1st Australian Innate Lymphocyte Symposium #AILS2015

12 June 2015
Walter and Eliza Hall Institute, Melbourne

Confirmed Speakers:
Gabrielle Belz
Andrew Brooks
Mariapia Degli-Esposti
Dale Godfrey
Phil Hansbro
Nick Huntington
Shaun McColl
Stephen Nutt
Mark Smyth
Sophie Ugolini
Eric Vivier
Wolfgang Weninger

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AILS2015 Program

8.30am: Arrival/Registration

8.45am: Welcome address.

Session 1 – The Menzies Foundation Session on Innate lymphocyte development

8.50am: Nick Huntington – Id2 governs NK cell homeostasis via IL-15 signalling.

9.05am: Cyril Seillet – Nfil3 drives the commitment of all ILCs

9.20am: Stephen Nutt – Peripheral Natural Killer Cell Maturation Depends On The Transcription Factors Aiolos and Blimp1

9.40am: Sophie Ugolini – Bcl2, What else?

10am: Gabrielle Belz – The role of IL-22 production by Ncr1+ ILC3 in intestine homeostasis

10.20am: Morning Tea.

Session 2 – ILCs in inflammation

10.45am: Eric Vivier – On Innate Lymphoid cell signature, plasticity and redundancy

11.10am: Wolfgang Weninger – IL-2 is a critical regulator of group 2 innate lymphoid cell function during pulmonary inflammation

11.35am: Phil Hansbro - Increased lung ILC1 and ILC3 in cigarette smoke-induced experimental chronic obstructive pulmonary disease

11.50am: Axel Kallies - The transcriptional regulator Blimp1 and its homologue Hobit cooperatively instruct tissue-residency of lymphocytes

12.05am: Mariapia Degli-Esposti – Trail mediated protection from auto-inflammation

Lunch 12.30pm

Session 3 – Innate-like T cells

1.30pm: Dale Godfrey – MR1-restricted T cell repertoire diversity engenders variable MR1-antigen recognition.

1.50pm: Liyen Loh - Anti-viral responses of human mucosal-associated invariant T cells to influenza-infected lung epithelial cells are modulated by monocytes.

2.05pm: Natalie Lister - Double-positive thymocytes select mucosal-associated invariant T cells
2.20pm: Shaun McColl – HOMEOSTATIC AND INFLAMMATORY MIGRATION OF $\gamma\delta$ T CELLS

2.40pm: Ben Roediger - IL-15-driven niche-filling by T cells and innate lymphoid cells in the murine epidermis

2.55pm: Andrew Brooks: Polymorphisms in KIR3DL1 dictate specificity for HLA-I allotypes

3.15pm: Afternoon Tea

**Session 4 – NK cell function**

3.40pm: Mark Smyth - Receptors and cytokines that control NK cell anti-tumor activity

4.00pm: Jai Rautela - The role of innate immunity in the treatment of metastatic breast cancer

4.15pm: Camille Guillerey - DNAM-1 controls immune responses against multiple myeloma.

4.30pm: Joanne Davis - A radio-resistant perforin-expressing lymphoid population controls allogeneic T cell engraftment, activation and onset of GVHD in mice.

4.45pm: Tessa Campbell - Manipulation of NK and NKT Cell Responses by Varicella Zoster Virus

5.00pm: Poster session/Drinks

7.00pm: End
Session 1 – The Menzies Foundation Session on Innate lymphocyte development

Id2 governs NK cell homeostasis by tuning their sensitivity to IL-15
Nicholas D Huntington
The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia and Department of Medical Biology, University of Melbourne, Victoria 3010 Australia.

The inhibitor of DNA binding 2 (Id2) is essential for NK cell and ILC development with its canonical role being to antagonize E-proteins and block T and B cell commitment. However, how this inhibitory function of Id2 promotes innate lymphocyte development and homeostasis remains enigmatic. Here we identify a key role for Id2 in regulating the threshold for IL-15 receptor signaling and subsequent homeostasis of NK cells by repressing the E-protein target gene Socs3. Deletion of Id2 in mature NK cells was incompatible with their survival and homeostatic proliferation. We demonstrate that this was due to impaired IL-15 receptor signaling. Remarkably, Id2-null NK cell homeostasis could be fully rescued in vivo by super-physiological IL-15 receptor stimulation or by genetic ablation of Socs3 in NK cells. Our results demonstrate that the interplay between Id2 and E-proteins modulates IL-15 receptor signaling to program NK cell development and homeostasis.

Transcriptional control of the innate lymphoid cell subsets development
Cyril Seillet¹, Lucille C Rankin¹, Joanna R Groom¹, Lisa A Mielke¹, Julie Tellier¹, Michael Chopin¹, Nicholas D Huntington¹, Gabrielle T Belz¹ & Sebastian Carotta¹
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Innate lymphoid cell (ILC) populations protect against infection and are essential for lymphoid tissue formation and tissue remodelling following damage. Nfil3 is implicated in the function of adaptive immune lineages and natural killer (NK) cell development but it is not yet known if Nfil3 regulates other innate lymphoid lineages. Here we show that all ILC subsets exhibited high Nfil3 expression and genetic deletion of Nfil3 severely compromised the development of all subsets. As ILCs play important roles in immune mucosal protection, we next sought to determine whether loss of ILC subsets that accompanies Nfil3 deficiency influences the capacity to mount protection in the lung and gut. Subsequently, Nfil3−/− mice were highly susceptible to protease allergen-induced inflammation or bacterial infection. All ILC derived from the common lymphoid progenitor (CLP) and adoptive transfer of Nfil3-deficient CLP failed to efficiently give rise to any ILC subsets suggesting that Nfil3 confers the ability of bone marrow progenitors to initiate ILC commitment. Thus, we demonstrate that Nfil3 is a key regulator of the development of bone marrow-derived ILC subsets essential for immune protection in the lung and gut.

Peripheral Natural Killer Cell Maturation Depends On The Transcription Factors Aiolos and Blimp1
Stephen Nutt
The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria, 3052, Australia.

Natural killer cells are an innate lymphoid cell lineage characterized by their capacity to provide rapid effector functions, including cytokine production and cytotoxicity. We have identified the Ikaros family member, Aiolos and the transcriptional repressor, Blimp1, as regulators of NK cell maturation. While the expression of Aiolos and Blimp1 is initiated early in NK cell ontogeny, both factors are required for the maturation in the spleen of CD11bhighCD27− NK cells. The differentiation block was intrinsic to the NK cell lineage and resembled that found in mice lacking T-bet, however, genetic analysis revealed that each factor acts independently. NK cells lacking Aiolos or Blimp1 are hyper-reactive to a variety of NK cell mediated tumor models, yet impaired in controlling viral infection, suggesting a possible regulatory function for CD27− NK cells in balancing these two arms of the immune response. These data place Aiolos and Blimp1 in the emerging gene regulatory network controlling NK cell maturation.
Bcl2 controls Natural Killer cell homeostasis by regulating cell death and proliferation

Charlotte Viant 1, Sophie Guia 1, Claire Bernat1, Eric Vivier 1, Nicholas Huntington 2 and Sophie Ugolini 1
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Natural killer (NK) cells are cytotoxic innate lymphoid cells (ILC) with important anti-microbial and anti-tumoral functions. We set up a genetic screen based on random germline mutagenesis using ENU (N-ethyl-N-nitrosourea) to identify genes involved in NK cell development and functions. A new mouse mutant with profound defect in NK cell homeostasis was generated and the causative hypomorphic mutation was identified in the Bcl2 gene. The analysis of this mouse mutant and the Ncr1-mediated deletion (Ncr1-Cre) of Bcl2 revealed a non-redundant intrinsic requirement for Bcl2 in the survival of NK cells in vivo. In both mouse models, the lymphopenia was more drastic in the more mature subset of NK cells. These data show that Bcl2 like the other anti-apoptotic protein Mcl1 is required to promote NK cell survival at steady state. However, in contrast to Mcl1-deficient NK cells, an enhanced survival and proliferation was observed for Bcl2-deficient NK cells upon in vivo IL-2 treatment. This IL-2-induced NK cell expansion was associated with an increase in Mcl1 expression in bcl2-deficient NK cells suggesting that Mcl1 can compensate Bcl2 deficiency in inflammatory conditions. Finally, we found that the survival defect in bcl2-deficient NK cell was associated with a higher proliferation capacity of the cells both in vitro and in vivo. Taken together, these data indicate that Bcl2 controls NK cell homeostasis by regulating cell death and proliferation.

The Innate Lymphoid Cell Network: Plasticity, Redundancy and Complementarity

Lucille Rankin1,2, Mathilde Giraud-Madoux1, Sophie Ugolini1, Nicholas Huntington1,2, Eric Vivier3, Gabrielle Belz1,2
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Innate lymphoid cells are an expanding family of lymphocytes that are prominently represented at mucosal surfaces and have been significantly implicated in the protection of epithelial barriers. Their precise role during immune protection is not yet clear. We have investigated how the transcription factor T-bet drives and maintains the identity of ILC3 that express the NKp46 natural cytotoxicity receptor (NCR) and have revealed that continuous T-bet expression is necessary for NCR+ ILC3 identity. Furthermore, conditional deletion of the key ILC3 genes Stat3, Il22, Tbx21 and Mcl1 demonstrated unexpectedly that NCR+ ILC3 were redundant for the control of colonic infections in the presence of T cells. Nevertheless, NCR- ILC3 were essential to maintain immune homeostasis, particularly in the caecum suggesting that T cells and ILC3 collaborate to form a fail-safe switch that is necessary for gut homeostasis.

Session 2 – ILCs in inflammation

On Innate Lymphoid cell signature, plasticity and redundancy

Eric Vivier & Sophie Ugolini
Centre d’Immunologie de Marseille-Luminy, Marseille, France

ILCs are a new type of lymphocyte, and their study is an emerging field in immunology that is having a major effect on our understanding of immune responses. However, much remains to be understood regarding the role of ILCs in humans and mice, particularly given the diversity of ILCs, which adds to the complexity of their analysis. ILCs can be classified into cytotoxic ILCs, such as NK cells, and helper-like ILCs, such as the ILC1, ILC2 and ILC3 subsets. The study of ILC2 cells has progressed considerably in recent years, due particularly to the development of mouse models selectively targeting these cells, such as RORa-deficient mice 2. By contrast, much remains unknown about the respective roles of NK, ILC1 and ILC3 cells. NK cells, ILC1 and NCR+ILC3 are known to express Nkp46 in humans and mice. We will present here recent findings on the transcriptomic signature of Nkp46+ ILCs, on their plasticity as well as on their role in natura.
Interleukin 2 is a critical regulator of group 2 innate lymphoid cell function during pulmonary inflammation

Ben Roediger¹,², Ryan Kyle¹, Szun S. Tay¹,², Andrew J. Mitchell¹,², Holly A. Bolton¹,², Thomas V. Guy¹,², Sioh-Yang Tan¹,², Elizabeth Forbes-Blom², Philip L. Tong¹,²,³, Yasmin Köller³, Elena Shklovskaya¹,², Makio Iwashima⁴,⁷, Kathy D. McCoy⁵, Graham Le Gros³,⁸, Barbara Fazekas de St. Groth¹,²,⁹ & Wolfgang Weninger¹,²,⁴,⁹

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Group 2 innate lymphoid (ILC2) cells have been implicated in the pathogenesis of allergic lung diseases, including bronchial asthma and bronchial hyper-responsiveness accompanying viral infections. The factors regulating the activity of ILC2 cells in these conditions are poorly defined. We show here that the cytokine interleukin-2 (IL-2) drives ILC2 cell expansion and production of IL-5 and IL-13, and is essential for the development of eosinophilic crystalline pneumonia (ECP), an idiopathic type 2 inflammatory lung disease in mice. We demonstrate that CD2⁺ ILC3 cells are an innate immune source of IL-2, and that ILC3 cells form clusters with ILC2 cells in the lung parenchyma. Finally, type 2 cytokine production by ILC2 cells during viral lung infection is critically dependent on IL-2. Together, our results reveal innate cell-derived IL-2 as a major driver of pulmonary type 2 pathology.

Increased lung ILC1 and ILC3 in cigarette smoke-induced experimental chronic obstructive pulmonary disease

Malcolm Starkey¹, Maximillian Plank¹, Prema Mono Nair¹, Tatt Jhong Haw¹, Paul Foster¹ and Philip M. Hansbro¹

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INTRODUCTION Chronic obstructive pulmonary disease (COPD) is the 3rd commonest global cause of mortality and there are no broadly effective therapies. It is often cause by cigarette smoking and is characterised by progressive chronic lung inflammation, emphysematous changes and lung function decline. The underlying mechanisms and immune responses that underpin COPD are incompletely understood. AIM To profile innate lymphoid cells (ILCs) in the lung in cigarette smoke (CS)-induced experimental COPD. METHODS Lung ILC subsets were characterised after 8 weeks of CS exposure that induces experimental COPD in wild-type (WT) C57BL/6 mice. In other experiments WT or RAG-deficient (RAG⁻) mice were subjected to 8-week CS. Hallmark features of COPD were assessed (pulmonary inflammation, emphysema and lung function). RESULTS Chronic CS-exposure increased the numbers of ILC1 (Lineage⁻CD90.2⁺CD45⁻IL-7Rα⁻T-bet⁻) and ILC3 (Lineage⁻CD90.2⁺CD45⁻IL-7Rα⁻Roryt⁻), but had no effect on ILC2 (Lineage⁻CD90.2⁺CD45⁻IL-7Rα⁻T-bet⁻Roryt⁻GATA3⁺ or Lineage⁻CD90.2⁺CD45⁻IL-7Rα⁻CD2⁺CD25⁻ST2⁺) in the lung of WT mice compared to normal air-exposed controls. CS also increased NK cell (CD45⁻T-bet⁻CD2⁻NK1.1⁻NKp46⁻) numbers in the lung. Chronic CS-exposure increased pulmonary inflammation, induced emphysema-like alveolar enlargement and impaired lung function (increased lung volumes, compliance and airway resistance). All these features were unaltered in RAG⁻ mice, suggesting a lack of adaptive immune cell involvement. CONCLUSION Our study is the first to show that ILCs are altered in CS-induced experimental COPD and that disease develops in the absence of adaptive immunity. This warrants further investigation into the role of ILC subsets in the pathogenesis of COPD.

The transcriptional regulator Blimp1 and its homologue Hobit cooperatively instruct tissue-residency of lymphocytes

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A novel immunoregulatory role of NK cells in non-lymphoid tissues: balancing anti-viral immunity and autoimmunity

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Natural killer (NK) cells are a prototypical component of innate immunity, known for promptly mediating cytotoxic and cytokine responses in settings of viral infection and malignancy. More recently, additional functions of NK cells have been recognized, including their role in immunoregulation. Although there has been a growing appreciation of the influence of NK cells on adaptive immune responses, in the settings examined to date, the physiological purpose of these activities has remained unclear, as they do not provide a survival advantage. We have recently defined a novel interaction between NK cells and CD4+ T cells during chronic murine cytomegalovirus (MCMV) infection in vivo. This interaction occurs in a nonlymphoid tissue, specifically involves TRAIL-expressing NK cells, and results in the elimination of activated CD4+ T cells. We show that through these interactions NK cells limit anti-viral immunity to curb the occurrence of autoimmunity.

Our studies provide novel evidence for the involvement of NK cells in the development of autoimmunity, and define the molecular interactions required for NK cells to balance the efficacy of protective adaptive immune responses with the risk of developing an autoimmune disease. These studies highlight a novel physiological purpose for the regulatory activity of NK cells.

Session 3 – Innate-like T cells

MR1-restricted T cell repertoire diversity engenders variable MR1-antigen recognition.

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Mucosal-associated invariant T (MAIT) cells are defined by expression of Vα7.2 (TRAV1-2)T-cell receptors (TCRs) that recognise riboflavin (Vitamin B2)-derivative antigens (Ag)s presented by MR1. Whether the MR1-restricted T cells repertoire extends beyond these cells is unknown. Here, we describe populations of highly diverse MR1-restricted human T cell populations encompassing TRAV1-2 and TRAV1-2 MR1-restricted T cells, some of which exhibited MR1 autoreactivity or reactivity towards folate (Vitamin B9)-derivative ligands. The TRAV1-2 cells utilised a broad range of TCRα and β chain genes suggesting that these cells may be able to recognise a diverse range of antigens presented by MR1. Structural analysis revealed that the autoreactivity and folate-derivative reactivity of TRAV1-2 TCRs was attributable to CDR3 loop-mediated effects. Furthermore, a TRAV1-2 TCR docked more centrally on MR1 in comparison to the TRAV1-2 TCRs, thereby adopting a markedly different molecular footprint. Taken together, diversity within the MR1-restricted T-cell repertoire leads to differential Ag recognition and functional responsiveness to MR1 and associated small molecule derivatives. This has important implications for the potential role for understanding MR1 restricted T cells and the role that they might play in non-microbial-based disease.

Anti-viral responses of human mucosal-associated invariant T cells to influenza-infected lung epithelial cells are modulated by monocytes.

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Non-classical innate T cells are rapid producers of inflammatory mediators that contribute to the cytokine milieu and modulation of other immune cell types in microbial infections. More recently, a new subset of MHC-Class 1 related (MR1)-restricted mucosal-associated invariant T cells (MAIT) has been identified. These lymphocytes comprise 1-10% of human CD3+ cells, and have potent anti-microbial properties. However, their role in viral infections remains unclear. We utilized an in vitro co-culture system of Influenza A Virus-infected (IAV-H1N1) human lung epithelial cells (A549) and human peripheral blood mononuclear cells (PBMC) with intracellular cytokine staining to assess the anti-viral effects of MAIT cells. We have demonstrated, for the first time, activation of MAIT cells (CD161+Vα7.2+ or MR1-5-OP-RU’Vα7.2+) in PBMC during co-culture with IAV-infected human lung epithelial cells. IFNγ production by MAIT cells was robust, although varied between donors (2-27% IFNγ+ N=9). Upregulation of CD69 was also observed on MAIT cells after IAV stimulation. In contrast, co-culture of PBMCs with mock-infected lung epithelial cells did not activate MAIT cells. The activation of MAIT cells was independent of MR1, given that the addition of anti-MR1 (clone 26.5) did not abrogate IFNγ production. However, depletion of CD14+ monocytes resulted in a significant reduction in IFNγ production by MAIT cells (N=5 P=0.01). This in part might be mediated by soluble factors produced by monocytes, given that an increase in TNF production was observed in CD14+ monocytes co-cultured with IAV-infected lung epithelial cells (N=4 P<0.002). Overall, our data demonstrate the capacity for MAIT cells to be activated by IAV in the presence of innate accessory cells and highlight their potential to contribute to universal immunity in influenza-infected individuals.

Double-positive thymocytes select mucosal-associated invariant T cells

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NKT and mucosal-associated invariant T (MAIT) cells express semi-invariant TCR and restriction by nonclassical MHC class I b molecules. Despite common features, the respective development of NKT and MAIT subsets is distinct. NKTs proliferate extensively and acquire effector properties prior to thymic export. MAIT cells exit the thymus as naive cells and acquire an effector/memory phenotype in a process requiring both commensal flora and B cells. During thymic development, NKTs are selected by CD1d-expressing cortical thymocytes; however, the hematopoietic cell type responsible for MAIT cell selection remains unresolved. Using reaggregated thymic organ culture and bone marrow chimeras, we demonstrate that positive selection of mouse iVα19 transgenic and Vβ8 transgenic MAIT cell progenitors requires MHC-related 1-expressing CD4+(+)CD8(+) double positive thymocytes, whereas thymic B cells, macrophages, and dendritic cell subsets are dispensable. Preincubation of double positive thymocytes with exogenous bacterial ligand increases MHC-related 1 surface expression and enhances mature MAIT cell activation in the in vitro cocultures. The revelation of a common cell type for the selection of both NKT and MAIT subsets raises questions about the mechanisms underlying acquisition of their specific features.
HOMEOSTATIC AND INFLAMMATORY MIGRATION OF γδT CELLS

Duncan R McKenzie, Ervin E. Kara, Iain Comerford and Shaun R. McColl
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Interleukin 17-producing γδ T cells (γδT17 cells) are innate-like lymphocytes that reside in mucocutaneous sites where they rapidly promote inflammation during bacterial and fungal infections. These cells also exist in secondary lymphoid organs and are recruited to inflammatory sites such as the central nervous system (CNS) during experimental autoimmune encephalomyelitis (EAE), and into tumours. Chemokine receptors are key mediators of selective leukocyte trafficking, yet the chemokine receptor-based migration of γδT17 cells is poorly understood. We demonstrate that γδT17 cells co-express functional CCR6 and CCR2, both of which are strongly implicated in EAE pathogenesis. However, recruitment of γδT17 cells into the CNS during early EAE was inhibited by deficiency of CCR2 but not CCR6. Further investigation revealed that γδT17 cells downregulate CCR6 upon activation in vitro and in vivo. In contrast, CCR6 but not CCR2 is an important regulator of γδT17 cell homeostasis, particularly in localisation to the dermis. We propose that CCR6 controls homeostatic migration while CCR2 alone drives inflammatory trafficking of γδT17 cells.

IL-15-driven niche-filling by T cells and innate lymphoid cells in the murine epidermis

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The epidermis in mice harbours a unique population of CD90+CD3+TCR Vγ5+ dendritic epidermal T cells (DETC) that are exclusive to rodents but not humans. DETC are generated from the thymus during embryonic development, occupy the epidermis at embryonic day 14 and expand in situ during the neonatal period. In TCRR5+ mice, which lack Vγ5+ DETC, the epidermis is populated by a polyclonal population of TCR aβ+ T cells that are predominantly CD4+CD8−, occasionally CD8+CD4+ but never CD8+CD4+. In RAG−/− and TCRR5−/−TCRδ−/− double knockout mice, which lack T cells, the epidermis becomes populated by CD90+CD3−CD2+ innate lymphoid cells (ILC). Vγ5 DETC, TCR aβ+ T cells and ILC are all absent from the epidermis of IL-15-deficient mice, indicating an essential role for this cytokine in lymphocyte occupation of the mouse epidermis. Further to this, we demonstrate ILC occupation in newborn mice and their replacement by DETC during the neonatal period in wild-type mice. Collectively, our results demonstrate that the murine epidermis provides a unique niche for IL-15-dependent lymphocytes under steady-state and post-inflammatory conditions, and whose occupancy is governed on a hierarchical basis.

Polymorphisms in KIR3DL1 dictate specificity for HLA-I allotypes

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The interaction between the Killer cell Ig-like Receptor 3DL1 (KIR3DL1) on Natural Killer cells and HLA class I molecules carrying the Bw4 epitope on target cells forms one of the key inhibitory receptor/ligand pairs of the NK cell immune response. Although the pivotal contacts between KIR3DL1 and HLA-Bw4 have been identified, the inhibitory capacity of given KIR3DL1 and HLA-Bw4 pairs differs, owing to the immense polymorphism of HLA-I molecules bearing the Bw4 motif and additionally, within KIR3DL1 itself. To better understand how polymorphism in KIR3DL1 impacts on specificity, we generated recombinant forms of KIR3DL1*005 and *015, representative KIR from each of the two inhibitory KIR3DL1 lineages, as well as the interlineage recombinant KIR3DL1*001. The binding of these recombinant KIR3DL1 molecules to a comprehensive panel of HLA class I molecules revealed distinct and different hierarchies of preferred HLA-Bw4 ligands for each KIR3DL1 allotype, with KIR3DL1*005 having a wider array of potential ligands than either the KIR3DL1*015 or *001 allotypes. These differences in binding were also reflected functional assays utilising NK cell clones expressing specific KIR3DL1 allotypes. Notably target cell lysis by clones expressing KIR3DL1*005 was markedly inhibited by the expression of HLA-A*24:02 and HLA-B*27:05 whereas these same HLA-I alleles had limited impact on clones expressing KIR3DL1*015 or *001. Finally we determined the crystal structures of KIR3DL1*005, *015 and *001 in complex with their ligands and showed that the broader specificity of KIR3DL1*005 was associated with the altered positioning of the D1 domain relative to the D2 domain as dictated by residue 283. Taken
together the data provide a rational basis to further refine our understanding of licensing and missing self models of NK cell activation in the context of infection and transplantation.

Session 4 – NK cell function

Receptors and cytokines that control NK cell anti-tumor activity

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Natural killer (NK) cells comprise a heterogeneous population of cells important for pathogen defense and cancer surveillance. However, the functional significance of this diversity is not fully understood. Here, we demonstrate through transcriptional profiling and functional studies that the activating receptor DNAM-1 (CD226) identifies two distinct NK cell functional subsets: DNAM-1+ and DNAM-1− NK cells. DNAM-1+ NK cells produce high levels of inflammatory cytokines, have enhanced Interleukin 15 signaling and proliferate vigorously. By contrast, DNAM-1− NK cells that differentiate from DNAM-1+ NK cells, have greater expression of NK cell receptor related genes and are higher producers of MIP1 chemokines. Collectively, our data reveal the existence of a functional program of NK cell maturation controlled through DNAM-1 expression. Using Vκ+MYC transgenic mice that spontaneously develop multiple myeloma (MM), we provided an in vivo demonstration that the immune system plays a critical role in the control of MM progression and response to treatment. By monitoring Vκ+MYC mice crossed with Cd226 mutant mice over a period of three years, we found that CD226 limits spontaneous MM development. We demonstrated that the CD226-dependent anti-myeloma immune response was mediated by both NK and CD8+ T cells through perforin and IFNγ pathways. Finally, we observed that standard drugs used in the management of MM patients, cyclophosphamide and bortezomib, required CD226 for optimal anti-myeloma efficacy and that anti-CD137 (4-1BB) monoclonal antibody exerted strong anti-myeloma activity. Progressive immunosuppression is associated with MM progression and strategies that aim to increase immune functions such as anti-CD137 monoclonal antibodies may have important therapeutic implications in MM.

The role of innate immunity in the treatment of metastatic breast cancer

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The long-term survival rate for women with triple negative breast cancer remains poor. Despite commonly displaying high levels of immunologically infiltrate, this particularly rampant subtype of the disease clearly evades immune control. Our previous data suggest that the suppression of tumour derived type-I interferon signaling may contribute to these mechanisms of immune evasion. The type-I interferons drive many aspects of innate and adaptive immunity, however, we hypothesized that innate effector cells may be optimally placed to respond to disseminated cells during the process of metastasis. Studies with our immune-competent models of metastatic breast cancer have revealed that the induction of a host-derived type-I interferon response can strongly suppress metastatic spread. In particular, we show that the use of the viral mimetic, Poly(I:C), potently activates the host NK cell population and confers the ability to eliminate breast tumour cells and prolong metastasis-free survival. The importance of these innate lymphocytes is further underscored by data showing enhanced rates of lung metastasis in animals lacking the NK cell population. Work on the therapeutic window for these interferon-based agents suggests that neo-adjuvant treatment is most efficacious in limiting breast cancer progression. We propose that along with our biomarkers that identify patients at risk of metastatic disease, therapies aimed at transiently activating the NK cell population may be a valuable anti-metastatic strategy in the clinic.
D Nadam-1 controls immune responses against multiple myeloma.

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Multiple myeloma is a blood cancer that arises in the bone marrow from malignant plasma cells. Although treatments have dramatically improved during the last decade, multiple myeloma remains an incurable disease responsible for more than 800 deaths per year in Australia. We investigated the role of DNAM-1 (CD226), a co-stimulatory molecule expressed by T cells and NK cells, in controlling multiple myeloma. Using transgenic mice that spontaneously develop multiple myeloma, we established that DNAM-1 limits tumour progression. Subsequent experiments demonstrated that both CD8 T cells and NK cells control the disease through IFN-γ- and perforin-dependent mechanisms. We showed that protection relies on DNAM-1 interaction with its ligand CD155 expressed on tumour cells. We also demonstrated that multiple myeloma development is associated with progressive immunosuppression; CD8 T cells and NK cells numbers are reduced, as is the expression of DNAM-1 on these cells. Interestingly, we found that DNAM-1 is necessary for optimal efficacy of cyclophosphamide and bortezomib, two chemotherapeutics currently used to treat multiple myeloma patients. Finally, we established that anti-CD137 mAbs therapy efficiently protects mice against multiple myeloma. Although, functional DNAM-1 was found dispensable for optimal efficacy of anti-CD137 mAbs, NK cells and CD8 T cells were required for its therapeutic effects. In conclusion, this work demonstrates for the first time the crucial role of DNAM-1 in controlling multiple myeloma and highlights the therapeutic potential of immunostimulatory strategies to treat this malignancy.

A radio-resistant perforin-expressing lymphoid population controls allogeneic T cell engraftment, activation and onset of GVHD in mice.

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Haematopoietic stem cell transplantation (HSCT) is an effective therapy for a number of blood cancers including leukaemia and lymphoma, by reconstituting a patient’s immune system with donor immune cells that mediate a graft versus tumour (GVT) effect. Immunosuppressive pre-transplantation conditioning is essential for donor cell engraftment in allogeneic BMT. The role of residual post-conditioning recipient immunity in determining engraftment is poorly understood. We examined the role of recipient perforin in the kinetics of donor cell engraftment. HLA-mismatched BMT mouse models demonstrated that both the rate and proportion of donor lymphoid cell engraftment, and expansion of effector memory donor T cells in both spleen and BM were significantly increased within 5-7 days post BMT in perforin-deficient (pfn−/−) recipients, compared with wild-type (WT). In WT recipients, depletion of natural killer (NK) cells prior to BMT enhanced donor lymphoid cell engraftment to that seen in pfn−/− recipients. This demonstrated that a perforin-dependent, NK-mediated, host-versus-graft (HVG) effect limits the rate of donor engraftment and T cell activation. Radiation-resistant NKT cells survived in the BM of lethally irradiated mice and may drive NK cell activation, resulting in the HVG effect. Furthermore, reduced pre-transplant irradiation doses in pfn−/− recipients permitted long-term donor lymphoid cell engraftment. These findings suggest that suppression of perforin activity or selective depletion of recipient NK cells prior to BMT could be utilized to improve donor stem cell engraftment in turn allowing for the reduction of pre-transplant conditioning.
Manipulation of NK and NKT Cell Responses by Varicella Zoster Virus

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Clinical evidence supports a crucial role for NK and NKT cells in the control of varicella zoster virus (VZV), the alphaherpesvirus responsible for varicella (chickenpox) and herpes zoster (shingles). It is accepted that extensive manipulation of the immune response occurs in order for productive infection to be established; however, despite their potent anti-viral capacity, research into how VZV interacts with NK and NKT cells remains surprisingly limited. Analysis by flow cytometry and immunofluorescence has revealed the first evidence that human NK and NKT cells are highly permissive to VZV infection. Crucially, both cell types were able to pass on infection to surrounding cells, suggesting a possible role for these innate lymphocytes in the dissemination of virus during host infection. A marked increase in the percentage of infected NK and NKT cells was also observed in the presence of interleukin 2, which is typically elevated during infection. Additionally, we have assessed the activation of NK cells in contact with VZV-infected target cells through detection of the degranulation marker, CD107a, which revealed restriction of NK cell activation. In complementary studies, investigation of NKG2D ligand modulation in VZV infected epithelial cells revealed significant decreases in cell-surface expression. Intriguingly, comparison with the closely related herpes simplex virus type 1 (HSV-1), revealed a strikingly different pattern of NKG2D ligand regulation. Our findings begin to unravel the complex interactions between VZV infection and the innate lymphocyte response.

Posters

Tcf-1 controls innate lymphoid cell differentiation and promotes lung immunity.
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Innate lymphoid cell (ILC) populations play a central role in conferring protective immunity at the mucosal frontier. We demonstrate that Transcription factor 7 (Tcf7, which encodes Tcf-1), a transcription factor important for T cell differentiation, is also expressed by all ILC subsets. Tcf-1 was intrinsically required for the differentiation of ILC1, ILC2 and NKp46+ ILC3 but not natural killer cells and NKp46+ ILC3. Of the remaining ILC2 in Tcf-1-deficient mice, expression of the IL-33R was consistently reduced, whereas other cell surface molecules commonly expressed on ILC2 remained unchanged, including Klrk1 and Icos. These results suggest that Tcf-1 not only influences development of ILC2 but also controls ILC2 function. Loss of Tcf-1 impaired inflammatory cytokine production IL-5 and IL-13 and resulted in crippled lung immunity in response to treatment with papain. In addition to Tcf-1, Gata3 and Rorcx play an important role in ILC2 development and function. Gene expression analysis revealed that both Gata3 and Rorcx expression were decreased in the absence of Tcf-1 suggesting that Tcf-1 is a critical factor in programing the ILC2 lineage.

Antibody-dependent cellular cytotoxicity against cells latently infected with HIV
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Introduction:
Current treatments for HIV infection are life-long as they do not diminish latent replication-competent HIV in long-lived resting CD4+ T cells. A major approach to an HIV cure is to reactivate the integrated HIV genome from latency and subsequently eliminate the cells harbouring reactivated HIV. We hypothesised that antibody-dependent cellular cytotoxicity (ADCC) could be a possible immune response to kill reactivated latently infected cells.

Methods:
We established in vitro assays to measure antibody-mediated killing by modifying the LDH-release cytotoxicity assay and the primary NK cell activation assay. The latently infected ACH-2 T cell line was reactivated using phytohaemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA). HIV reactivation was confirmed via intracellular p24 staining.

Results:
A CD4+ T cell line (CEM.NKr-CCR5 cells) pulsed with the HIV envelope glycoprotein gp120 and a chronically HIV-infected T cell line (8E5/LAV cells) elicited high levels of antibody-mediated NK cell activation and were susceptible to ADCC-mediated killing. However, we found that reactivated latently infected ACH-2 cells, though eliciting HIV-specific antibody-mediated NK cell activation, were not susceptible to ADCC-mediated killing. The reactivated cells expressed high levels of gp120 (as high as or higher than gp120-pulsed cells), but did not express CD4, likely due to down-modulation by the HIV accessory proteins Vpu and Nef.

Conclusion:
Our studies suggest that reduction in CD4-induced antibody epitopes at least partially protects reactivated latent infected cells from ADCC. These studies need to be confirmed in primary cell models of latency and in vivo studies. Future studies have to assess whether inhibition of Vpu and/or
Nef can render reactivated latently infected cells susceptible to ADCC-killing. Our results highlight a previously under-appreciated problem for the proposition that ADCC antibodies can assist in an HIV cure.

The structure of the TIGIT-Nectin2 interaction
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Natural killer (NK) cells constitute a crucial arm of the innate immune response and as such are the front line defence against viral infections. NK cell function is dictated by signals received via a wide array of inhibitory and activating receptors families such as the Nectin receptor family, which comprises two inhibitory receptors (TIGIT and CD96) and a single activating receptor (DNAM1). TIGIT, CD96 and DNAM1 possess between one and three extracellular immunoglobulin-like (Ig) domains, the most membrane distal of which allows them to engage nectin adhesion molecules, namely Nectin2 and Necl5, on the surface of adjacent cells to facilitate cell-cell adhesion and NK cell function. In order to understand the molecular mechanisms behind the recognition of nectin adhesion molecules by NK cells, we have determined the structure of TIGIT in complex with domain-1 of Nectin2 and used a targeted mutagenesis approach to interrogate the energetic basis underpinning the TIGIT-Nectin2 interaction. Altogether our data reveal that TIGIT and Nectin2 associate via a complimentary lock and key interaction, in which the apical residue of the key and those that cap the pocket forming the lock contribute significantly to the binding. This binding mode has also been observed in the TIGIT-Necl5 interaction as well as nectin-nectin homo- and heterodimers, suggesting a high degree of conservation in the binding interface by Nectin receptors and their ligands.

The structure of the atypical Killer Cell Immunoglobulin-like Receptor, KIR2DL4
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Novel non-canonical role of STAT1 in Natural Killer cell cytotoxicity
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STAT1 is an important regulator of NK cell maturation and cytotoxicity. Although the consequences of Stat1-deficiency have been described in detail the underlying molecular functions of STAT1 in NK cells are only partially understood. Here we describe a novel non-canonical role of STAT1 that was unmasked in NK cells expressing Stat1-Y701F. This mutation prevents JAK-dependent phosphorylation, subsequent nuclear translocation and cytokine-induced transcriptional activity. As expected Stat1-Y701F mice displayed impaired NK cell maturation comparable to Stat1−/− animals. In contrast Stat1-Y701F NK cells exerted a significantly enhanced cytotoxicity in vitro and in vivo in absence of detectable nuclear activity. Consistently, immunofluorescence studies uncovered the recruitment of STAT1 to the immunological synapse during NK cell killing. A Stat1−/− mouse expressing FLAG-tagged STAT1a was used to study the STAT1a interactome in NK cells. Mass spectrometry revealed that STAT1 directly binds proteins involved in cell junction formation and proteins associated to membrane or membrane-bound vesicles. We propose a novel function for STAT1 in the immunological synapse of NK cells regulating tumor surveillance and cytotoxicity.

Role of Regulatory T cells in Citrobacter rodentium infection.
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CD4+CD25+Foxp3+ Regulatory T cells (Treg) have been implicated in the maintenance of immune homeostasis in large intestine, the most common site for inflammatory bowel disease (IBD). More recently, microbial infection is increasingly associated with the development of IBD. To determine the role of Treg cells during microbial induced IBD, we examined the course and outcome of Citrobacter rodentium infection in mice depleted of Treg cells. Interestingly, we found that mice depleted of Treg cells are more susceptible to mucosal C. rodentium infection than non-
depleted mice. Treg-depleted mice exhibited exacerbated weight loss, significantly higher bacterial burden in the colon, increased dissemination to systemic tissue, increased epithelial permeability and colonic pathology. Compared to non-depleted mice, at 5 days after infection depleted mice showed altered expression of inflammatory cytokines and antimicrobial peptides in the colon, which have been shown to be protective against C. rodentium infection and important for maintaining epithelial integrity. These data indicate that Treg cells are important in maintaining the host immune response in the colon and the possible role of Treg cells in regulating the innate immune epithelial barrier which will provide new therapeutic insights for IBD.

Plasticity in CD1d-lipid antigen recognition by non-canonical NKT cells
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Natural Killer T (NKT) cells are specialised lymphocytes that recognise lipid antigens presented by the MHC Class I-like molecule CD1d. Following activation, they rapidly secrete a broad range of immunoregulatory cytokines that can influence other mediators of immune responses and therefore represent a promising therapeutic target for cancer and other diseases. The most extensively studied are type 1 NKT cells, which recognise a marine sponge derived glycolipid alpha-galactosylceramide (α-GalCer), express a semi-invariant T cell receptor (TCR) and their role in the immune system is well established, however, much less is known about type 2 NKT cells, which do not recognise α-GalCer and express a diverse TCR repertoire. Using a panel of CD1d mutants, we reveal that different type 2 NKT cell hybridomas can adopt multiple ways to interact with CD1d, and furthermore, we identify a new population of type 2 NKT cells that specifically recognizes the microbial derived lipid-antigen alpha-glucuronosyl-diaclylglycerol (α-GlCα-DAG). Single cell sequencing of these cells reveals a TCR repertoire distinct from type 1 NKT cells. Collectively, our results suggest that type 2 NKT cells express highly diverse TCRs and rely on different mechanisms than type 1 NKT cells to recognize distinct antigens. The knowledge obtained from these studies increases the scope of antigens recognized by NKT cells and provides valuable insight in how these cells can be manipulated for therapeutic gain.

The Role of Interferon Epsilon in the Regulation of Innate-like Immune Cells in the Female Reproductive Tract
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In the female reproductive tract (FRT), homeostasis is maintained to enable embryo implantation and development in parallel with priming of the immune system, to protect against localised infection from mucosal pathogens including sexually transmitted infections (STIs). Chlamydia trachomatis is one of the most common bacterial STIs with infectivity and pelvic inflammatory disease, however, the mucosal immune response to Chlamydia infection is not well understood. Type I Interferons (IFNs) are important cytokines that regulate host innate immune response to infections, including STIs. IFN epsilon (ε) is a novel type I IFN that was discovered in our laboratory and its expression is most abundant in the epithelial cells of the uterus, where there is constitutive expression unlike other type I IFNs. Importantly, using IFNe\textsuperscript{-/-} mice we have demonstrated that this novel cytokine protects mice from experimental models of STIs - Chlamydia muridarum and HSV-2, and regulates levels of NK cells. We now extend our mechanistic studies to assess the role of IFNe in the induction and regulation of innate-like immune cells including innate lymphoid cells (ILCs) and mucosal associated invariant T (MAIT) cells. ILCs have been demonstrated to be key regulators/effectors of the mucosal immune response in the lung and the intestine. However, the role of these cells in the physiology and infection of the FRT has not been fully elucidated. We now characterise these innate-like immune cells in the FRT of wildtype and IFNe\textsuperscript{-/-} mice. We are currently determining the role of IFNe in the regulation of ILCs in response to Chlamydia muridarum FRT infection. Investigation of the role of IFNe in the orchestration of the mucosal immune response in the FRT may identify new therapeutic strategies for targeting and manipulating this innate immune response in disease.

NK cells require IL-28R for optimal in vivo activity
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Natural killer (NK) cells are naturally circulating innate lymphoid cells that protect against tumor initiation and metastasis and contribute to immunopathology during inflammation. The signals that prime NK cells are not completely understood and although the importance of IFN type I is well recognized, the role of type III IFN is comparatively very poorly studied. IL-28R-deficient mice were resistant to LPS and cecal ligation puncture-induced septic shock, and hallmark cytokines in these disease models were dysregulated in the absence of IL-28R. IL-28R-deficient mice were also more susceptible to growth of the NK cell-sensitive lymphoma, RMAs. Specific loss of IL-28R in NK cells transferred into lymphocyte-deficient mice resulted in reduced LPS-induced IFN-g levels and enhanced tumor metastasis. Therefore, by employing IL-28R-deficient mice, which are unable to signal type III IFN-I, we demonstrate for the first time, the ability of IFN-I to directly regulate NK cell effector functions in vivo, alone and in the context of IFN-ab.

**Human NK cell development**

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Despite extensive progress in our understanding of the pathways and regulation of murine haematopoiesis, our knowledge of these processes, in particular, lymphopoiesis, in humans remains rudimentary. Here, we describe the isolation of three progenitor subsets from human foetal bone marrow. We describe a process of differential commitment to the B, T and NK cell lineages as differentiation progresses through these subsets, traceable by a decline in expression of CD117, mirroring the murine system. We further demonstrate the presence of subsets similar in both phenotype and function in umbilical cord blood and the bone marrow of humanised mice, validating these as appropriate sources for the investigation of human haematopoiesis. Overall, we describe several stages in the process of lymphopoiesis that will form the basis of investigating the regulators of this process in humans.

**Innate immunodeficiency following genetic ablation of Mcl1 in Natural Killer cells**

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The cytokine IL-15 is required for Natural Killer (NK) cell homeostasis, however the intrinsic mechanism governing this requirement remains unexplored. Here, we identify the absolute requirement for myeloid cell leukemia sequence-1 (Mcl1) in the sustained survival of NK cells in vivo. Mcl1 is highly expressed in NK cells and regulated by IL-15 in a dose-dependent fashion via STAT5 phosphorylation and subsequent binding to the 3'UTR of Mcl1. Specific deletion of Mcl1 in NK cells results in the absolute loss of NK cells from all tissues owing to a failure to antagonizing pro-apoptotic proteins in the outer mitochondrial membrane. This NK lymphpenia results in mice succumbing to multi-organ melanoma metastases, being permissive to allogeneic transplantation and being resistant to toxic shock following polymicrobial sepsis challenge. These results clearly demonstrate a non-redundant pathway linking IL-15 to Mcl1 in the maintenance of NK cells and innate immune responses in vivo.