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Kate Schroder

Inflammasomes: from fundamental biology to new therapeutics

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Inflammation drives many devastating human diseases for which patients have no disease-modifying drugs. Indeed, inflammation contributes to the susceptibility or severity of all top 10 causes of human deaths; accounting for 55% of global deaths. The inflammasome pathway is a key driver of pathological inflammation in this context, contributing to several common human diseases (e.g. metabolic disease, neurodegenerative diseases, cancers).

Inflammasomes are signalling hubs that provide an activation platform for the zymogen protease, caspase-1. Upon activation, caspase-1 triggers the maturation and secretion of potent pro-inflammatory mediators (interleukins (IL)-1β and -18) and induces cell lysis, culminating in immune system activation. Here, we reveal the mechanisms by which inflammasomes signal, and how a small molecule inflammasome inhibitor can silence pathological inflammasome signalling for therapeutic management of several human diseases.

Mark Hampton

Redox biology and immunology: exploring new connections

Mark Hampton

Department of Pathology and Biomedical Science, University of Otago, Christchurch Campus

Kylie Quinn

Metabolic adaptations in ageing T cells

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Ageing leads to the accumulation of differentiated, unconventional T cell populations. One such cell type is the virtual memory T (T_{VM}) cell, which are semi-differentiated but antigen-naïve CD8 T cells. T_{VM} cells have increased survival compared to conventional naïve CD8 T cells (T_N) and accumulate in number with age, but become dysfunctional. Our work identified age-related shifts in T cell metabolism that did not correlate with function but may support the preferential survival of certain T cell subsets with age. With increased age, memory phenotype cells such as T_{VM} cells exhibited increased mitochondrial load and spare respiratory capacity, despite a marked reduction in classic T cell functions (proliferation, IFNg production and cytotoxicity). In both mouse and human CD8 T cells, increased SRC was associated with heightened sensitivity to IL-15 and blocking IL-15 could reduce SRC in T_{VM} cells. Our model suggests that IL-15 sensitivity and signalling increases in memory phenotype T cells in general and T_{VM} cells in particular with age, to support increased SRC and cell survival. However, SRC is not a consistent positive predictor of conventional T cell function across the lifespan. In our ongoing work, we are using our model of T cell survival, function, metabolism and ageing to optimise the development of T cell-based therapies for older patients.

Session II

Investigating the role of TLR2 within 1928T2z CAR T-cells

Nouri Y[1,2], Dasyam N[1], Li P[4], Hermans I[1], Perret R[1], Weinkove R[1,2,3]
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Chimeric antigen receptor (CAR) T cell therapy is becoming a standard of care for certain relapsed and refractory B-cell lymphomas and myeloma. However, only 50% of recipients of clinically-approved 'second-generation' CAR T-cells have a complete response, indicating a need for CAR T-cells with improved activity. The Malaghan Institute is enrolling to a phase 1 clinical trial ('ENABLE') of locally-manufactured third-generation CAR T-cells containing a Toll-like receptor 2 (TLR2)-derived co-stimulatory domain alongside CD28 and CD3ζ, targeted against CD19 (1928T2z). Third-generation CAR T-cells incorporating the TLR2 signalling domain show improved cytotoxic activity in comparison to a second-generation CAR T-cell construct, however, the intracellular mechanisms involved in CAR signalling via the TLR2 domain are not yet fully defined.

Using the TLR2 inhibitor ortho-Vanillin, as well as CARs with targeted mutations in the TLR2 signalling domain, we explored the contribution of TLR2 function to 1928T2z CAR T-cell activity. We show that when TLR2 signalling is inhibited, CAR T-cell killing of antigen-expressing target cells is impaired, as is the expression of activation markers and cytokine production. Furthermore, preliminary investigations into protein signalling show phosphorylation differences between constructs with and without a functional TLR2 signalling domain.

Characterising the mechanism and function of 1928T2z CAR T-cells will aid in identifying the most promising CAR designs and clinical applications for future trials. CAR T-cell technology has the potential to significantly advance cancer therapy both within and beyond New Zealand, leading to long-term remissions in people with cancers that currently have poor outcomes.

A combined genomic approach to neoantigen discovery for T cell adoptive immunotherapy.

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Immunotherapy is considered the most promising approach to treating advanced cancers, but despite recent successes, many patients do not respond or only experience a partial response. Now, it is becoming increasingly evident that the effectiveness of immunomodulating treatments are reliant on pre-existing immune responses to the tumour. Thus increasing immune recognition of the tumour is a critical goal in advancing immunotherapy.

Neoantigen targeting represents an effective and safe option for tumour-specific immune therapy. Neoantigens arise from any genomic mutation altering protein sequence and are a common feature in advanced tumours. Targeting neoantigens can elicit a robust tumour-rejection response, particularly because the T cell pool recognising these antigens are not affected by central tolerance. Furthermore, there is now mounting evidence that immune recognition of neoantigens is key to successful Immune Checkpoint Inhibitor (ICI) therapy.

By utilising a combinatorial genomic approach, we identified neoantigens able to bind MHC class I in a patient with stage 3 melanoma. Whole-genome sequencing of tumour and germline tissue allowed us to identify non-synonymous mutations for which we computationally predicted MHC binding. From our pipeline, we have been able to identify 4 neoantigen targets that bind our patient's MHC Class I molecules (HLA-A2) in vitro where the germline equivalent does not. These neoantigens represent an excellent target for recognition by the immune system.

Characterising Chimeric antigen receptor (CAR) T-cell products for Aotearoa's first CAR T-cell clinical trial (NCT04049513).

Dasyam N[1]
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Session II, Castle 1 Lecture Theatre, July 7, 2022, 13:30 - 15:45

CAR T-cells are generated via the introduction of a transgene into T-cells allowing the surface expression of the CAR, which allows for targeting an antigen of choice. CARs have evolved in a modular manner over time from 1st, 2nd, 3rd and next generation CARs, which has led to hundreds of clinical trials around the world. These treatments are currently most efficacious in patients with haematological malignancies, specifically B-cell malignancies, though more challenging solid tumour indications are also being targeted in clinical trials. Our construct (1928T2z) uses an antigen recognition domain derived from the scFv of the murine FMC63 antibody allowing recognition of CD19, along with a CD28 transmembrane domain as well as CD28, TLR2TIR and CD3ζ costimulatory and signalling domains. While CAR T-cell therapies have proven to be effective, there remains room to improve both in efficacy as well as safety. Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are commonly reported adverse events and due to the introduction of a transgene there is an associated risk of insertional mutagenesis. While we are involved in mechanistic studies, which can help design more effective CARs; characterising the product that gets infused into to patients provides valuable information allowing us to draw correlations in patients responses and safety. In this presentation I will cover the early work involved in characterising our clinical product via spectral flow, qPCR and co-culture assays. In addition I will also present early work to identify transgene integration sites.

Association of Cancer-Associated Fibroblast Phenotype and Frequency with Two-Point Immunoscore in Colorectal Cancer Patients

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Colorectal cancer (CRC) is prevalent in New Zealand with over 3100 annual cases. 26% of CRC patients in New Zealand are diagnosed at the emergency department. The Immunoscoretm measures CD3+ and CD8+ T cell infiltrate in CRC and can improve the prognostic accuracy of the traditional staging system. We have validated a two-point Immunoscoretm in a cohort of ~500 Dunedin CRC patients. Immunoscorehigh patients show higher disease-free survival compared to Immunoscorelow patients. We have also shown that inclusion of effector T regulatory cells can improve prognostic accuracy of the Immunoscoretm. I hypothesise that use of the Immunoscoretm in determining patient outcome is improved further by inclusion of stromal cell types of the tumour microenvironment (TME). Cancer-Associated Fibroblasts (CAFs) are immunomodulatory cells dominant in the TME that express mesenchymal, fibroblastic and inflammatory markers, and are independently associated with CRC prognosis. CAFs are desmoplastic cells whose phenotype, function and spatial localisation varies by CRC stage. Mesenchymal CAF (myoCAF; PDGFRα+/PDGFRβ+/αSMA+) and inflammatory CAF (iCAF; PDGFRα+/PDGFRβ+/αSMA-) population frequency in CRC were analysed via Flow Cytometry. iCAF (Vimentin+/CD34+/αSMA-) and myoCAF (Vimentin+/CD34+/αSMA+) populations were validated and analysed for cell location via immunohistochemistry in fixed-formalin paraffin-embedded CRC tissue. Future objectives will evaluate the frequency of myoCAFs and iCAFs with Immunoscorehigh and Immunoscorelow patients in the Dunedin CRC cohort to determine the impact of CAF phenotype on T cell infiltrate and patient outcome. Quantification of CAF phenotypes will improve accuracy in combination with the Immunoscoretm to stage CRC patients for treatment and prognosis in a New Zealand setting.

Ex Vivo Culture of Patient-Derived Endometrial Cancer Tumour for Immunological Characterisation and Exploration of Levonorgestrel Resistance

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Endometrial cancer (EC) is the most common gynaecological cancer in the developed world and its incidence is fastest rising in Aotearoa, particularly in pre-menopausal women.
Early studies indicate that the use of the levonorgestrel (LNG)-releasing intra-uterine system (IUS) can inhibit the growth and development of early-stage EC. However, response rates are as low as 43%, and research into the cause of non-response is lacking. The long-term ex vivo culture of patient tissue is a novel technique for exploring the effects of LNG on an unmanipulated piece of EC tissue. Typical cell culture techniques involve breaking down the natural 3D architecture, subsequently reducing the clinical relevance of the model. In contrast, ex vivo patient-derived explant models retain the spatial relationship of the tissue. We aim to establish a methodology to maintain explants taken from patients with early-stage EC. Using the model, we further aim to investigate the effects of LNG on tissue survival and changes in the composition of the immune compartment.
EC explants are capable of surviving in ex vivo culture for up to 21 days. Flow cytometric analysis has identified CD3+ lymphocytes as the predominant constituent of CD45+ cells present within explants. These CD3+ cells remain viable in the explant model for up to 7 days. Further investigation will elucidate the phenotype and function of specific tissue-resident T-cell sub-populations in response to LNG.
Data from this project will provide a foundation for immunological phenotyping of the EC tumour microenvironment and elucidate potential immune mechanisms of LNG resistance.

Specific targeting of synthetic cancer vaccines to human dendritic cells via CLEC10A

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Dendritic cells (DCs) are critical regulators of immunity and offer promising targets for cancer immunotherapy. DCs are able to initiate protective adaptive immunity and have an emerging role in regulating the T cell response within tumours, yet cancer vaccines have so far shown limited efficacy. We aim to develop self-adjuvanting peptide vaccines to specifically target human DCs for the stimulation of anti-cancer T cells. Using in-house optimised peptide chemistry, we generated a synthetic vaccine construct targeting the pattern recognition receptor CLEC10A (CD301, MGL) expressed by human DC populations. We confirmed the specific binding of our vaccine constructs by human blood CD1c+ DCs from healthy donors via CLEC10A using high-dimensional flow cytometry. Vaccine-bound cells comprised the classical DC2 subset and the newly identified DC3 subset, which have recently been assigned a unique role in generating anti-cancer T cell responses. Transcriptional profiling revealed that CLEC10A engagement alone does not alter the activation state of CD1c+ DCs, identifying CLEC10A as a useful target for the specific yet inert delivery of cargo to human DCs. Our ongoing work is aiming to characterise the effects of vaccine binding through CLEC10A on DC maturation and assess the implications for intracellular processing of the vaccine cargo. This class of vaccines has the potential to specifically and effectively target subsets of human DCs, and can be manufactured at large scale with routine peptide chemistry, promising to become a valuable tool for cancer immunotherapy.

Drug and auto-inducible promoters to improve CAR T cell efficacy and patient safety in solid tumour treatment

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Chimeric antigen receptor (CAR) T cell therapy has been shown to be a successful immunotherapy treatment option for blood borne human cancers. However, the efficacy of CAR T cell therapy for treatment of solid tumours is decreased by the inhibitory, pro-apoptotic tumor microenvironment (TME). Gamma-chain cytokines provide a way to enhance T cell survival in the TME, however inflammatory or anti-apoptotic regulation of CAR T cells may impact on safety of the procedure. Drug or tumour antigen-inducible promoters target gene expression to the tumour site in a temporarily and anatomically restricted manner to improve patient safety. The use of transcription factor-based auto-inducible promoters have been previously proposed for use in CAR T cell therapy. However basal leakiness and poor dynamic promoter performance has limited the application of these promoter for clinical use. In this study, drug or auto-inducible promoters were designed utilizing T cell activation-associated transcription factor binding sites, together with the addition of short enhancer elements. Lentivirus and salmonid-based Sleeping Beauty transposon was utilised to facilitate gene transfer into primary human T cells. Two auto-inducible constructs provided a 70-fold increases in gene expression upon antigen-triggering. In parallel, drug-inducible promoters were also refined to promoter gene expression or repression to control T cell activation as a safety-switch. Selected promoter-enhancer constructs are currently being tested for conditional expression of genes of interest in primary human CAR T cells for treatment of breast cancer in a humanised (NSG) mouse model.

Dissecting the integration of cytokine signals by DC2s that contribute to Th2 priming

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Dermal type 2 Dendritic Cells (DC2s) survey the microenvironment for antigens to present to CD4+ T-cells in the draining lymph node. Antigen presentation, cytokine production and co-stimulation by DC2s are highly influenced by TLR ligands and cytokines at the site of antigen exposure, directly affecting the CD4+ T-cell response following priming.
The development of the TH2 response is elusive, with no single factor yet shown to be TH2-polarising. Although several signals have been identified that individually impact on TH2 polarisation by DC2s, it is not yet understood how each signal contributes overall to the response.
By simultaneously measuring the proliferation of CD4+ T-cells in response to allergen immunisation and their differentiation into TH2 cells, this project sought to distinguish the signals that promote TH2 development by increasing T-cell proliferation and survival, from those that specifically drive their differentiation into TH2 effectors. A division-dye labelled CD4+ T-cell adoptive transfer system was utilised in cre-flox mouse strains to delete specific cytokine receptors in DCs, while preserving direct cytokine effects on T-cells. Results from these experiments implicate the signalling of several cytokines in conditioning DC2s to instruct CD4+ T-cell proliferative capacity independently from instructing TH2 differentiation.

MAIT cells activate dendritic cells to promote T follicular helper cell differentiation and induce humoral immunity

Pankhurst T[2]
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Protective immune responses against respiratory pathogens, including SARS-CoV-2 and influenza virus are initiated by the mucosal immune system. However, most licensed vaccines are administered parenterally and are largely ineffective at inducing mucosal immunity. The development of safe and effective mucosal vaccines has largely been hampered by the lack of a suitable mucosal adjuvant. In this study we explore a novel class of adjuvant that harness mucosal-associated invariant T (MAIT) cells. We show evidence that intranasal immunisation of MAIT cell agonists co-administered with protein, including the receptor-binding domain from SARS-CoV-2 and haemagglutinin from influenza A virus, induced potent humoral immunity and IgA production. MAIT cell adjuvant activity was mediated by CD40L-dependent activation of dendritic cells and subsequent priming of CD4+ T follicular helper cells. In summary, we show that MAIT cells are promising vaccine targets that can be utilised as cellular adjuvants in mucosal vaccines.

Session V

Primary dendritic cells cultured in the presence of Flt3L or GM-CSF and IL-4 can dramatically affect the transduction efficiency of lentiviral vectors.

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Dendritic cells (DCs) are essential antigen presenting cells responsible for the initiation and activation of innate and adaptive immune responses. In response to antigen exposure, DCs upregulate or downregulate genes specific to the antigen encountered. To further understand the role of these genes in the initiation of allergic responses, we aim to introduce a lentiviral vector system to specifically delete the differentially expressed genes using CRISPR-Cas9 technology. Primary dendritic cells were cultured from the bone marrow of Cas9 expressing mice and cultured in the presence of Flt3L or GM-CSF and IL-4, transduced with lentiviral vectors containing sgRNA's targeting differentially expressed genes. While Flt3L BMDCs resemble *in vivo* splenic DCs, GM-CSF and IL-4 BMDCs are more alike to monocytes, making the former more favourable for *in vitro* work. Surprisingly, successful lentiviral integration was highly dependent on the growth and transduction conditions for each type of bone marrow derived dendritic cell (BMDC) where Flt3L BMDCs were more difficult to transduce. However, with careful optimisation, it is possible for up to 73% of cells in culture to successfully integrate the lentiviral vector containing a CD86 targeting sgRNA, resulting in a downregulation of CD86 expression. For further application it is imperative to test sgRNA's targeting other genes in the hope to uncover the functional aspect of the genes already identified as potential candidates involved in Th2 development.

Reducing toxicity of CAR-T cell therapy using stimuli-responsive “Pro-tags”

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Chimeric antigen receptor (CAR) engineered T cells have shown revolutionary success in treating refractory hematologic malignancies. However, there are still challenges in translating CAR-T cell therapy into solid tumours, for example potential on-target-off-tumour toxicity. The aim of this project is to improve the safety profile of CAR-T cell therapy via universal CAR-T cells. Universal CARs recognising a generic tag linked to specific tumour ligands, have been proposed as an option to simplify this process. This means that only one universal CAR DNA construct needs to be developed and this can be used to transfect cells from any patient. The universal CAR T cells can then be used in combination with many different specific tumour-ligand-tag constructs to treat cancer

The first step of the development of this approach involves the design and synthesis of folate-biotin and glucose-biotin constructs containing a biotin tag attached to molecules overexpressed by tumours. To further enhance this technology and allow more precise control over our universal CAR-T cells therapeutic tumour targeting activity, we synthesised masked versions of pro-tags, containing a bulky group that only released upon exposure to a stimulus present specifically in the tumour microenvironment, for example hydrogen sulphide. With this approach, CAR-T cells should preferentially attack tagged tumour cells and not those healthy cells that express the same targeting ligand. Here, we will present data on synthesis and in vitro evaluation of the tag and pro-tag constructs. Overall, this project will contribute to the advancement of CAR-T cell therapy in treating solid tumours.

Development of an arrayed CRISPR-Cas9 genetic screen to study Ccl17 transcriptional control in primary mouse BMDCs, and unveil genes involved in Th2 immune responses.

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Allergic immune responses are characterised by the presence of CD4+T cells polarised to a Th2 phenotype. Conventional dendritic cells (cDCs) are essential antigen presenting cells for Th2 priming, but there is no consensus as how DCs might drive the development of Th2 cells.

CCL17 is a chemokine associated with type 2 inflammation that is upregulated by migratory DCs during Th2 priming in vivo . CCL17 binds to a receptor, CCR4, that is predominantly expressed by CD4 T cells of Th2 phenotype. Together, these data suggest a role for DC-derived CCL17 in Th2 polarisation.

To unveil Ccl17 transcriptional control, we performed an arrayed CRISPR-Cas9 screen in mouse bone marrow DCs (BMDCS) generated from Ccl17-eGFP reporter mice expressing the endonuclease Cas9. Using replication-defective lentiviral vectors, BMDCs were transduced with guide-RNAs (gRNAs) targeting 34 genes that are up-regulated in DCs exposed to allergen in-vivo, and the modulation of Ccl17-eGFP expression was visualised by flow cytometry.

So far, we have delivered gRNAs with a consistent efficiency of 20 to 40%. A control Cd86 gRNA was effective in downregulating the CD86 membrane protein in transduced BMDCs. The delivery of gRNAs targeting known regulators of Ccl17 expression induced the expected modulations, with Stat6, Klf4 and Irf4 correctly identified as positive regulators. Additional gRNAs are currently being assessed.

Altogether, we have developed a robust method that enables us to evaluate the role of genes expressed by DCs in the regulation of Ccl17 expression and potentially uncover new mechanisms involved in Th2 polarisation.

Short-Chain Fatty Acids – boosting the anti-tumour function of CD8+ T cells

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CD8+ T cells are vital mediators of anti-tumour immunity. Their production of cytotoxic molecules, such as IFN-γ and TNF, directly trigger tumour cell death and their abundance within tumours is associated with better patient outcomes. However, many CD8+ T cells have impaired function within tumours due to immunosuppression and the lack of metabolic substrates in the environment. Thus, finding ways to enhance CD8+ T cells to overcome these factors within tumours, is of great interest to improve patient prognosis.
Recent studies suggest that microbial short-chain fatty acids (SCFAs) (acetate, propionate, and butyrate) may enhance CD8+ T cell function by increasing their metabolic flexibility and production of IFN-γ. This study aims to fully characterise the effect of SCFAs on CD8+ T cells phenotype, function, and anti-tumour capacity using a combination of in vitro and in vivo cell culture and flow cytometric analysis. This research will then evaluate if SCFAs have use as an immune-boosting agent and potential additive to current immunotherapies to improve patient outcome. Preliminary in vitro results show an increased frequency of CD8+IFN-γ+ T cells when activated peripheral blood mononuclear cells are cultured with butyrate and propionate but not acetate. Butyrate-treated cells had a greater frequency of several effector populations than acetate and propionate treated cells including CD8+CD25+IFN-γ+ and CD4+IFN-γ+IL-2+ cells. These early results suggest butyrate may be a strong candidate for increasing effector function in both CD4+ and CD8+ T cells.

Helminth infection model reveals novel subpopulations of granulocytes

Noble S[1]
[1]*Malaghan Institute of Medical Research*

Granulocytes are historically considered to be pro-inflammatory, cytotoxic effector cells, however increasing evidence suggests granulocytes are more heterogeneous than previously appreciated. There exist multiple functional subtypes of granulocytes, with roles ranging from maintaining immune homeostasis and inhibiting pathogens, to exacerbating pathology. I am using a helminth infection model to investigate granulocyte heterogeneity, specifically focusing on eosinophil subtypes.

Helminth parasites reside in the gastrointestinal tract and are able to regulate the host immune system. The immunoregulatory properties of helminths give them huge potential as a therapeutic for autoimmune and inflammatory conditions. Eosinophilia is a hallmark of helminth infection and helminths may modulate eosinophil subtypes. This makes helminth models an invaluable tool for investigating granulocyte heterogeneity and has crucial ramifications for hookworm therapy.

I have used high-dimensional flow cytometry for granulocyte immunophenotyping in a pre-clinical helminth infection model. I will present data showing that helminth infection elicits novel tissue-specific eosinophil subpopulations that are identifiable by alterations in surface receptors. These eosinophil subpopulations could be a mechanism by which helminths regulate inflammatory responses.

Furthermore, I will present results from a human clinical trial showing that hookworm-infected participants exhibit peripheral eosinophilia driven by synchronized changes in peripheral cytokine levels. Hookworm infection also altered levels of eosinophil degranulation products in stool, indicating functional changes to gastrointestinal eosinophils. These results contribute to our understanding of the heterogeneity of granulocytes, as well as illuminating the fascinating interplay between parasite and host and providing valuable insight into the therapeutic potential of helminths for gastrointestinal diseases.

Complexity and Risk: The Push and Pull of SARS-CoV-2 Vaccine Design

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To date, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has infected 505 million people and killed 6.2 million people. Several vaccine candidates have been developed to stop the spread of this deadly virus, however every candidate has its drawbacks. This seminar will discuss the push and pull in SARS-CoV-2 vaccine design: between increased complexity, and increased risk; and decreased complexity, and decreased risk. Coronaviruses derive their name from the crown of Spike proteins, speckling the membrane of the virus, which are used to enter human cells. The most simplistic models of antibody-mediated neutralisation involve the antibody physically disrupting the Spike protein's association with its receptor on human cells, by binding the receptor binding domain (RBD). Any vaccine strategy will elicit a mixture of neutralising and non-neutralising antibodies, and so the proportion of neutralising antibodies can be increased by focusing on the RBD alone as an immunogen. However restricting the immunogen to the RBD restricts the available T cell epitopes. While increasing a scope of targetable epitopes in a SARS-CoV-2 vaccine will provide a larger, broader T cells response, this will lead to a less focused humoral response. This work dissects the benefit of increasing antigen complexity on the magnitude and affinity of the RBD targeted humoral response as the breadth of the T cell response increases. We present a novel subunit vaccine candidate, focused on the RBD epitope with restricted additional spike derived epitopes, which is well suited as a booster vaccine to provide protection against current and future SARS-CoV-2 variants.

Session VI

Investigating different ligand and adjuvant combinations for development of a polyvalent Staphylococcus aureus vaccine in a murine model of infection

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Staphylococcus aureus is an opportunistic human pathogen that can cause infections varying from superficial skin infections to more severe morbidities like bacteraemia. It is a major cause of nosocomial infection. In recent years multi-drug resistant strains have emerged prompting the need to investigate other avenues for protection such as vaccines. An enticing option for patients at risk of nosocomial infection would be a prophylactic vaccine that subsequently reduces S. aureus disease burden, severity, or even colonisation. Our group is currently developing a polyvalent vaccine comprised of three different, highly conserved proteins. These proteins are immune evasion factors important in establishing initial infection. Earlier studies show vaccination of mice with the polyprotein results in neutralising antibodies to these vaccine targets, as well as significantly increased protection in a S. aureus intraperitoneal murine model of infection.
My research looks to further optimise the immune response via the addition of different ligand adjuvants to the vaccine. These ligands are pattern recognition receptor agonists and their addition has been shown to enhance protection and immunogenicity in other vaccine models. Total specific antibody response to the three proteins, as well as functional assays, to determine the ability of generated antibodies to neutralise these targets were measured. It was found that the addition of some ligands resulted in significantly reduced S. aureus burden in tissues, as well as improved animal welfare during infection compared to their non-spiked counterpart. Additional studies will investigate the cellular response that is prompting this enhanced protection from the ligand spiking.

Inflammatory T cells result in epithelial damage in patients with Crohn's disease

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Crohn's disease (CD) is a chronic inflammatory bowel disease with many contributing factors: the immune response, genetic susceptibility, environmental factors, intestinal epithelial barrier, and the host microbiome. Current treatments aim to control excessive inflammation but are not always effective and patients often lose responsiveness. We have studied the contribution of the immune response and the epithelial barrier using intestinal organoids, which can account for patient heterogeneity. We developed a 2D intestinal monolayer transwell system which represents the lumen (mucosal side) and lamina propria (serosal side) of the gut. We used 2D human intestinal organoids to model the gut immune responses in people with CD compared to healthy controls (HC), as well as the effect of bacteria and immune cells from the same patient on the epithelial integrity. Addition of peripheral blood mononuclear cells (PBMCs) to CD monolayers resulted in reduced epithelial resistance compared to monolayers derived from HC donors. We developed a model of graded inflammation using HC monolayers and polyclonally active immune cells at varying degrees of activation. The degree of immune cell activation was correlated with reduced epithelial integrity. Epithelial integrity was restored with the addition of anti-TNFa antibodies. This model allows us to compare and test the effect of probiotics e.g. Escherichia coli Nissle 1917 on epithelial integrity. These data highlight the importance of investigating T cell populations, and the interaction of gut bacteria, immune cells and the intestinal epithelial cells to understand the pathogenesis of CD.

Investigating lipid changes linked to remyelination in experimental autoimmune encephalomyelitis (EAE)

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Multiple sclerosis (MS) is an autoimmune disease characterized by damaged myelin sheets. These sheets facilitate fast and efficient signal transduction and become damaged by infiltrating immune cells. Remyelination, the process of restoring damaged myelin sheets, can be enhanced by stimulating oligodendrocyte precursor cells (OPC) to migrate into the damaged area and to mature into oligodendrocytes, the cells that produce myelin sheets. This maturating process can be tracked using different markers such as O4. Recently, studies showed that O4 is a sulfatide, a specific class of lipids. Moreover, during development of OPCs to mature oligodendrocytes, the lipid profile changes from short chain sulfatides to sulfatides with longer fatty acid chains. This makes sulfatides an interesting target to investigate remyelination in a MS-like disease setting such as EAE. Here we present a method to readily detect sulfatides using mass spectrometry imaging in spinal cord tissue sections and compare lipid profiles from healthy and diseased animals.

Poster Session

High-throughput ELISA-based Screen to Identify Novel DC2 to Th2 Cell Interactions

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New Zealand has one of the highest rates of allergic disease in the developed world. Allergic responses are characterised by an inappropriate response to innocuous environmental antigens mediated by T helper (Th) 2 cells. Differentiation into a Th2 cell lineage is thought to require signals derived from dendritic cells (DCs) and appears to be mediated by a specialised, yet unknown function of the DC2 subset. The majority of communication between DC2s and Th2 cells occurs via receptor ligand interactions, however, it remains unclear what interactions drive Th2 differentiation. Therefore, we aimed to identify novel protein-protein interactions between DC2s and Th2 cells by implementing a protein-binding ELISA-based screening method. Target proteins were identified through bulk RNA sequencing of DC2 subsets carrying florescent antigen and IL-4 producing T cells isolated from mice treated with Th2-inducing, Nippostrongylus brasiliensis. Additionally, Th0 proteins were included from a published protein atlas. The DC and T cell differentially expressed genes were converted into fusion proteins through transfection of the Expi293F mammalian cell line with custom-made plasmids containing the target gene fused with either the Fc domain of human IgG (DC2 gene) or alkaline phosphatase (T cell gene). Each DC2 fusion protein was screened against each T cell fusion protein, totalling over 25,000 potential protein-protein interactions. Initial results indicate over 100 protein-protein interactions many of which appear to be novel. These results are the first step to identifying potential receptor ligand interactions between DC2s and Th0 cells, leading to Th2 differentiation and allergic disease.

Feasibility of using a luminescence-based method to determine serum bactericidal activity against Neisseria gonorrhoeae

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Development of a vaccine to limit the impact of antibiotic resistant Neisseria gonorrhoeae is a global priority. Serum bactericidal antibody (SBA) is a potential indicator of protective immunity to N. gonorrhoeae, but conventional assays measuring colony forming units (CFU) are time-consuming and labour intensive. We determined the feasibility of measuring viability of N. gonorrhoeae using a high throughput, same day luminescent assay.

The BacTiter-Glo Microbial Cell Viability Assay (ATP assay) was modified for use in estimating viable bacterial numbers. A strong correlation between CFU and luminescence was seen across a range of 50 – $5x10^6$ CFU, for all strains (r=0.9). Normal Human Serum (NHS) was screened for serum sensitivity and combined with murine anti-sera to determine bactericidal titres versus CFU readings. NHS from healthy individuals were screened in sensitivity assays - values were significantly reduced with the ATP method for N. gonorrhoeae strains FA1090 and MS11, whereas P9-17 data were comparable for all donors. Mouse anti-P9-17 SBA titres to P9-17 were similar with both methods (r=0.97).

Quantification of N. gonorrhoeae ATP using a commercial reagent shows utility as an alternative approach to manual enumeration of CFU for measuring SBA to N. gonorrhoeae. In contrast, the ATP method under-estimated serum sensitivity for two of the three strains, suggesting that it is unsuited to this assay. We are currently applying this method to additional strains of N. gonorrhoeae. The ATP assay may be advantageous for directly reading antibody-mediated killing, independent of alternative mechanisms that kill the gonococci over a longer period.

Sleeping Beauty Transposon-Based Vector Kit-Sets for the Manufacture of Artificial Antigen Presenting Cells for CAR NK or T cell therapy

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Artificial antigen presenting cells (aAPC) offer a cost-effective and convenient tool for the expansion of antigen specific T cells, and chimeric antigen receptor (CAR) - bearing T cells and NK cells. aAPC are particularly useful for the expansion of low frequency antigen-specific lymphocytes due to their ability to expand antigen-reactive lymphocytes in bulk cultures. The most commonly used aAPC is K562 which lacks most MHC expression and is therefore useful for the expansion of T cells without triggering allogeneic responses. In addition, the lack of MHC-I on K562 is a potent trigger for NK cell expansion. Difficulty in accessing existing aAPC lines, and the requirement of time-consuming and iterative lentiviral single-gene transfers with antibody-mediated sorting selection, led us to develop publicly-available SB-based vectors with antibiotic selection options. Each of these vectors contains two to three genes comprised of surface antigen or MHC, costimulatory molecules, and membrane-bound cytokines. This allows for selection of up to six transgenes with simultaneous transposition using only two antibiotics. Our new vector system offers human vectors CD19, Her2, HLA-A2 or disulphide-trapped HLA-E, together with constitutive and linked expression of 4-1BB, CD86 and membrane-bound IL-15 or IL-21.

The role of MAIT cells in the Atopic March

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The atopic march is a progression of allergic diseases that develop over the course of infancy and through childhood. It begins soon after birth with IgE-mediated allergen sensitisation and the associated the development of atopic dermatitis (AD). The sensitisation then primes other mucosal sites, such as the gut and lung, eventually leading to food allergies, rhinitis, and/or asthma . It is estimated that 50% of new-borns who develop AD will eventually develop asthma. To determine the potential link between the atopic march and the activation of mucosal associated invariant T (MAIT) cells, a T cell subset found in allergen-exposed tissues, we developed a preclinical mouse model of the atopic march consisting of a epicutaneous sensitisation and subsequent lung challenge with the common allergen house dust mite. We found that cutaneous MAIT cell inhibition by topical application of a MAIT cell antagonist during the sensitisation phase mice are protected from airway eosinophilia upon intranasal challenge. Our data thus indicates that MAIT cells are mechanistically involved in the spreading of allergic immune responses across mucosal sites.

Tumour-infiltrating CD4 T cells express the chemokine receptors CXCR4 and CCR4 in human colorectal cancer

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T cell migration into the tumour microenvironment is essential to the anti-tumour immune response and is reliant on the production of chemokines and expression of chemokine receptors. T regulatory cells (Tregs) can have diverse phenotypes within colorectal cancer (CRC) and their use as a prognostic marker of patient outcome is variable. One major subtype of Tregs are 'effector' Tregs, which have been associated with a positive patient prognosis in CRC. The aims of this study were to determine chemokine receptor expression on T cell populations within the tumour, peripheral blood, and non-tumour bowel. Using mass cytometry and high-dimensional analysis, we have shown an enrichment of CD4 T cells (n=3. P= <0.001, Freidman test) and Tregs (n=3, p=<0.05, Freidman test) expressing the transcription factor GATA-3 and chemokine receptors CCR4 and CXCR4 in the tumour compared to non-tumour bowel of the same patients. We propose that expression of these chemokine receptors on Tregs promotes co-localisation in tissues with their effector T cell counterpart, allowing subset-specific suppression. We are currently assessing whether expression of the chemokines that bind these receptors cause migration towards tumour cells. These data will provide insights as to why effector Tregs are associated with a positive prognosis in CRC.

Predicting anti-TNF Responses in Inflammatory Bowel Disease Patients Using Intestinal Organoids

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Inflammatory bowel diseases (IBD), including Crohn's disease (CD), affect 30,000 people in New Zealand and costs the healthcare system over NZD 245 million per year. Treatment with anti-tumour necrosis factor (anti-TNF) monoclonal antibodies is effective in some patients but has a non-response rate between 13 – 40%. Whether a patient will respond or not is unknown before treatment and therefore represents sunk cost and unnecessary treatment side-effects. Human intestinal organoids (HIOs) are miniature three-dimensional organs grown in culture that more accurately represent the epithelium of the gut compared to traditional cell culture. We hypothesise that HIOs cultured from primary colon biopsies can be used to measure intestinal permeability and anti-TNF response at an individual level. HIOs from healthy individuals (HI) and patients with CD were successfully grown in vitro. Following culture for 4 days with recombinant TNF, differences in HIO growth were observed. At low dose (1 ng/mL), an increased quantity of organoids grew in both HI and CD cultures. Interestingly, at high dose (100 ng/mL), this number was reduced. Uptake of FITC-dextran was used as a measure of permeability, demonstrating that CD organoids were 'leakier' than HI organoids and were more susceptible to increased 'leakiness' following TNF treatment. Future immune co-culture experiments will determine the effect of TNF-producing immune cells on HIO barrier integrity and culture with anti-TNF will determine whether the observed effects can be reversed. Results from this research could help in more accurately determining effective treatment schedules for IBD patients.

Development of Adjuvant-free House Dust Mite Model of Allergic Airway Inflammation

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House Dust Mite (HDM) preparations from the arthropod Dermatophagoides pteronyssinus can be used to generate allergic airway inflammation in animal models of asthma. HDM is one of the most common allergens in humans globally, affecting 1-2% of the population, making it a relevant model allergen.
We aimed to develop an adjuvant-free HDM-induced mouse model of allergic airway inflammation, whereby animals are initially sensitized to the allergen and after subsequent airway challenge, hallmarks of allergy are observed. These features include airway eosinophilia, increased mucus production in bronchioles and elevated serum IgE levels.
We found the nature of the HDM preparation, be it whole crushed mites, or soluble extract, affected the magnitude and cellular profile of airway inflammation.
Interestingly, we observed a marked difference when sensitizing mice intranasally or intradermally at a distant site (ear), with skin sensitization inducing the greatest numbers and frequencies of eosinophils in the bronchoalveolar lavage (BAL) fluid following challenge.
We have studied the fate of administered allergen at the sensitization stage in draining lymph nodes from these two sites (lung and skin), and observed distinct compositions of dendritic cell subsets amongst the antigen-presenting cells harbouring allergen.
Using mouse models deficient in various dendritic cell subsets, we addressed this phenomenon.
Our results support the skin being a tissue particularly well suited to generate Type 2 immune responses. This may be related to the different phenotypes and transcriptional profiles of dendritic cells in skin compared to lung and their draining lymph nodes.

T cell-mediated mechanisms behind the heparan sulphate mimetic HS16-35 in a mouse model of multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune disease characterized by infiltration of pro-inflammatory immune cells into the brain and spinal cord; hence, modulating the trafficking of immune cells into the central nervous system (CNS) is a successful therapeutic approach in MS. CNS trafficking is regulated by heparanase-dependent cleavage of heparan sulphates (HS), highly sulphated glycosaminoglycans expressed on the surface of cells and within the extracellular matrix at the blood–brain barrier. HS16-35 is an HS mimetic that strongly inhibits heparanase activity and is currently explored in our group as an MS therapy. This work aims to delineate the mechanisms by which HS16-35 reduces CD4+ T cell infiltration into the CNS and ameliorates disease severity in a mouse model of MS, experimental autoimmune encephalomyelitis (EAE).
To determine whether HS16-35 administration has lasting effects on CD4+ T cells, female C57BL/6J mice were immunized with myelin oligodendrocyte glycoprotein ($MOG_{35–55}$) peptide to induce a MOG-specific CD4+ T cell response and treated with vehicle (PBS) or HS16-35 on days 5–7. On day 8, spleens and lymph nodes were collected, and cells were cultured with vehicle or HS16-35 in the presence of antigen. After 96 hours, CD4+ T cell activation and cytokine responses (IL-17A, IFN-γ, IL-6, IL-10) were analyzed by flow cytometry to determine the effect of HS16-35 on CD4+ T cell phenotype and function.
Our findings will aid in determining whether treatment with the HS mimetic, HS16-35, directly regulates CD4+ T cell function in addition to altering immune cell trafficking in a mouse model of MS.

High-Dimensional Spectral Cytometry Optimization of an Immunophenotyping Panel for a Longitudinal Human Hookworm Infection Model

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The parasitic field is lacking in studies that profile the long-term effects of low-dose human hookworm infection on the immune system of the hosts. Understanding these long-term effects could be the key to unlocking the potential of human hookworm as a therapy for a range of inflammatory diseases such as IBD, asthma and allergies. We conducted a year-long clinical study with multiple timepoints of peripheral blood sampling to answer this exact gap in the parasitic immunology field. Results of the rigorous optimization process and the solutions to the challenges of longitudinal clinical studies will be shown. Large numbers of interesting cell types and markers, day-to-day machine variation, staining variation and inter-participant variation are just some of the challenges to overcome with such a study design. Solutions to these challenges included implementing a standardized protocol that ensures the performance of all markers, including specific controls to assess and correct for staining and machine performance, assessing inter- and intra- participant autofluorescence profiles, and quality checking the panel using high-dimensional techniques. The sum of these solutions is a high-quality panel that is replicable, allows for data normalisation and provides the capability to profile in-depth, the changes in the human immune system from low-dose human hookworm infection.

Clinical Safety and Tolerability of a Controlled Human Hookworm Infection Model

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It has been long suggested that a lack of endemic helminth infection in developed nations may contribute to increasing rates of inflammatory conditions such as inflammatory bowel disease, asthma and allergy. This concept is embodied by the hygiene hypothesis, which simply states that people from developed countries experience less infectious diseases, especially parasitic diseases, but more autoimmune and allergic diseases. It is well established that gastrointestinal parasites such as the human hookworm Necator americanus can modulate the immune system of their hosts to promote their own survival. For this reason, extensive profiling of the effects of a low-dose controlled human hookworm infection on the host immune system could lead to its potential use as a therapeutic agent for these diseases. We conducted a year-long study of healthy volunteers infected with human hookworm to assess the safety and tolerability of the infection. Variables assessed included frequency and severity of symptoms, ferritin levels and platelet and other blood cell counts. To determine patency of hookworm infection, eggs present in the faeces were quantified with further confirmation through visualisation of worms in the small intestine using capsule endoscopy. The hookworm infection was found to be well tolerated by the majority of the study participants, with no changes of concern among the safety variables measured. All participants also showed an established, viable hookworm infection. This therefore shows great promise in moving toward the trial of a potential novel therapy for inflammatory disease.

Using gene editing to fine-tune gene expression and dissect the immune checkpoint inhibitor response in melanoma

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Immune checkpoint inhibitors (ICIs) have revolutionised the treatment of cancers such as melanoma by unleashing immune cells to kill cancer cells. One type of ICI works by blocking the interaction of the PD-1 inhibitory receptor on T cells with its ligand, PD-L1, on cancer cells. ICIs targeting PD-1/PD-L1 are an effective treatment in approximately 30% of melanoma patients but the underlying biology of efficacy remains poorly understood.

We hypothesise that there are optimal expression levels of PD-1/PD-L1 that tip the balance towards either immune escape or cancer cell killing. To examine this we are using a unique application of CRISPR/Cas9 gene editing to fine-tune endogenous gene expression of both PD-1 and PD-L1/PD-L2 (in T cells and melanoma cells respectively) at physiologically relevant levels. Fine-tuning of gene expression will be achieved via insertion of synthetic microRNA response elements of varying strengths into the 3'UTR of the target genes[1].

To date we have demonstrated up to 98% targeted insertion of a miRNA response element into target genes in melanoma cells. Flow cytometric analysis of PD-L1 edited melanoma cells demonstrates a concurrent decrease in cell surface protein expression indicative of fine-tuned gene expression. Additionally, we have generated highly efficient (>90%) PD-L1 and PD-L2 knockout melanoma cells. Our knock out editing rates in primary human T cells are also highly effective (>90%), with targeted knock in rates achieving 65% efficiency. Experiments are underway to assess the functional impact of melanoma PD-L1/PD-L2 modulation on melanoma-specific T cells. Upon successful completion, this research aims to guide the selection of patients who may respond positively to ICI treatment and to aid the development of optimal T cell based immunotherapies.

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Spatial localization of CCL17+ CD301b+ cDC in the murine lymph node during Th2 responses

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Previous work in our laboratory has shown upregulation of the two CCR4 ligands: CCL22 and CCL17 in skin migratory cDC2 during Th2 responses induced by Nippostrongylus brasiliensis.
Given that CCR4 is the primary chemokine receptor for both Th2 T-cells and CD4+ Tregs, the localization of CCR4 ligand-producing migratory cDC2 in the draining lymph node is of considerable interest. Using various fluorescent reporter mouse models, including a homozygous CCL17EGFP knockout reporter strain and a heterozygous counterpart, we can identify the localization of cDC2 in the draining lymph node as well as T-cell subsets. With this, we can identify lymph node microenvironments where T-cell priming by these specific cDC2 subsets can occur, and potentially identify DC2 subsets responsible for various aspects of the Th2 response.

Using spectral flow cytometry to profile immune cell metabolism

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It is becoming increasingly evident that engagement of specific metabolic pathways regulates immune cell function and fate. However, identifying how specific changes in cell metabolism alter immune responses in humans has been somewhat limited due to the large number of cells required for commonly used methodologies. To address this issue, we are developing high-dimensional spectral flow cytometry assays to interrogate both functional phenotypes and metabolic characteristics of immune populations at a single cell level. Using this technique we demonstrate the ability to identify metabolic heterogeneity in human T cell subsets and highlight the influence substrate exposure can have on metabolic pathways in immune cells.

Inhibition of inflammatory immune cell infiltration into the CNS using a novel heparan sulfate mimetic

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Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS) where immune cells attack the myelin sheath surrounding axons. Targeting immune cell trafficking into the CNS is an effective treatment for MS. However, complete abolishment of immune surveillance by some drugs, like natalizumab, can cause deadly side effects such as progressive multifocal leukoencephalopathy. Normally, the tightly controlled blood brain barrier (BBB) protects the CNS from inflammation by restricting, but not completely inhibiting, the entrance of immune cells. In MS, enzymes released at the BBB contribute to its breakdown. One such enzyme, heparanase, has been suggested as a therapeutic target for drug development in neuroinflammatory disorders, as it can be selectively inhibited by heparan sulfate (HS) mimetics. Historically, HS-mimetics have been inappropriate drug candidates due to their complex synthesis and anti-coagulant effects. The novel structure of our mimetic prevents it from exhibiting these issues. Our lab has found that therapeutic administration of this mimetic significantly reduces disease in experimental autoimmune encephalomyelitis (EAE), a murine model of MS. By maintaining integrity of the BBB, entrance of immune cells into the CNS is significantly reduced. Interestingly, this inhibition of inflammatory trafficking does not correspond with reduced homeostatic trafficking into the CNS as quantified by flow cytometry. Moreover, in vivo migration studies suggest that treatment with this HS-mimetic maintains chemokine gradients, which may enhance homeostatic trafficking in healthy animals. These findings suggest that this HS-mimetic selectively reduces neuroinflammatory trafficking, whilst maintaining the immune surveillance that is essential for CNS homeostasis.

Dietary modulators of the aryl hydrocarbon receptor influence GPR15 expression on human CD4+ T cells

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Diets high in fruit and vegetable are perceived to be beneficial for intestinal homeostasis and inflammatory bowel diseases (IBDs). Recent breakthroughs in the field of immunology have highlighted the importance of the ligand-dependent aryl hydrocarbon receptor (AhR) as a critical regulator of many aspects of immune homeostasis and function relevant to IBDs. Importantly, the AhR has been demonstrated to directly regulate the expression of GPR15 on CD4+ T cells.1,2 GPR15 is an important gut homing marker whose expression on CD4+ T cells in the peripheral circulation is elevated in patients suffering from ulcerative colitis, relative to healthy individuals.3 At present, no information is available on whether physiologically-relevant forms and concentrations of fruit and vegetable-derived metabolites can impact CD4+ T cell gut homing, via GPR15, in an AhR-dependent manner. Here we show that physiologically-relevant vegetable-derived metabolites exhibit AhR regulating properties and influence the level of GPR15 expression on human CD4+ T cells, as assessed by flow cytometry

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Breastfeeding Promotes Early Neonatal Regulatory T Cell Expansion and Immune Tolerance of Non-inherited Maternal Antigens

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Background: Breastfeeding is associated with long-term health benefits, such as a lower incidence of childhood infections, asthma, obesity and autoimmune disorders. However, little is known regarding how the maternal and neonatal immune systems interact after parturition when the neonate receives nutrition from maternal breast milk.

Methods: We undertook a comparative analysis of immune repertoire and function at birth and 3 weeks of age in a cohort of 38 term neonates born by caesarean section grouped according to feeding method (breast milk versus formula). We used flow cytometry to study the immune phenotype in neonatal and maternal blood samples and mixed lymphocyte reactions to establish the proliferation response of neonatal versus maternal lymphocytes and vice versa. The microbiome of neonatal stool samples was also investigated using 16S rRNA sequencing.

Results: We show that the proportion of regulatory T cells (Tregs) increases in this period and is nearly two-fold higher in exclusively breastfed neonates compared with those who received formula milk only. Moreover, breastfed neonates show a specific and Treg-dependent reduction in proliferative T cell responses to non-inherited maternal antigens (NIMA), associated with a reduction in inflammatory cytokine production. We also observed the enrichment of short chain fatty acid producing taxa (Veillonella and Gemella) in stool samples of exclusively breastfed neonates.

Conclusion: These data indicate that exposure of the neonate to maternal cells through breastfeeding acts to drive the maturation of Tregs and 'tolerizes' the neonate towards NIMA.

Exploiting the intrinsic insolubility of T cell and NK cell-activating cytokines and chemokines for Escherichia coli expression

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Two γ-chain cytokines (IL-2 and IL-15) and the CXCL11 chemokine were prepared using E. coli inclusion bodies (IB) to maximise yield and purity. The expression of IL-2, IL-15 and CXCL11 in IB allowed the application of harsh washing steps to deplete host contaminants. Solubilisation of washed IB in 6-8 M guanidine allowed capture in denaturing conditions on Ni-NTA columns with a rapid refolding technique using column equilibrium to native conditions with a simple REDOX shuffle. The activity of purified IL-2 and IL-15 was similar to commercially obtained stocks. The highly-basic CXCL11 peptide presented additional challenges, including that the N-terminal phenylalanine must be exposed for full agonist activity on CXCR3-expressing cells. However, the use of a PelB signal sequence immediately prior to the N-terminal phenylalanine did not generate recoverable functional CXCL11 in the E. coli periplasm or extracellular media. Successful expression of functional CXCL11 was achieved using a cleavable SUMO tag. The SUMO tag allowed for sufficient insolubility of the highly basic CXCL11 for harvest from IB, but facilitated sufficient solubility for SUMO-protease cleavage in native conditions. Following SUMO-protease cleavage, immediate precipitation of CXCL11 could be exploited to facilitate depletion of the water-soluble SUMO-tag and protease. The activity of purified CXCL11 was confirmed using competitive migration assays using 1:1 admixtures of GFP (control) and RFP-tagged Jurkat cells expressing CXCR3. These rapid and improved E. coli expression and protein purification protocols demonstrate that the inherent insolubility of clinically-relevant cytokines can be an asset to their purification from E. coli.