4 hours. Following this, floating (non-adherent) and adherent cell fractions are separately recovered from the upper chamber and transmigrated cells from the lower chamber and underside of the insert. Following addition of compatible counting beads, monocyte subset analysis and quantification as well as endothelial cell surface analysis are simultaneously performed on all fractions by multi-colour flow cytometry.
Results to date confirm distinct adhesion and migration characteristics for Classical, Intermediate and Non-Classical monocytes as well as for the DR^{mid} and DR^{hi} intermediate monocyte subsets. In ongoing work, the system will be adapted to compare monocyte subsets with and without exposure to atherogenic lipids and lipoproteins.

Leukocyte Fractionation by Centrifugal Elutriation to Facilitate Fluorescent Activated Cell Sorting (FACS) of Human Monocyte Subsets

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Monocytes are immature cells of myeloid lineage that are produced in the bone marrow and transit via the blood to organs and tissues where they differentiate into specific effector cells. Monocytes represent a vital link between the innate and adaptive components of the immune system with roles in tissue homeostasis and repair. Based on CD14 and CD16 expression, classification of human blood monocytes can be divided into 3 distinct subpopulations, namely $CD14^{++}CD16^{-}$ "Classical", $CD14^{++}CD16^{+}$ "Intermediate" and $CD14^{+}CD16^{++}$ "Non-Classical" subsets. Recently, we have shown that the human intermediate monocyte subset can be further subdivided based on HLA-DR (MHC II) and CD16 surface expression, producing two additional subsets, which we refer to as DR^{mid} and DR^{hi} intermediate monocytes. In order to further characterize the DR^{mid} and DR^{hi} intermediate subsets, FACS may be employed to generate pure populations of each. However, sorting cell populations of low frequency consumes time and resources and may be associated with suboptimal purity. Thus, pre-enrichment techniques which concentrate cell populations of interest while also removing other unwanted cells prior to FACS may be of value. However, for pre-enrichment techniques, it is essential that the specific cells of interest are not depleted or functionally altered in the process.
In this study, we compared a commercial "no touch" magnetic activated cell sorting (MACs) kit with centrifugal elutriation (CE) for efficiency of total monocyte and monocyte subpopulation enrichment from human peripheral blood mononuclear cells (PBMCs) prepared by standard Ficoll gradient. In multiple experiments, CE resulted in enrichment of the monocyte fraction from a mean of 13.1% to 65.2% of total cells with excellent cell viability. Importantly, CE provided consistent enrichment of all monocyte subsets including both DR^{mid} and DR^{hi} intermediates. In contrast, the MACS protocol, while providing comparable enrichment of total monocytes from unfractionated PBMCs, was associated with selective loss of DR^{mid} intermediate monocytes. Importantly, monocytes enriched by CE were not activated by the elutriation process, as evidenced by lack of pro-inflammatory cytokine production in subsequent culture.
We conclude that CE is a consistent and highly cost efficient method for enriching monocytes which faithfully preserves each monocyte subpopulation and does not result in cellular activation.

Peripheral blood B cell maturation patterns are a useful diagnostic and prognostic marker for disease severity in paediatric primary antibody deficiency.

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Flow cytometry is a fundamental laboratory tool in the diagnosis of Primary Immune Deficiencies (PIDs). Primary Antibody Deficiency (PAD) is the most common form of PID in adults and children. Common Variable Immune Deficiency (CVID) is an idiopathic permanent antibody deficiency with low IgG and low IgA or/and IgM. CVID is complicated by recurrent infections and is treated with long term Immunoglobulin replacement therapy. Other PADs include isolated IgG subclass deficiency and specific antibody deficiency, were patients have poor specific antibody

vaccine responses. These other antibody deficiencies can rarely evolve into CVID but usually occur as milder conditions less often requiring immunoglobulin therapy.
Various abnormalities in B cell subpopulations frequencies have been found in CVID. *Piątosa et al* proposed using B cell maturation patterns, as identified by immunophenotyping of peripheral blood, to subgroup CVID patients.
We wished to explore if B cell maturation patterns could be useful markers to predict PAD diagnosis, severity and requirement for therapy in children with PAD.
Methods: B cell immunophenotyping including transitional, naïve, memory B cells and plamsablasts, was performed on peripheral blood samples from 25 children with primary antibody deficiency. Patients were subgrouped in 6 groups using a modified *Piątosa et al.* B cell maturation classification system and then analysed in relation to diagnosis and treatment.
Results: All patients with a CVID diagnosis had an early B cell maturation block prior to or atthe germinal centre (Group 1-3). Only 2 (12.5%) of the non-CVID antibody deficiency group had a pre-germinal centre block (Group 1-2). All patients receiving IgG replacement therapy had an early Group 1-3 block. The 2 non-CVID antibody group patients with Group 1-2 block were the only individuals in that group that are on Immunoglobulin replacement therapy.
Conclusions: Peripheral blood B cell maturation patterns seem to help differentiate between CVID and other PADs. Early blocks in B cell maturation correlate with a more severe antibody deficiency and a requirement for long term antibody replacement therapy.

Compensation-free dead cell exclusion by a simple multi-beam excitation (MBE) approach

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In flow cytometry and cell sorting the reliable exclusion of dead/damaged cells is often a requirement. DRAQ7 is a far-red fluorescing biomarker of cell viability designed for this application. This water-soluble molecule selectively binds nuclear dsDNA with high affinity but is cell membrane impermeant. Accordingly,it only labels cells with compromised plasma membranes (due to poor storage, mechanical damage, cytotoxicity, apoptosis induction, serum starvation, etc.). DRAQ7 has been robustly tested and exemplified(e.g. Akagi, et al. and Smith, et al., Cytometry Part A 2013) for compatibility with multi-colour experiments and uniquely for long-term, real-time analysis. It has been utilised to monitor cell viability in 2-D and 3-D spheroid/micro-tissue assays.
Multi-colour flow cytometry is limited by the narrow spectral space available and by the propensity to exploit very bright chromophores to label antibodies. Although brightness may be desirable for sensitivity it can increase need for compensation. DRAQ7can benefit panel design due to its emission profile. It has no overlap with GFP/FITC or R-PE and minor compensation with APC. Due to its specificity it provides clear signal-to-noise in membrane-compromised cells. DRAQ7 is optimally excited by orange / red wavelengths (with peaks at 599 nm & 644 nm) yet it is possible to excite DRAQ7 with blue, green or yellow laser lines, allowing use on simple cytometry platforms.It emits in the far-red, unshiftedirrespective of excitation light wavelength.
In principle, this means that it will occupy more than one fluorescence channel which may be undesirable. Paradoxically, however, this enables selection of a unique population of membrane-compromised cells; plotting pairs of far-red channels from different laser excitations against each other one can choose a suitable pairing that gives the best separation of a double-positive population and chromophore panel. The double-positive population is then defined as the dead-cell gate and these events are entirely excluded from all other channels avoiding any compensation between DRAQ7™ and other chromophores. This can be achieved during acquisition or post-acquisition. The technical principles of this simple approach and exemplar data will be presented.

Infection with a respiratory pathogen attenuates IL-17 mediated CNS autoimmunity through IL-10 induction

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Epidemiological studies have described an association between infection with certain bacteria or viruses and development of autoimmune diseases, such as multiple sclerosis (MS). Conversely, infection with helminth parasites has been associated with a reduced incidence and severity of autoimmunity. Whooping cough is a re-emerging vaccine-preventable infectious disease caused by the bacteria *Bordetella pertussis*. Clearance of the bacteria is

associated with the induction of Th1 and Th17 cells. However, the infection is persistent and is also associated with the induction of and recruitment of IL-10-secreting Treg cells to the lungs during the acute stage of disease. In this study we have used a murine model of MS, experimental autoimmune encephalomyelitis (EAE), to examine the effect of infection on development of CNS inflammation. The data reveal that concurrent infection with *B. pertussis* significantly attenuates clinical disease and weight loss in mice with EAE. This reflected a significant reduction in the frequency of infiltrating IL-17^{+}, IFN-γ^{+} and IFN-γ^{+}IL-17^{+} CD4 and γδ T cells into the central nervous system (CNS) of *B. pertussis* infected compared with uninfected mice with EAE. Furthermore analysis of T cell populations in the spleens revealed that infected mice had higher numbers of IL-10-producing Foxp3^{+} cells as well as IL-17^{+}IFN-γ^{+} CD4 T cells. An examination of antigen-specific T cell responses in lymph nodes and spleen revealed that systemic IL-17A and IFN-γ production was enhanced in infected mice with EAE. The attenuation of EAE by *B. pertussis* was lost in IL-10$^{-/-}$ mice, suggesting that IL-10 induced by *B. pertussis* suppresses T cell responses that mediate CNS inflammation. Our findings demonstrate that a bacterial infection of the respiratory tract can attenuate the course of EAE in mice and this reflects the suppressive effect of pathogen-induced IL-10 produced by T cells or innate immune cells, which appear to be capable of preventing Th1 and Th17 cells from entering the CNS.

Quantification of Intracellular QDs and Caused Immune Responses by Multi-Color Flow Cytometry: Gains and Limits

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Quantum Dots (QDs) are fluorescent semiconductor nano-crystals which have been proposed as convenient tool for multi-color bio-diagnostics due to their broad absorbance and easy excitation by fitted lasers. Surface modification is required to reduce QD toxicity.

Unless all benefits provided by QDs, the problem of their intracellular quantifying is unresolved due to complex and not clearly investigated nanoparticles interaction with cellular compartments. The main goal of this work is to find best range QDs parameters for flow cytometry quantification and their effect on cell function. RAW264.7 murine macrophage cell line was used in the study due to its quick QDs ingesting and broad expression of CD80/86 markers. Range of QDs with different size and composition in three concentrations were tested.

Materials and methods: Thioglycolic acid (TGA)- and TGA-gelatin-covered red (5.1 and 5.3 nm) and green (2.1 and 2.3 nm) CdTe QDs were obtained by water-based synthesis. RAW264.7 murine macrophage cell line was used in study. Cells were cultured under standard conditions. Apoptosis and viability assay was performed by Annexin V. Level of CD80/86 expression was measured. Cells were co-incubated with or without nanoparticles for 12 or 24 hours. Control samples remained untreated. Samples were harvested on the day of analysis.

Results and discussion: Maxima shift of fluorescence peak on histogram (signal intensity vs. count) was observed at 100 nM concentration. Highest signal amplification had red particles (up to 10 folds); green particles demonstrated only 2-4-folds fluorescence increase. Exposure to 100 nM QDs led to apoptosis and down-regulation of CD80/86 pro-inflammatory markers (less than 50% of control). Red and green QDs have different uptake dynamics – smaller particles were internalized and digested faster. At 12 hours observation point highest uptake had green TGA-covered dots (>80 % of population) with significantly induced apoptosis (86% of FITC positive cells); green gelatinated particles showed mild toxicity (<4%) within 49% uptake. Red QDs demonstrated 42% and 53% internalizing for TGA-gelatin/ TGA-alone capping without affecting cell viability. Drastic changes recorded after 24 hours exposure – green QDs dropped fluorescent response down to 20%; red nano-crystals were fully ingested and formed bright swallowed structures.

Conclusion: We developed fast and simple strategy for semi-quantitative analysis of QDs internalizing, investigated uptake dynamics and cellular responses on population level. Red-emitting QDs are promising diagnostic tool due to reduced auto-fluorescence and prolonged intracellular remaining time. Green gelatinated QDs could be suitable as fast working cellular assays. Apoptosis depends on uptake rate; QDs size and composition are secondary parameters.

DRIVING THE BARRETT'S TO OESOPHAGEAL ADENOCARCINOMA SEQUENCE: IMPACT OF THE TISSUE MICROENVIRONMENT ON T-CELL PROFILE

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Background:
The premalignant lesion, Barrett's oesophagus (BO), is associated with an increased risk of oesophageal adenocarcinoma (OAC). BO is known to have a chronic inflammatory component and an important role for T-cells in the initiation of oesophageal inflammation has been identified. It is therefore important to delineate the role of T-cells at each disease stage to identify potential immunotherapeutic targets to halt disease progression.
The aim of this study was to examine T-cell profile in normal, BO and OAC tissue using *ex-vivo* patient samples and to examine factors released into the tissue microenvironment which may impact T-cell activation.
Methods:

1) Oesophageal biopsies were enzymatically digested for T-cell phenotyping by flow cytometry. Expression of CD4, CD8, CD45RO, CD62L and CD69 was examined.
2) Biopsies were cultured to generate tissue conditioned media (TCM) and levels of cytokines were assessed by ELISA.
3) PBMCs were pre-treated with supernatant from Het-1A, QH, GO and OE33 cell-lines. Cytotoxic activity was assessed by measuring CD107a expression by flow cytometry.

Results:
Infiltrating $CD4^+$ and $CD8^+$ T-cells decreased from normal to BO and OAC tissue. Expression of CD45RO significantly decreased from normal to OAC. A decrease was also observed CD69 expression. Levels of IL-6 significantly increased in BO compared to normal TCM. IL-6, GM-CSF, IL-8, IL-1β, TNF-α and IL-10 were significantly increased from BO to OAC. Levels of IL-12p70, IFN-γ and IL-2 were significantly increased in OAC TCM compared to control. Cytotoxic activity of $CD8^+$ cells significantly decreased following pre-treatment with BO (QH) and OAC (OE33) cell-line supernatant.
Conclusion:
These results suggest that T-cell recruitment and activation in diseased tissue may be diminished. The increase in IL-6 in BO suggests an inflammatory response possibly providing a driving force for disease progression. The presence of both pro- and anti-inflammatory cytokines in OAC may promote mixed T-cell populations, possibly creating an environment conducive to tumour development. The reduction in cytotoxic activity suggests that as the disease progresses the ability of these $CD8^+$ T-cells to kill malignant or premalignant tissue may be diminished again suggesting a diminished activation phenotype.

Monocytes and T Cells in Haemochromatosis

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Haemochromatosis (HH) is an iron overload disorder where too much dietary iron is absorbed through the intestine. The body has no way of removing the excess iron so it accumulates initially in the liver parenchymal cells and then in other organs. Studies have shown that $CD8^+$ T lymphocyte numbers can be correlated with the severity of clinical expression of HH. The aim of this study was to explore whether monocyte and T cell numbers are altered in HH patients and in particular which subsets were affected.
In this study, blood was taken from 300 HH patients and healthy volunteers then prepared according with EuroFlow Consortium SOPs and tested within 3 hours. To measure monocyte and T cell populations and differentiate their subsets the samples were stained according: monocytes - CD45 (PerCP Cy5.5), HLA-DR (PE), CD14 (APC), CD16 (FITC) and T cells – CD45 (PerCP Cy5.5), CD3 (FITC), CD4 (APC), CD8 (PE). The samples were acquired in the BD Accuri C6 flow cytometer and the analysis of the number of cells per ml of blood was performed with Infinicyt software (Cytognos).
In our results there was a significant decrease in the number of classical and non-classical monocytes, however there was no noticeable difference in intermediate monocytes. There was no significant difference found between T cell numbers although there was a trend of increasing cell numbers. The data also suggests a subset of HH patients have a significant increase in $CD8^+$ T cells. Furthermore elevated numbers of $CD4^-/CD8^-$ T cells were seen in some HH patient samples; these could be NK T cells, but this has to be determined. In conclusion HH patients' monocytes and T cells are altered in HH patients.

A novel method for glycoprofiling live cells using Lab in a Trench

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Lab in a Trench (LiaT) is a microfluidic system that allows repeated probing of cells captured in a shear-free environment. We have demonstrated a novel method of sequentially glycoprofiling live B-cells using LiaT and an epifluorescent microscope. Lectins are carbohydrate binding proteins that can identify specific glycans on a cell surface. Cells were captured with LiaT and incubated in appropriate buffer. Commercially available plant lectins were labelled with DyLight 488, introduced into the system and allowed time to bind to the cells. These lectins were then eluted using the appropriate free sugar and further lectins were used to probe the cell. Lab in a Trench analyses can be carried out in parallel, with the analysis of up to four trenches of cells at a time. Each trench captures between 5 and 30 cells. The compact platform is compatible with standard laboratory reagents and microscopes. Measurements of the cell fluorescence were taken at various timepoints after addition of the lectin probe and its elution by sugar. Images were analysed using ImageJ. Sequential images were overlaid to demonstrate localisation of the lectins and the glycans to which they bind. Image analysis allows semi-quantitative comparison of glycan density on cells. This is the first demonstration of sequential analysis of glycan distribution on the surface of live cells.

Human Vγ9Vδ2 T cells can selectively promote T_H1 or T_H2 responses via interactions with dendritic cells or B cells *in vitro*

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The Vγ9Vδ2 subset of human γδ T cells can induce maturation of dendritic cells (DC) into antigen-presenting cells (APC) and B cells into antibody-secreting plasma cells. Since B cells are capable of presenting antigens to T cells, we investigated if Vγ9Vδ2 T cells can influence antigen presentation by these cells. Vγ9Vδ2 T cells, B cells and monocytes were isolated from human blood. Vγ9Vδ2 T cells were expanded by phosphoantigen stimulation and monocytes were induced to differentiate into immature DC. Vγ9Vδ2 T cells were co-cultured with equal numbers of DC or B cells. Expression of APC markers, production of cytokines and antibodies, and stimulation of T cells by the DC or B cells was then measured using flow cytometry and ELISA. Vγ9Vδ2 T cells induced expression of CD40, CD86 and HLA-DR and the secretion of IFN-γ, IL-6 and TNF-α by DC. They augmented the ability of DC to stimulate proliferation and IFN-γ production by antigen-specific and alloreactive T cells. In contrast, Vγ9Vδ2 T cells induced CD86 and HLA-DR expression by B cells and they promoted the release of IL-4, IL-6, TNF-α, and IgG, IgA and IgM by B cells. B cells matured with Vγ9Vδ2 T cells stimulated proliferation but not cytokine secretion by conventional T cells. These data suggest that Vγ9Vδ2 T cells can induce maturation of DC and B cells into APCs, but while they prime DC to stimulate T_H1 responses, they prime B cells to stimulate T_H2 responses.

B lymphopoiesis in pregnant women

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B cell development and haematopoises are very complex processes. Associate this development to a normal pregnancy we have exceptional events happening in the human body. In mice, it is known that during pregnancy, B cell production is temporary interrupted due to a decrease in IL-7 availability (1) that is controlled by sex hormones and the reduction of IL-7 is enough to temporally reduce bone marrow B lymphopoiesis in mice. In man, however B lymphopoiesis does not seem to be IL-7 dependent.
Our aim was to determine if there is also a reduction in B lymphopoiesis in pregnant women and to look for different B cell subpopulations.

By flow cytometry, using a Beckman Coulter IntraPrep kit and an 8 colour staining protocol, we analyzed intracytoplasmically in plasmoblasts and memory B cells IgA1 (PE), IgG (FITC) and IgM (APC) immunoglobulin classes. To identify B cells and plasmoblasts we used CD19 (PE Cy7), CD20 (eFluor 450) and CD38 (APC eFluor 780). CD 38 and CD27 (PE Cy5) were used to distinguish immature, naïve and memory B cells.
Our preliminary results show that, contrary to what happens in mice, we did not observe a drastic reduction in immature B cells during any stage of human pregnancy. However comparing samples from pregnant women with non-pregnant controls, our results suggest that the percentage of IgA^+ and IgG^+ plasmoblast as well as memory IgM^+ is higher during pregnancy.
We still need to do further studies to identify, characterize and understand these changes. This is the first time that a systematic analysis of b lymphopoiesis has been carried out in human pregnancy. Our results indicate that unlike mice, B lymphopoiesis is not drastically perturbed during pregnancy.
(1) Nabil Bosco, Rhodri Ceredig and Antonius Rolink; "Transient decrease in interleukin-7 availability arrests B lymphopoiesis during pregnancy"; 2008; European Journal of immunology; 38: 381-390

Development of a flow cytometry-based potency assay for the immunomodulatory properties of Mesenchymal Stromal Cells

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MSC have been described as possessing immunomodulatory properties, having several effects on immune cells. However, there is a commercial need to develop reliable, rapid, quantifiable assays to assess the potency of allogenic human Bone Marrow Mesenchymal Stromal Cells (hBM MSC). For these propose, using flow cytometry, we developed a rapid screening whole blood assay. The aims of this assay are to determine the effects of hBM MSC on the production of TNF-α and IL-12 by LPS-stimulated monocytes.
A number of factors were considered prior to optimising the assay, including: 1) the type of anticoagulant (Heparin; EDTA or Citrate), 2) incubation time (4H, 6H, 8H and 24H), 3) blood dilution (1/2; 1/5; 1/10; 1/15; 1/20 and 1/30) and 4) LPS concentration (0.5ng/ml, 1ng/ml, 2ng/ml, 5ng/ml, 10ng/ml and 20ng/ml).
We used the IntraPrep kit as intracellular staining protocol from Beckman Coulter according to the manufacturer's instructions. We analysed the intracellular expression of TNF-α PE or IL-12/IL-23 p40 PE. After staining samples were acquired using the BD Accuri C6 four color flow cytometer.
Heparin was the best anticoagulant in terms of cell activation. This was because anticoagulants containing divalent cation chelating agents removed Ca^{2+} that was found necessary for optimal monocyte activation.
Detection of TNF-α production was optimal at 6H while IL-12 became optimally detectable by 24H. We detected optimal cytokine production using a 1/10 blood dilution and 1ng/mL of LPS.
Our results also show that hBM MSC have the capability of suppressing monocyte activation. Different ratios of Monocyte:MSC were used and after 6H incubation, we detected a dose-dependent suppression of TNF-α expression by monocytes at the higher concentrations of hBM MSC.
In conclusion, hBM MSC have the capacity to immunoregulate monocytes activation in a dose-dependent fashion, and we have established a rapid and quantifiable assay to determinate the effects of hBM MSC on monocytes.

Monocyte subpopulations express interferon lambda receptor.

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Background
Interferon lambda (IFN-λ) or Type III IFN is an anti-viral cytokine family important in the resolution of HCV infection [1]. The three subtypes of IFN-λ: IL-28A, IL-28B and IL-29, mediate their effects via the IL- 28R1/IL-10R2 receptor

complex. Unlike the receptor for IFN-α or IFN-β (Type I IFNs), IFN-λ receptor expression appears to be limited to certain populations of cells including epithelial cells and some immune cells.

Objectives

In order to identify immune cell subsets responsive to IFN lambda we examined IL- 28R1 and IL-10R2 receptor expression on NK cells, T cells as well as monocyte subpopulations in whole blood using flow cytometry.

Results

Whole blood samples were obtained from 9 healthy donors. Expression of the IL-28R1 and IL-10R2 receptor chains was examined on CD3+ T cells, CD56+ NK cells and CD14+ monocytes (n=3). T cells and NK cells showed no expression of IL-28R1 or IL-10R2. In contrast, CD14+ cells were positive for both IL- 28R1 and IL-10R2. In 6 healthy donors, classical (CD14++, CD16-) monocytes expressed an average % positivity for IL-28R1 of 48.5% and IL-10R2 of 81.8%. Intermediate (CD14++, CD16+) expressed an average % positivity of 70.4% and 88.9% for IL-28R1 and IL-10R2, respectively. Finally, non-classical (CD14+, CD16++) monocytes expressed an average % positivity of 33.4% for IL-28R1 and 96% for 1L-10R2.

Conclusion

Expression of the IFN-λ receptor by subpopulations of monocytes suggests they are capable of IFN-λ signalling *in vivo.* Elucidating the exact mechanisms of IFN-λ signalling is relevant in studying the immune response to HCV infection for the development of new therapeutics and vaccines.

References

1. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. (2009) Nature 461, 399–401.

From single cell to 'holoclone'? Prostate Cancer cells produce colonies of multiple discrete morphologies when single cell cloned by flow cytometry.

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In developed countries Prostate Cancer (PCa) is the most common malignancy among men. Many men with PCa are treated with androgen deprivation therapy but unfortunately up to 40% of these succumb to castrate resistant prostate cancer (CRPC) which has poor survival rates with a median survival of just 14 months. Within the tumour some cells become insenstive to androgens and these have been proposed to have stem cell properties. The cancer stem cell (CSC) hypothesis postulates that tumour masses may arise from a single cancer cell with stem-like characteristics and that these CSC's survive in the harsh tumour environment, display treatment resistance and are therefore are the origins of lethal metastatic disease. In this way CSCs have been implicated strongly in disease progression. To investigate the mechanisms of drug-resistance by CSC and to develop novel targetted therapies there have been a number of attempts to re-capitulate the formation or selection of prostate CSC by *in vitro* generation of 'holoclones'. Here, we sought to use 'flow cytometry' to single cell clone PCa cells to investigate their capacity to form 'holoclones' which may comprise of CSCs. Using a BD FACSAria IIIu Cell Sorter, androgen dependent LNCaP and androgen independent LNCaP-abl PCa cell lines were sorted into 96 well plates as single cells at 1 cell per well. The cells were then incubated for 2 weeks. We show that LNCaP cells single cell cloned in this way did not have the capacity to grow. In contrast, androgen-resistant LNCAP-abl cells (which more resemble CRPC) grew and produced colonies which we classified into three distinct morphologies (A, B and C). Morphology C colonies resembled 'holoclones' and hence potentially comprise prostate CSCs. To determine their self-renewal capacity (a defining feature of CSCs), cells from these distinct morphologies were re-plated and incubated for a further 2 weeks. When cells from each of the three morphologies were re-plated it was established that they possessed different capacities for self-renewal. Cells from colonies of morphology A were not able to self-renew while those of morphologies B and C were. Importantly, the number of morphology C colonies was increased substantially in this way providing a method by which potential prostate CSC's may be obtained for detailed molecular and phenotypic characterisation. Further work is needed to clarify whether the morphology classified here as C are indeed 'holoclones' and comprised of prostate CSCs. Clearly, a robust method to isolate potential prostate CSC's will support on-going detailed molecular analysis of their properties and the identification of molecular targets by which they may selected for destruction.

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Optimisation of synergic drug treatment via a dual gradient microfluidic chip

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Background: We present the usage of a newly designed microfluidics chip for the detection of synergistic effects of drug co-treatment. The chip can expose adherent cells to linear diffusion gradients that lie orthogonal to each other. It is suitable for live cell imaging. As primary model, we test the synergy of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and cycloheximide (CHX) in inducing apoptotic cell death in HeLa cells.
Methods: On-chip experiments were performed on a spinning disc microscope. One micromolar TO-PRO®-3 or 2 □g/ml propidium iodide were added for cell death detection. Resulting images were analysed via Image J. For comparison, cell response was analysed on an Accuri C6 platform via flow cytometry.
Results: Changes in cellular cell death response after TRAIL treatment as analysed by flow cytometry were strongest between 3 and 10 ng/ml TRAIL. Addition of CHX was effective at a concentration range of 0.3-1 □g/ml. When reproducing this concentration range on the dual gradient chip, cell death levels varied according to the chip's quadrants. Three hours after treatment start, cell death was highest in the quadrant of TRAIL and CHX co-treatment, followed by TRAIL-only (73.6 % and 46.7 % respectively). In contrast, cell death in the quadrants of CHX-only and Sham were very low (7.0 % and 3.5 %, respectively).
Conclusion: The dual gradient chip experiments successfully replicated the synergistic interaction of TRAIL and CHX–induced cell death. This dual gradient chip thus allows us to identify concentration profiles with an optimal synergistic treatment effect.

The effects of anti-TNF antibody therapy on blood monocytes in patients with inflammatory bowel disease

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Monocytes, recently classified as CD14++/CD16- (classical), CD14++/CD16+ (intermediate) and CD14+/CD16++ (non-classical), play an as-yet poorly characterized role in inflammatory bowel disease (IBD) pathogenesis. In this study, proportions and absolute numbers of circulating monocyte subsets were determined by flow cytometry of fresh PBMC samples from 4 groups: **A**. Healthy controls (n=11); **B**. Crohn's disease (n=17) and **C.** Ulcerative colitis patients not receiving infliximab (n=12) and **D**. IBD patients receiving infliximab (n=29). For Group D, samples were analyzed before and after anti-TNF-α infusions.
Total monocyte numbers were higher in Group B and C compared to groups A and D, (17.7±9.9 and 22.8±8.5; 15.1±6.1 and 19.0±10.8 $x10^4$ cell/ml respectively; $p<0.001$, one way ANOVA). Group D total monocytes were reduced to 7.3±4.9 $x10^4$ cells/ml following infliximab ($p<0.001$, paired t-test).
Subset analysis showed increased classical monocytes in ulcerative colitis patients compared to the other groups (Group A 8.6±3.6; Group B 10.2±7.1; Group C 13.7±5.7; Group D 6.7±5.2 pre-infliximab and 2.6±2.4 $x10^4$ cells/ml post-infliximab, $p<0.001$). Similar trends were present for the non-classical monocytes (Group A 10.8±6.2; Group B 12.5±13.0; Group C 15.6±12.8; Group D 10.2±8.5 pre-infliximab and 6.0±6.04$x10^3$ post-infliximab, $p<0.001$). The intermediate subset was increased in crohn's disease patients and reduced following infusion of infliximab (Group A 10.8±5.8; Group B 34.7±25.7; Group C 32.6±43.0; Group D 51.7.0±60.9 pre-infliximab and 17.2±19.0 $x10^3$ post-infliximab, $p<0.001$).
Total lymphocyte numbers were significantly reduced in Group D following infliximab therapy (pre 53.2±38.8 and post 26.2±26.5$x10^4$ cells/ml). However, Group D responded to infliximab with a significant increase in their granulocyte numbers (pre therapy 19.3±8.3 and post therapy 28.7±17.2 $x10^5$ cells/ml).
Freshly isolated PBMCs from Group A showed preferential binding of Infliximab to the Classical and Intermediate monocyte populations. Following anti-TNF therapy PBMCs from Group D show no increased intracellular cleaved caspase 3. Functional assays carried out on Group D also show blunted IL-12 production when stimulated with LPS following infliximab infusion in comparison to healthy controls.
Thus, inflammatory bowel disease is associated with an increase in total monocytes in the circulation compared to healthy controls and infliximab infusion leads to a significant reduction in total monocyte numbers and subsets but also lymphocyte counts. Granulocyte numbers however are significantly increased following this therapy. Infliximab

preferentially binds to particular subsets of PBMCs and following treatment, causes a blunt pro-inflammatory cytokine production by LPS stimulated monocytes.

Hypoxia Enhances the Radio-resistance of Mouse Mesenchymal Stromal Cells

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Mesenchymal stromal cells (MSCs) are radio-resistant bone marrow progenitors that support hematopoiesis and its re-constitution following total body irradiation. MSCs reside in hypoxic niches within the bone marrow and tumor microenvironments. The DNA Damage Response (DDR) represents a network of signaling pathways that enable cells to activate biological responses to DNA damaging agents. Hypoxia-mediated alterations in the DDR contribute to the increased radio-resistance of hypoxic cancer cells, limiting therapeutic efficacy. The DDR is important in mediating mouse MSC radio-resistance. However, the effects of hypoxia on MSC radio-resistance are currently unknown. We have shown that hypoxia (i) increases MSC proliferation rate and colony size; (ii) increases long-term survival post irradiation and (iii) improves MSC recovery from IR-induced cell cycle arrest, the latter by using flow cytometric techniques to study cell cycle progression. DNA DSB repair in MSCs was up-regulated in hypoxia, accelerating the resolution of highly genotoxic IR-induced DNA double-strand breaks (DSBs). In addition, HIF-1α was found to contribute to this enhanced DSB repair by regulating (i) the expression of DNA ligase IV and DNA-PK_{cs} and (ii) Rad51 foci formation in response to DNA DSBs in hypoxic MSCs. We have demonstrated, for the first time, that hypoxia enhances mouse MSC radio-resistance *in vitro*. These findings have important implications for our understanding of MSC functions in supporting allogeneic bone marrow transplantation and in tumorigenesis. We are currently using these techniques to investigate the DDR of cortical and medullary thymic stromal cell lines.

Human beta defensin-3 activates Langerhans cells and reverses the suppressive effect of Vitamin D on IL-23 expression in psoriasis

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Psoriasis is an immune-mediated inflammatory skin disorder. IL-23 plays a central role in psoriasis by directing the development of T helper (h) 17 cells which contribute substantially to disease. Beta defensins are small antimicrobial peptides which are chemotactic for a variety of immune cells and can participate in the initiation of the adaptive immune response. The expression of human beta defensin-3 (HBD3) is enhanced in psoriasis and it has been reported that HBD3 induces the maturation of DC and other innate immune cells by binding to Toll like receptor (TLR)-2. However, the effect of HBD3 on DC function in psoriasis is unknown. Here we demonstrate that the HBD3 enhanced the expression of TLR2 and induced IL-23 production by Langerhans cells (LC). The serum expression of HBD3 was enhanced in psoriasis and both epidermal and highly purified monocyte-derived LC from psoriasis patients produced significantly more IL-23 in response to photbol myristate acetate and zymosan activation respectively, compared with LC from healthy controls ($p<0.01$). Moreover, whilst vitamin D_3, 1,25-dihydroxyvitamin D_3(1,25 $(OH)_2D_3$) suppressed IL-23 by epidermal and monocyte derived LC from healthy controls ($p<0.01$) via IL-10 ($p<0.05$), addition of HBD3 abrogated this suppressive effect ($p<0.01$). Moreover, 1,25 $(OH)_2D_3$ failed to regulate IL-23 production by epidermal and monocyte derived LC from psoriasis patients. HBD3 inhibited the suppressive effect of 1,25 $(OH)_2D_3$ in psoriasis since neutralization of HBD3 enhanced the suppressive effect of 1,25 $(OH)_2D_3$ on IL-23 production by LC from psoriasis patients ($p<0.05$). This study suggests that HBD3 plays a pathogenic role in psoriasis by promoting the expression of IL-23 by LC and by interfering with the ability of 1,25 $(OH)_2D_3$ to regulate its expression. Thus, targeting HBD3 may increase the therapeutic efficacy of 1,25 $(OH)_2D_3$ in psoriasis.

Effects of Non-target dose on DNA Damage in Normal Prostate Cells

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Introduction: Advanced radiotherapy treatments utilise high intensity modulated fields to achieve a uniform dose to the target. However, the non-target dose received by surrounding healthy tissue is a cause for concern. Recent *in vitro* studies have demonstrated DNA damage in cells positioned in areas outside the primary treatment field following exposure to modulated radiation fields. However, whether these effects are directly caused by non-target radiation or bystander effects due to factors secreted by neighbouring irradiated cells is not fully understood. The aim of this study was to determine DNA damage levels in normal prostate cells positioned out of the target field following irradiation using a 6 MV photon beam, in the presence or absence of bystander factors.
Methods: Human prostate cancer cells (LNCap) and normal prostate cells (PNT1A) were irradiated simultaneously. Cells were placed in a specially designed phantom whereby prostate cancer cells were positioned in the centre of the radiation field with normal prostate cells 1 cm away from the field edge, mimicking a clinically relevant set up. A dose of 2 Gy was delivered to the prostate cancer cells with a 10x15 cm^2 field with a 6 MV photon beam using an Elekta Precise linac. The average dose to the normal cells was measured to be 0.2 ± 0.03 Gy using Gafchromic film. Radiation-induced double stranded DNA damage was determined using the phosphorylation of histone 2A (γH2AX) assay, detected by confocal microscopy and flow cytometry, while clonogenic assays determined post-irradiation cell survival. Experiments including normal cells treated with conditioned media collected from irradiated cancer cells were also performed to evaluate the role of bystander factors in the non-target area.
Results: As previously reported, a 2 Gy dose in field is sufficient to cause a dramatic increase in γH2AX foci in prostate cancer cells. Interestingly, exposure to non-target radiation induced γH2AX foci formation in normal prostate cells, even in the absence of cellular communication with cells irradiated in field. Additionally, a statistically significant reduction in cell survival of the normal prostate cells was observed post-irradiation, in the absence of cellular communication with cells irradiated in field.
Conclusion: This study shows that non-target radiation alone can cause significant early DNA damage in normal prostate cells. These results provide evidence that non-target effects in other healthy tissues need to be further investigated. A better understanding of the radiobiological response in normal cells outside the primary treatment field would assist in the planning of radiotherapy treatments.

The viability, permeabilisation and autolytic response of a cheese starter culture *Lactococcus lactis* subsp. *cremoris* AM2 to various salt levels measured using Flow Cytometry

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In this study the response of AM2 cheese starter culture to various salt treatments was investigated. Starter culture viability was evaluated using standard plate count method. Flow cytometry (FCM) following differential staining by SYTO9 and PI was used to determine sub-populations of live, permeabilised/dead cells. Cell autolysis was monitored by the measurement of released intracellular marker enzyme (LDH) during growth and also two weeks storage at 8 ^{o}C. Salt treatments applied in this study included 0, 0.9, 1.2, 1.8 and 3.0% NaCl (v/v), which corresponds to 0, 0.81, 1.62 and 2.7% salt-in-moisture (S/M) levels respectively. Treatment of starter culture with various salt concentrations has shown to impact culture viability, with considerable differences in viable counts and degrees of permeabilisation among various salt treatments. Higher viability was maintained at lower salt concentrations. However, significantly lower viability was noticeable for samples containing 3% (v/v) NaCl. FCM data revealed similar trends to those of a standard plate counts. Prior to salt additon, percentages of intact/live cells for all samples were between 85-90% with only 10 to 15% cells classified as dead or highly permeabilised. Number of live/ intact cells were relativelly high for 0% (v/v) salt addition rate where AM2 retained a high viability (~85- 90%) up to day 7 of storage, with a slight decrease to 75 % on day 14. FCM revealed no significant effect of 0.9 % NaCl (v/v) treatment on AM2 starter permeability. Further increase in salt concentration affected viability and permeabilisation of cheese culture. On day 1 of storage number of intact/live cells decreased for 1.2% and 1.8% salt addition level to 70 and 60%, respectively. The greatest decrease of intact/live cells was recorded after 3% (v/v) salt addition level. On day 1 of storage intact/live cells constituted 50% of total cell profile. Autolytic behaviour of AM2 was unaffected by above salt treatments as indicated by very low intracellular LDH release during growth and storage period. Overall a decrease in culture viability with increased permeabilisation after low salt treatments may suggests potential of AM2 for production of healthier cheese with reduced salt content.