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Poster # 1

LUNG ILC2 RESPONSE TO VIRAL AND ALLERGEN CHALLENGE REQUIRES IL-7Rα SIGNALING

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Group2 Innate lymphoid Cells (ILC2s) are a group of immune cells found to be important in controlling airway inflammation and damage induced by diverse challenges. Proper development and function of these immune cells is necessary for recovery from infection and return to homeostasis. These cells are controlled by immune hormones called cytokines. Interleukin-7 (IL-7) is a cytokine produced by stromal cells or primary lymphoid tissues and is known for its importance in the development of various immune cells including ILC2s, however the mechanism by which it controls their development is unclear. Thymic stromal lymphopoeitin (TSLP) is a related cytokine produced primarily by epithelial cells in mucosal tissues. It signals ILC2s to produce cytokines and factors important for repairing damaged tissues. Using multiple loss-of-function and gain-of-function approaches we showed that IL-7 controls the expression of a critical transcription factor, GATA3, which is known to play an important role in the development of ILC2s in the bone marrow. We have also found that IL-7 and/or TSLP are important in ILC2 mediated immune response during influenza/A infection, with impaired cell numbers and cytokine production in IL-7Rα mutant and TSLPR KO mice. These findings demonstrate important roles for IL-7 and TSLP in controlling development of airway inflammatory responses mediated by ILC2s.

This research was supported by a grant to NA from the Canadian Institutes for Health Research (MOP-126060 and PJT-156119)

Poster # 2

Mutual Antibody T cell Engagers (MATEs): Driving Chimeric Antigen Receptors (CARs) toward improved safety and flexibility

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Chimeric Antigen Receptor (CAR) T cell therapy has achieved revolutionary success with some blood cancers, yet problems with response durability and toxicity remain. Our lab is developing a novel strategy called Mutual Antibody T cell Engagers (MATEs) that splits the CAR into two proteins (the extracellular antigen-binding domain and the intracellular signaling domain) and uses a leucine zipper to allow their interaction. With this approach, we anticipate MATE T cells to function only in the presence of the extracellular component in a dose-dependent manner, allowing fine-tunable control over MATE T cell activity *in vivo*.

Poster # 3

IL-6 receptor expression on T cells is required for the development of donor-specific antibodies in transplantation

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Organ transplantation is life-saving procedure for individuals with end-stage organ failure but its success is limited by immune-mediated rejection. Transplant rejection is caused by the activation of T cells towards allogeneic antigens expressed by graft cells. It is known that the inflammatory cytokine interleukin -6 (IL-6) plays an important role in activation of allogeneic immune responses but the cellular and signalling mechanisms by which IL-6 contributes to rejection are poorly understood. We have examined the role of IL-6 receptor (IL-6R) expression on T cells in controlling immune responses that cause transplant rejection by generating mice that lack IL-6R specifically in T cells (IL-6R-TKO). These mice lack IL-6R expression in CD4 and CD8 T cells but maintain expression of this receptor on myeloid cells. Aortic interposition transplants were performed from Balb/c (H-2 d) mice into the infrarenal aorta of wild-type and IL-6R-TKO C57Bl/6 (H-2 b) recipients. The activation of peripheral T cell responses, as indicated by CD4 and CD8 T cell accumulation in transplanted artery segments, was not affected in IL-6R-TKO graft recipients. The accumulation of macrophages in transplanted artery segments was also not affected in IL-6R-TKO mice. However, eliminating IL-6R in T cells markedly and significantly reduced the production of donor-specific antibodies toward allogeneic antigens. These findings suggest that IL-6R expression on T cells is not required for the activation of peripheral T cell responses but is needed for the development of antibody responses in transplantation.

Poster # 4

Optimization of CRISPR/Cas9-mediated homology-directed repair in human Tregs

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Targeted genome editing by CRISPR/Cas9 in tandem with homology-directed repair (HDR) allows the precise insertion of transgenes at specific genomic sites. This technology holds great promise not only as a research tool but also in ushering in the next generation of gene-edited Treg cell therapies with context-specific functions for autoimmunity and transplant rejection. Although CRISPR/Cas9-mediated HDR has recently been developed in human T cells, the experience in human Tregs is limited. Our approach is to combine CRISPR/Cas9 genome editing via ribonucleoprotein (RNP) electroporation with adeno-associated virus (AAV) to knock-in a reporter gene and achieve genome-edited Tregs that can be isolated by flow sorting. We adjusted T cell preactivation, electroporation voltages, and RNP format to achieve a gene knockout efficiency of 60-90% in human Tregs, as assessed at the genomic or protein level. Design and testing of guide RNAs targeting Treg-associated genes and the safe harbour locus *AAVS1* (control) revealed several candidates with high editing efficiency in Tregs. Corresponding HDR DNA templates encoding GFP were constructed and their performance validated in a cell line. We are currently producing recombinant AAV6 as HDR donors for human Tregs; future work will optimize AAV6 delivery in the context of RNP electroporation and cell expansion. Successful optimization of CRISPR-mediated HDR in human Tregs will enable the investigation of proteins critical for human Treg function and pave the way for gene-edited Treg cell therapies.

Poster # 5

Donor-specific chimeric antigen receptor Tregs restrain humoral alloreactivity

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Background: Cell therapy with autologous regulatory T cells (Tregs) is feasible and safe in solid organ transplantation. Phase II trials which aim to combine Treg therapy and immunosuppression minimization are planned but the ability of Treg cell therapy to limit the increased risk of humoral alloreactivity that comes with lower immunosuppression is unknown. We analyzed the effect of irrelevant or donor specific chimeric antigen receptor (CAR) Tregs on humoral alloreactivity in immunocompetent mice receiving skin allografts.
Methods: Tregs expressing an anti-HLA-A2-specific CAR (donor specific) or an anti-HER2 CAR (irrelevant) were administered to Bl/6 mice at the time of transplanting an HLA-$A2^+$ Bl/6 skin graft in the absence of immunosuppression in naive or sensitized mice. After three weeks, the amount of antigen-specific B cells, T cells and donor-specific antibodies were evaluated.
Results: We found that donor-specific CAR-Tregs, but not irrelevant CAR Tregs, significantly delayed skin allograft rejection. Moreover, only treatment with donor-specific CAR-Tregs resulted in diminished levels of transplant-induced donor-specific antibodies (DSAs) and frequencies of DSA-secreting B cells. Donor-specific CAR Tregs-treated mice also had weaker DSA-specific recall antibody response, but normal responses to an irrelevant antigen, demonstrating antigen-specific suppression. However, donor-specific CAR Tregs were not effective in previously sensitized mice.
Conclusion: These data show that via their ability to restrain both T cell and B cell-mediated rejection in immunocompetent recipients, donor-specific CAR Tregs have the potential to minimize antibody-mediated allograft rejection.

Poster # 6

Regulation of innate immune responses through mTOR during fetal life

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Pattern Recognition Receptor (PRR) responses are profoundly attenuated in human myeloid cells below 33 weeks of gestation. We have recently provided evidence that these responses are regulated by low cellular bioenergetics and reduced mTOR function resulting in limited translation of immune response proteins. Our data further indicate an important role for the negative mTOR regulator DDIT4L, which appears to be selectively upregulated in early ontogeny. We hypothesize that in conditions of low cellular bioenergetics, DDIT4L acts to limit translation of energetically costly, dispensable immune response genes.
To address this question, we engineered a stable THP-1 cell line to express the human DDIT4L gene "on-demand" from a tetracycline-inducible promoter, using a lentiviral transduction vector. We also assayed the developmental regulation of DDIT4L in human monocytes isolated from preterm (<33 weeks), term cord blood, and adult peripheral blood.

Using bioinformatics and genome-wide gene expression data, we found that expression of *DDIT4L* after LPS stimulation of monocytes strongly inversely correlates with expression of genes encoding mitochondrial and ribosomal components. We show that the *DDIT4L* gene is selectively upregulated in neonatal monocytes, upon LPS, and more so at lower gestational age and when cellular energy production is constrained by hypoxia and low glucose. We generated a series of transduced THP-1 clones that express graded levels of DDIT4L, which will be used to study the dose-dependent impact of this molecule on mTOR function, glycolysis, mitochondrial stability and translation.

Collectively, our results support an important role for DDIT4L in regulating innate immune responsiveness during human fetal ontogeny. Experiments are underway to confirm the effect of DDIT4L on selectively preventing translation of inflammatory genes in human myeloid cells. We speculate that this mechanism allows cells to fine-tune their immune response machinery to prevent overt inflammation during development *in utero*.

Poster # 7

SspH2 Ubiquitination of Nod-like receptors and the role it plays in innate immune modulation

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Salmonella enterica serovar *typhimurium* is a Gram-negative bacterium that replicates in mammalian host cells and causes food-borne illness in approximately 87 500 Canadians every year. Essential to *Salmonella* pathogenesis is the needle-like Type III Secretion System, which injects proteins into host cells to facilitate bacterial invasion and survival. *S. enterica* has several novel E3 ubiquitin ligase (NEL) effectors, that when absent, compromise bacterial growth in the host. NEL effectors such as SspH2, attach ubiquitin protein to lysine residues on host proteins to modify their functions or cause their destruction. SspH2 and host SGT1 interacts with host NOD-like receptors (NLRs) to enhance host innate immune mediated inflammation. This is beneficial for *S. enterica* because this inflammation kills commensal bacteria in the gut and releases nutrients from the host, enabling more efficient host colonization.

I hypothesize that SspH2 ubiquitinates NLRs directly on the lysine residue in their P-loop to increase innate immune response activity. HEK293T cells have been co-transfected with NOD1/NOD2 and SSpH2/ SSpH2 C580A (catalytic mutant) alongside Myc-ubiquitin to determine bacterially mediated ubiquitination of host NLRs. My data illustrates reciprocal binding of host NLRs with SspH2, alongside SspH2 induced ubiquitination. Additionally, HeLa cells have been transfected with NOD2 and NOD1 in addition to SspH2/SspH2 C580A to determine IL-8 secretion. My data indicates that there is an increased level of IL-8 secretion found with and without NOD2 agonist. Additionally, when SspH1 and SspH2 are present together, the pro-inflammatory SspH2 phenotype is sustained. This data suggests that SspH2 acts on multiple NLRs to induce a pro-inflammatory response by host cells. This knowledge will help elucidate the fundamentals of NEL effector biology and how NELs induce an altered host response. This is important for understanding mechanisms that can be utilized for antimicrobial interventions.

Funding: New investigator start up grant and Li Ka Shing institute of Virology

Poster # 8

Transcriptomic analysis of host immune responses for refining the diagnosis of neonatal sepsis

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Introduction: Neonatal sepsis represents a major cause of mortality worldwide. Diagnosis is challenging since clinical signs are nonspecific. Blood cultures are often unreliable in infants and can be either falsely negative in >50% of cases or contaminated. As a result, we seriously lack accurate data on the incidence of bacterial sepsis in low-income countries. This can lead to overtreatment with antibiotics, which encourages antimicrobial resistance. The transcriptomic signature of the host immune response to an infection may provide an accurate alternative in identifying newborns truly infected with bacterial pathogens.

Methods: Prospective cohort of 300 infants under 3 months evaluated for neonatal sepsis at the Kamuzu Central Hospital in Lilongwe (Malawi). Standardized clinical data is captured electronically and synchronized in a REDCap database in Vancouver. Biological samples (blood culture, complete blood count and RNA protected whole blood) are collected. Whole blood RNA will be extracted and sequenced on an Illumina NextSeq 500. RNA-seq data will be used to generate a gene-based classifier, which will identify the optimal number of features (genes) that best discriminate infants with bacterial sepsis versus those with non-bacterial sepsis or other sepsis-like syndromes.

Results: Enrollment began in June 2018 and 274 infants have been enrolled so far. Data have been captured electronically for 256 of these infants (median 3 days of life; 10% mortality). Of these, 57 (23%) have a positive blood culture. RNA protected whole blood has been collected in 92% of infants. Microbial etiologies are diverse, with a majority of gram-positive bacteria.

Future directions: Enrollment is expected to close on August 30th, 2019. This study will be the first to validate the incidence of bacterial sepsis and the data obtained from blood cultures in a low-income country. We hope that these data will inform policies to use antibiotics more judiciously in these areas of the world.

Poster # 9

Regulatory T cell-specific deletion of the insulin receptor prevents diet-induced and age-associated metabolic syndrome

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Adipose tissue (AT)-resident regulatory T cells (Tregs) are important regulators of local and systemic inflammation and metabolism. We have previously found that insulin receptor signaling inhibits Treg suppressive function in vitro, and hyperinsulinemia is associated with alterations in visceral AT Tregs in vivo. To directly test the role of Treg-intrinsic insulin receptor signaling, we generated *Foxp3*^{cre}*InsR*$^{fl/fl}$ mice and fed them either chow or high-fat diet (HFD) to induce hyperinsulinemia and obesity. Compared to *Foxp3*^{cre} mice, *Foxp3*^{cre}*InsR*$^{fl/fl}$ mice had improved glucose tolerance and insulin sensitivity after 13 weeks of HFD. This protective metabolic effect was associated with increased brown AT ST2^{+} Treg numbers and functions and dampened AT inflammation in *Foxp3*^{cre}*InsR*$^{fl/fl}$ mice, rather than changes in food consumption or energy expenditure. Moreover, *Foxp3*^{cre}*InsR*$^{fl/fl}$ mice were protected from age-associated glucose intolerance and insulin resistance as determined in 52-week-old mice. Unlike in the HFD cohort, visceral AT Treg numbers and functions were greatly reduced in aged *Foxp3*^{cre}*InsR*$^{fl/fl}$ mice, leading to increased inflammation in the visceral AT. Surprisingly, elevated AT inflammation was associated with improved metabolic outcomes. Gene expression analysis revealed differential gene signatures in AT Tregs and total AT inflammation, suggesting that the underlying mechanisms contributing to the metabolic syndromes were distinct between the two insulin resistance models. Together, these data suggest that hyperinsulinemia contributes to metabolic disease in part by differentially affecting Treg control of AT inflammation in diet-induced versus age-associated metabolic syndrome.

Poster # 10

Combined Analysis of Flagellin-Specific Immune Responses and Microbiome Diversity in IBD Patients

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BC Children's Hospital

Inflammatory bowel disease (IBD) is a chronic intestinal inflammation of the gut, consisting of two closely related diseases, Crohn's Disease (CD) and Ulcerative Colitis (UC). Both diseases are partly driven by pathogenic T cell responses to components of commensal bacteria, with evidence that flagellin may be a key antigen driving IBD pathogenesis. Chronic inflammation in IBD leads to a microbial imbalance in the gut, resulting in a loss of key beneficial bacteria, further perpetuating the disease. Using stool and peripheral blood samples from IBD patients and healthy controls, we investigated the relationship between the gut microbiome composition and adaptive immune responses to *Lachnospiraceae*-derived A4-Fla2 and *E. coli* H18 FliC flagellin antigens. We used a flow cytometry-based assay to detect circulating flagellin-specific $CD4^+$ T cells following antigen stimulated upregulation of CD25 and OX40, and detected anti-flagellin IgG and IgA by ELISA. We observed that, compared to healthy controls, IBD patients had enriched proportions of Fla2-specific $CD4^+$ T cells and anti-Fla2 antibodies. Microbiome analysis utilized 16s rDNA sequencing, with IBD patients and healthy controls clustering significantly separately by -diversity analysis. As expected, we observed a reduced Shannon's diversity in IBD patients, with lower diversity correlating with greater disease severity and higher proportions of circulating flagellin-specific $CD4^+$ T cells. Differential abundance analyses confirmed enrichment of previously reported bacterial species in IBD patients. Importantly, we show that the relative abundance of these enriched bacteria positively correlated with immune responses to flagellin antigen. These data are the first report of relationships between gut bacteria and flagellin-specific immune responses in IBD patients.

Poster # 11

Pharmacological inhibition of RORγt preserves human Th17-like regulatory T cell stability

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Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, are incurable chronic conditions that result from uncontrolled gut inflammation. Pathogenic Th17 cells, characterised by production of IL-17 in the absence of IL-10, are thought to contribute to this inflammation, but in humans, antibody-mediated blockade of IL-17 is an ineffective IBD therapy. We hypothesized that anti-IL-17 antibodies may not completely disable Th17 cells, and moreover could have deleterious effects on Th17-like FOXP3^{+} regulatory T cells (Th17-like Tregs). We investigated whether pharmacological inhibition of RORγt, the Th17 cell lineage-defining transcription factor, was an alternate approach to inhibit Th17 cells. Addition of BMS-336, a small molecule RORγt inhibitor, to human peripheral Th17 (CXCR3^{-}CCR4^{+}CCR6^{+}) and Th17.1 (CXCR3^{+}CCR4^{+}CCR6^{+}) cells inhibited expression of RORγt target genes in a dose-dependent manner. Similarly, IL-17 production by laminar propria mononuclear cells, isolated from IBD and non-IBD subjects, was significantly inhibited by BMS-336. BMS-336 also inhibited expression of RORγt-regulated genes in Th17-like Tregs (CD4^{+}CD25^{hi}CD127^{lo}CXCR3^{-}CCR4^{+}CCR6^{+}) without affecting expression of FOXP3 or their suppressive function. Interestingly, RORγt inhibition significantly increased production of IL-10 in Th17-like Tregs. When cultured under proinflammatory conditions, Th17-like Tregs were destabilised, as evidenced by loss of FOXP3 expression and re-methylation of the Treg specific demethylation region; this destabilisation was repressed by BMS-336. Overall, these results demonstrate that inhibition of RORγt is a promising approach to selectively inhibit Th17 cells, and in parallel enhance the function Th17-like Tregs by increasing IL-10 production and restraining their lineage instability in the presence of inflammation.

Poster # 12

Reduction of oncogene-regulated CCL5 production in lung cancer cell leads to an altered immune microenvironment and decreased tumor burden

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Lung cancer development is driven by the expression of mutant oncogenes, with EGFR and KRAS being the most frequent mutations in lung adenocarcinoma (LUAD). Tumor cells can produce cytokines that alter the local immune environment and influence tumor development and progression. To identify cytokines that are regulated by oncogenic signaling during early lung tumorigenesis we used normal cells expressing doxycycline inducible mutant $KRAS^{G12V}$, $EGFR^{L858R}$ or wild-type EGFR and analyzed cytokine production with a multiplex assay (LUMINEX). Induction of $KRAS^{G12V}$ or $EGFR^{L858R}$ expression in normal cells rapidly increased CCL5 and CCL2 production. In KRAS mutant lung cancer cells, CCL5 production was decreased following disruption of oncogene signaling with a MEK inhibitor (Trametinib) or by KRAS knockdown. To test the effects of tumor-derived CCL5 on the immune environment and tumor growth *in vivo*, we knocked down CCL5 in murine lung tumor cells and injected the cells intravenously into mice. Mice injected with CCL5 knockdown cells had decreased macrophages and regulatory T cells (Tregs) within the lungs and reduced tumor burden. We used CIBERSORT to quantify 22 immune cell types in over 300 human LUAD and 100 matched normal lung tissues. Inflammatory macrophages (M1, M0), T follicular helper cells, plasma cells, and immunosuppressive Tregs were significantly enriched in early stage LUAD tumors. These data suggest that oncogenic signaling regulates expression of CCL5 in lung tumor cells, and that reducing tumor-derived CCL5 production leads to disruption of tumor-induced immune suppressive environment in the lungs and decreased lung tumor growth. Therefore, targeted inhibition of CCL5 may represent therapeutic strategies to block the recruitment of immune suppressive cells to the tumor microenvironment, thereby enhancing the anti-tumour immune response.

Poster # 13

A novel mouse model based on the NR1H3 R415Q mutation linked to progressive MS in families presents with a proinflammatory phenotype and worse disability secondary to demyelinating neurodegeneration.

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Background: Multiple sclerosis (MS) is a neurodegenerative disease of the central nervous system characterized by inflammatory demyelination and disability secondary to axonal loss. 15% of patients present with primary progressive MS (PPMS) where disease worsens without recovery, and most patients with relapsing MS ultimately progress. Unfortunately, the lack of etiologically relevant models of progressive MS has hindered the development of effective treatments. The nuclear receptor subfamily 1 group H member 3 (*NR1H3*) R415Q mutation was identified in two Canadian families linked with severe and rapidly progressing PPMS. NR1H3 transcriptionally regulates immune cell function and proteins required for lipid and cholesterol homeostasis. We developed a mouse model of progressive MS carrying a homologous *NR1H3* mutation to characterize the immunological processes driving risk and progressive disease in MS.

Methods: Spleen, liver, brain, and spinal cord from heterozygous (HET) and homozygous (HOM) *NR1H3* mutant and wild-type (WT) mice were analyzed by whole transcriptome sequencing. Serum was screened using enzyme-linked immunosorbent assays (ELISA) and flow cytometry to assess immune profiles. *NR1H3* mice were immunized to induce the experimental autoimmune encephalomyelitis (EAE) model of MS, and the mice were scored over 50 days for clinical disease. Immunohistochemistry was performed on spinal cord sections to characterize inflammatory neurodegeneration.

Results: Transcriptome analysis showed significant differences in gene expression in the spleen and liver of *NR1H3* HOM compared to WT mice including CD5 antigen-like (*Cd5l*), which is a key regulator of Th17 pathogenicity and lipid homeostasis. Serum CD5L levels in *NR1H3* HOM mice tended to be lower than WT. *NR1H3* mice also had increased serum IL-17A, IL-16, and IP-10 levels compared to WT. *NR1H3* mice spleens had reduced polarization of macrophages with a reparative M2-like phenotype. *NR1H3* EAE mice had more severe disability, spasticity, earlier disease onset, and weight loss compared to WT EAE mice; this difference was most significant in male mice. Histologically, *NR1H3* EAE showed increased microglia activation, gliosis, demyelination, axonal damage, and lipid accumulations in the spinal cord compared to WT EAE.

Conclusions: The *NR1H3* mutation dysregulates immune and lipid clearing pathways, which alters key mediators of inflammation and repair in MS. This contributes to increased clinical and immunopathological disease severity associated with progression that provides support to validate this mouse as a valuable model of progressive MS.

Poster # 14

The MicroRNA-17~92 Cluster in CD8 T Cell Function and Response to Lung Adenocarcinoma

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The microRNA-17~92 (miR-17~92) cluster was shown to be upregulated in patients with lung adenocarcinoma (LAC), the most common form of non-small cell lung cancer (NSCLC). High expression of microRNA-17 from the cluster in serum of LAC patients was associated with decreased survival. Additionally, the miR-17~92 cluster was shown in T cells to regulate interleukin-7 (IL-7) signaling, the PD-L1/PD-1 axis, and in promotion of CD8+ T cell cytotoxic activity. Tumor-infiltrating CD8 T cells are essential for tumor clearance but are suppressed by inhibitory receptor blockade resulting in downregulation of activation-induced cytokines and proliferation. In this study, we investigated the role of miR-17~92 in lung tumor cell mediated suppression of infiltrating CD8 T cells. We hypothesize that upregulation of miR-17~92 cluster in LAC tumor cells, lead to a Th2 skewed tumor microenvironment, and induce reduction in CD8 T cell IL-7 responsiveness and anti-tumor function. We initially showed alteration to lymphocyte cell populations in LAC patients with high expression of microRNAs in the miR-17~92 cluster. Next, we used in vivo models of LAC to assess whether infiltrating populations of CD8+ T cells with low IL-7 receptor alpha (IL-7Rα) and high checkpoint receptor expression have alteration to the miR-17~92 cluster. Understanding lung tumor cell upregulation of the miR-17~92 cluster, and their putative regulation of IL-7 signaling and checkpoint receptor expression on infiltrating CD8 T cells in the tumor microenvironment would aid in selection of efficacious immunotherapy treatment.

Poster # 15

Cytoskeleton-dependent processes in B-Lymphocytes are regulated by the carboxyl tail of Connexin43

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Cytoskeleton-dependent processes such as adhesion, extension of protrusions, membrane spreading, immune synapse formation, motility, and directed migration are necessary for normal B cell development and immune responses. Signaling by the B cell antigen receptor (BCR), integrins and chemokine receptors results in cytoskeletal rearrangements that are required for these cellular events. We have previously shown that the gap junction protein, Connexin43 (Cx43) is important for B cell adhesion, spreading, motility, directed migration, and extravasation through endothelial cell layers. Further work has shown that the carboxyl tail (CT) of Cx43 is critical for these effects. However, whether the effects of the CT are independent of the rest of the protein as well as the specific domains and residues responsible for such effects were not understood. Using a chimeric protein composed of the CT of Cx43 fused to the extracellular and transmembrane portions of CD8, we were able to express the chimera at the plasma membrane. Then we showed that the CT localized at the plasma membrane, was sufficient to support BCR-mediated cell spreading independent of the rest of Cx43. Truncations of the CT defined the region of the CT between amino acids 246-307 as important for supporting B cell spreading. In addition, a series of point mutations of the tyrosine (Y) and serine (S) residues revealed their differential importance in B cell spreading. Specifically, Y247F, Y265F and Y267F, as well as S279A and S282A mutations impeded cell spreading, hence found important for membrane spreading, while S255A and S262A had no effect on spreading. We propose that this reflects unique roles for these serine residues as potential recruitment sites for protein complexes involved in the remodeling of the actin cytoskeleton. Current studies include determining the effects of these key sites on cell motility and chemokine directed migration. Future work includes identifying the proteins that interact with the Cx43 CT and how these interactions influence the dynamics of the actin network and possible interplay with other cytoskeleton elements. The results may reveal critical interactions that are nucleated by the Cx43 CT and link receptors to molecular mechanisms that control the cytoskeletal architecture that are important for B-lymphocyte immune responses.

Poster # 16

Creating reovirus variants with improved oncolytic potency

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Reovirus is a nonpathogenic virus that naturally inhabits the enteric tract of humans. Reovirus can also selectively infect and replicate in tumor cells and is therefore a candidate for cancer therapy. Given that wild-type reovirus is naturally adapted to enteric environments (rather than tumors), our laboratory used positive selection to isolate 13 reovirus variants that replicate more efficiently in tumor cells relative to wild-type reovirus (T3wt). Since these 13 reovirus variants have 1-4 mutations each, we did not know which mutations were important or dispensable. To identify the mutations critical for enhanced oncolysis, I introduced 24 mutations into the reovirus reverse genetics plasmids using site-directed mutagenesis. Then, I generated viruses with either single mutations, or various combinations of 2 or 3 mutations. Using plaque size to reflect the proficiency of reovirus replication in tumor cells, I found that beneficial mutations can have additive effects on oncolytic potency. The oncolysis-promoting mutations predominated in reovirus structural protein lambda 2 and the sigma 1 cell attachment protein. I found that most viruses with increased oncolytic activity than T3wt had reduced sigma 1 protein levels, a previously well described mechanism of increased oncolysis. Interestingly, there are some variants with mutations in sigma 1 that have similar sigma 1 levels to T3wt, which could indicate a new and different mechanism.

Concurrently, by using a syngeneic mouse model of breast cancer, I observed that T3wt reduced tumor growth and improved survival as expected. In future animal experiments, I will compare the oncolytic activity of reovirus variants to identify the most-oncolytic reovirus platform and how they induce innate and adaptive anti-tumor immune responses. My experiments will provide a better understanding of reovirus biology and how to improve reovirus as a cancer therapeutic.

Poster # 17

Multi-parameter Monitoring of the Immune Response in Transplantation

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Aim: To enable rapid and innovative monitoring of the dynamic changes in the recipient immune response in organ and cellular transplantation through bioinformatics integration of precise convergent assays for deep phenotyping and cellular bioenergetics.

Methods: Peripheral blood of transplant recipients and controls was collected and deep phenotyping was performed using multichannel CytoFLEX cytometry (Coulter) and mitochondrial and cytosolic bioenergetics of T lymphocytes using the Seahorse XFe96 Analyzer (Agilent). Additionally, RNA was stored for transcriptomics.

Results: Panels of 55 antibodies provided precise cytometric multichannel definition of innate and adaptive immune cells, for which T-distributed Stochastic Neighbor Embedding (tSNE) and Hierarchical Consensus Clustering enabled automated visualization of surface molecule expression and machine representation of an exceptional array of memory, helper, regulatory and other critical cell subsets. Mitochondrial and cellular respiration were quantitated by simultaneous measurement of the extracellular acidification rate (ECAR), an indicator of glycolysis and the oxygen consumption rate (OCR) an indicator of oxidative phosphorylation, with and without the use of agents targeting the electron transport chain to determine an array of metabolic parameters including basal respiration, ATP production, maximum respiration and spare respiratory capacity.

Conclusion: The combination of deep phenotypic and functional measurements provides a novel foundation for understanding of the changes in the immune system in transplantation. Further phosphoproteomics and transcriptomics will complete multi-parameter monitoring, using bioinformatics integration and visual analytics to streamline clinical interpretation.

Poster # 18

Homozygosity for a novel *CARD11* mutation causes profound combined immunodeficiency, inflammatory gastrointestinal disease, and complete abrogation of MALT1 activity

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BACKGROUND: The CARD11–BCL10–MALT1 (CBM) complex is a critical signalling adaptor that regulates lymphocyte activation, proliferation, and survival. Primary immunodeficiencies (PIDs) affecting each component result in broad clinical manifestations ranging from combined immunodeficiency (CID) to lymphoproliferation. We present the laboratory and clinical findings of the 4th and 5th ever cases of CARD11 deficiency in two Canadian First Nations patients found to be homozygous for a novel *CARD11* mutation.

RESULTS: We have identified an 8-month-old boy who presented with a severe case of entero/rhinovirus bronchiolitis with interstitial lung disease and a 17-year-old boy with a history of severe pulmonary infections, chronic sinusitis, candidiasis, invasive bacteremia, and severe ileo-colitis and oral ulceration requiring total colectomy. Both patients lacked Tregs and memory B cells, and possessed hypogammaglobulinemia. Sequencing both patients revealed homozygosity for the same novel variant of *CARD11* (c.2509C>T; p.R837*), which rendered CARD11 protein undetectable by immunoblot. To confirm CARD11 deficiency, we stimulated patient B cells with phorbol 12-myristate 13 acetate (PMA) and ionomycin across a time-course and immunoblotted for various signalling proteins in the NF-κB and MAPK pathways and cleavage substrates of the MALT1 paracaspase. NF-κB and JNK activation were completely absent, MALT paracaspase activity was lost, and the CBM complex could not be assembled.

CONCLUSIONS: These two cases highlight the crucial role of CARD11 in regulating lymphocyte development, function, and humoral responses. In addition, we have identified the oldest known living individual with CARD11 deficiency and he presented uniquely with inflammatory gastrointestinal disease in addition to CID, thus further broadening the spectrum of phenotypes associated with CARD11-related PIDs.

Poster # 19

The ATP-Binding Cassette Gene ABCF1 Functions as an E2 Ubiquitin-Conjugating Enzyme Controlling Macrophage Polarization to Dampen Lethal Septic Shock

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Sepsis is a bi-phasic inflammatory disease that threatens approximately 30 million lives and claims over 14 million annually, yet little is known regarding the molecular switches and pathways that regulate this disease. Here, we have described ABCF1, an ATP-Binding Cassette (ABC) family member protein, which possesses an E2 ubiquitin enzyme activity, through which it controls the Lipopolysaccharide (LPS)-Toll-like Receptor-4 (TLR4) mediated gramnegative insult by targeting key proteins for K63-polyubiquitination. Ubiquitination by ABCF1 shifts the inflammatory profile from an early phase MyD88-dependent to a late phase TRIF-dependent signaling pathway, thereby regulating TLR4 endocytosis and modulating macrophage polarization from M1 to M2 phase. Physiologically, ABCF1 regulates the shift from the inflammatory phase of sepsis to the endotoxin tolerance phase, and modulates cytokine storm and interferon-b (IFN-b)-dependent production by the immunotherapeutic mediator, SIRT1. Consequently, ABCF1 controls sepsis induced mortality by repressing hypotension-induced renal circulatory dysfunction.

Poster # 20

Mortality and Morbidity in Scleroderma Renal Crisis: A Systematic Literature Review

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***Objectives*:** The objective of this study was to systematically review the mortality and morbidity associated with scleroderma renal crisis (SRC) and to determine temporal trends.

***Methods*:** We searched Medline, EMBASE and the Cochrane Database of Systematic Reviews from database inception to January 15, 2019. Bibliographies of selected articles were hand-searched for additional references. Data were extracted using a standardized extraction form. Study quality was assessed using the Newcastle-Ottawa scale. Results were analyzed qualitatively.

***Results*:** Twenty studies with 14,059 SSc subjects, of which 854 had SRC and 4095 had SSc-associated end stage renal disease (SSc-ESRD), met inclusion criteria. Study quality was generally moderate. Cumulative mortality in the post-angiotensin converting enzyme (ACE) inhibitor era was approximately 20% at 6 months, 30-36% at 1 year, 19%-40% at 3 years and almost 50% at 10 years from SRC onset. Although the introduction of ACE inhibitors in the early 1970s resulted in a 50% improvement in SRC mortality, there was no further improvement thereafter. SRC mortality rates were proportionally higher than mortality rates associated with other SSc organ involvement. The rate of permanent dialysis after SRC in the post-ACE inhibitor era ranged from 19-40%. Three to 17% of SSc patients underwent renal transplant. Survival was better in patients post-renal transplant (54-91%) compared to those on dialysis (31-56%). Graft survival improved over time and appeared similar to that of patients with other types of ESRD.

***Conclusions*:** SRC mortality and morbidity remain high. Novel treatments are required to improve outcomes of SRC.

Poster # 21

Tumour-derived G-CSF dysregulates hematopoiesis and leads to impaired responsiveness to immunotherapy due to heightened immunosuppression

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Immunotherapy has improved patient outcome and survival in a subset of human malignancies. However, particular types of tumours and a significant number of patients do not respond to current therapies highlighting the need to better understand the interactions between tumours and the immune system. Phagocytes, such as dendritic cells (DCs), macrophages and myeloid-derived suppressor cells (MDSCs) are critical in orchestrating or antagonizing innate and adaptive immune responses against tumours. In turn, phagocyte activity and development are heavily dependent on tumour-derived factors.

We identified granulocyte colony-stimulating factor (G-CSF) as a potent tumour-derived cytokine able to alter phagocyte development and function, resulting in tumour-induced immunosuppression. Using Cytometry by Time-of-Flight (CyToF) we show that tumour growth, and associated G-CSF expression, leads to a systemic accumulation of a complex mixture of immature myeloid cells, and perturbations in a wide variety of signalling pathways throughout the immune system. Our results show that G-CSF impairs DC maturation and induces the expansion of immature myeloid cell progenitors and MDSCs. Interestingly, G-CSF is particularly powerful in terms of its ability to block the development of a subset of DCs ($CD103^+CD11b^-$), the abundance of which, has been associated with improved cancer treatment outcome and strong $CD8^+$ cytotoxic T cell activation potential.

Our *in vivo* data using syngeneic breast cancer models show that tumour-secreted G-CSF poses a barrier to adoptive T cell therapy. Neutralization of G-CSF in the AOM/DSS spontaneous colon cancer model reduces tumour burden, MDSC accumulation and normalizes colonic immune cell composition. Together our data suggest that modulating tumour-secreted G-CSF could be key to improving immunotherapy efficacy in cancers associated with G-CSF-induced myeloid lineage perturbations and immunosuppression.

Poster # 22

Bacterially derived oligomannose mimetics for the elicitation of glycan-specific neutralizing antibody responses to HIV-1

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The recovery of many human broadly neutralizing antibodies (bnAbs) specific for a patch of oligomannose-type glycans on the HIV spike has highlighted it as a vaccine target [1]. However, immunogens that readily evoke bnAbs to this patch have not been forthcoming. One possible reason may be immune tolerance restrictions related to the host origin of the target glycans. To overcome these potential restrictions, we are exploring the concept of molecular mimicry, using bacterially derived analogs of mammalian oligomannose, to elicit the desired bnAb responses. Using as template a bacterial oligosaccharide backbone that resembles the D1 arm of oligomannose, we have synthesized oligomannose mimetics with a D3 arm-like extension and shown that a BSA conjugate of the lead mimetic evokes modest tier 2-level nAb responses upon immunization of human-antibody transgenic animals [2]. We have now elaborated on the design of the published conjugates. First, our lead mimetic has been conjugated to CRM197, a more clinically apt carrier protein than BSA, at various glycoside densities. Second, a derivative of the lead mimetic has been synthetized integrating a bacteria-specific Kdo-lipid A moiety. We have pursued detailed antigenic characterization of these new conjugates with various oligomannose patch-specific bnAbs and their inferred germline precursors by ELISA, SPR and flow cytometry. Results with a CRM197 conjugate show greater nAb affinity relative to the equivalent BSA conjugate. This affinity seems to increase with a greater glycoside density. Results show also binding of the estimated germline precursors of the different nAbs tested, which is encouraging for further ongoing immunogenicity studies *in vivo*.

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[2] Pantophlet *et al., Nat. Commun.* 8, 1601 (2017).

Poster # 23

Identifying Secreted Proteins that Promote Immune Evasion during Malignant Lung Transformation

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Lung cancer is the leading cause of cancer-related deaths in Canada, with lung adenocarcinoma as the most common subtype. High mortality is partially attributed to late diagnosis. To decrease mortality, earlier detection is needed. Diagnostic biomarkers, such as secreted proteins, are a sensitive method for detection. I aim to identify secreted protein changes during malignant transformation of lung epithelial cells to lung adenocarcinoma *in vitro*.

Immortalized human bronchial epithelial cells (HBEC) were infected with lentiviral vectors expressing oncogenic mutations EGFR L858R or KRAS G12V. To promote transformation, this will be done in conjunction with a c-terminal p53 construct. Cell transformation will be confirmed with anchorage-dependent and independent growth assays. Cells will then be grown to confluency in standard serum-free media. Media will be switched to one with reduced supplements for 72 hours. Collected media will be spun, filtered, and then concentrated with a 3kDa cut-off filter. Concentrated media will be analysed with tandem mass tag mass spectrometry. Secreted proteins that show significant relative-fold change will be validated with ELISA. Validated proteins will be assessed for effects on immune cells in phenotypic assays.

HBEC cells expressing either mutation suggest greater clonogenic potential, relative to the vector control. However, cells fail to show anchorage-independent growth. Oncogenic mutations EGFR L858R and KRAS G12V appear to be insufficient to confer HBEC transformation. Future work will focus on establishing a concrete, transformed phenotype *in vitro* to examine secreted proteins.

Poster # 24

Analysis of HLA Allele and Eplet Distributions in BC's Kidney Transplant Patient and Recipient Populations

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AIM: This study presents HLA allele and eplet frequencies in BC patient and donor populations to explore the likelihood of matching by each method as a foundation for a national eplet-matching program in kidney transplantation.

METHODS: Next Generation Sequencing was performed in 1846 patients and donors for all 11 classical HLA genes. Alleles were converted to eplets with HLAMatchmaker, and frequencies were compared across donor and patient groups using statistical models and k means clustering.

RESULTS: 361 alleles were identified (206 class I, 155 class II) and translated to 148 eplets (59 class I, 89 class II); a 59% reduction in complexity. There was a complex relationship between alleles and eplets: eplet 193PV was encoded by 51 alleles whereas B*07:02 allele encoded 6 eplets. Allele frequencies across all 4 groups of transplanted patients, pre-transplant patients, living donors, and deceased donors were lower than eplet frequencies. Only one allele (DPA1*01:03) occurred in more than 48% of subjects, whereas eplet frequencies were more homogeneous, with some eplets occurring in >98% of subjects (i.e. 85VG). Overall allele frequencies differed significantly between patient and donor groups ($p<0.0001$) but eplet frequencies were comparable in all groups (p=N.S). Class I eplets formed several discrete clusters associated with specific alleles: cluster 1 included several eplets coded by HLA-A*01 and cluster 2 eplets coded by HLA-A*02, while cluster 3 contained neither of these nor other key eplets observed in cluster 1 or 2.

CONCLUSION: Even in the highly ethnically diverse population of BC, eplet typing markedly reduces the complexity of HLA identity by comparison with allele typing. Eplet frequency profiles are comparable between patient and donor populations, and subdivide into a small number of principal clusters. These results suggest that eplet typing may simplify histocompatibility matching for kidney transplantation.

Poster # 26

Expansion of thymus-derived regulatory T cells using GMP-compatible reagents

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Animal and early clinical studies have shown that regulatory T cell (Treg) therapy can prevent graft-versus-host disease (GVHD) following hematopoietic stem cell transplant. Despite these promising results, challenges remain for Treg therapy including the difficulty in isolating and expanding sufficiently pure cells. We previously found that the thymus, routinely removed during pediatric cardiac surgery, is a plentiful source of Tregs that can be easily isolated and prevent xenoGVHD in mice. To use this therapy in humans we are now developing methods for isolation and large-scale expansion of thymic Tregs using GMP-compatible reagents. $CD4^+CD8^-CD25^+$ thymic Tregs were isolated from pediatric thymuses using GMP-compatible magnetic bead-based separation. Tregs were expanded in the presence of IL-2 and rapamycin using anti-CD3/CD28 or anti-CD3/CD28/CD2 antibody tetramers, anti-CD3/CD28 expansion beads or artificial antigen-presenting cells. Thymic Treg numbers expanded *in vitro* when cultured with GMP-compatible expansion reagents. The highest expansion was obtained using anti-CD3/CD28 expansion beads. Cells cultured with these reagents expressed high levels of FOXP3, maintained expression of Treg-characteristic markers, produced low levels of inflammatory cytokines and suppressed the proliferation of anti-CD3/CD28 stimulated T cells. Thymic Tregs can be isolated and expanded with GMP-compliant expansion reagents, maintaining FOXP3 expression and suppressive function. Once the protocol is established, we will transfer our protocols to a GMP facility for scale-up. We are the first group to pursue clinical application using allogeneic thymic Tregs, providing an opportunity for an “off-the-shelf” therapy to treat GVHD.

Poster # 27

Composition of the Gut Microbiota and its Metabolites Regulate Neutrophil Responses Towards Vascular Allografts

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Background: The gut microbiota influences immune development and activation, in part through the properties of short chain fatty acids (SCFAs; acetate, propionate, butyrate) produced by the bacterial fermentation of dietary fiber. Disruption of the gut microbiota with antibiotics early in the life of murine graft recipients exacerbates neutrophil responses towards vascular allografts. We have examined how the gut microbiota is involved in exacerbating neutrophil responses in this setting.
Methods: Rejection of vascular grafts was examined using a murine aortic interposition model. The gut microbiota was disrupted by administration of an antibiotic cocktail (ampicillin, vancomycin, metronidazole, neomycin sulfate) for the first 3 weeks of life in graft recipients. Gut microbiota in antibiotic-treated and untreated mice was normalized by co-housing and the role of the SCFA acetate determined by delivery of this metabolite through the drinking water.
Results: Disruption of the gut microbiota in graft recipients by treating them with antibiotics for the first 3 weeks of life resulted in increased neutrophil accumulation in allograft arteries. This effect was not associated with an increase in circulating neutrophils or in acute neutrophil activation as determined by neutrophil infiltration in an air pouch model of acute inflammation. Cohousing of antibiotic treated with untreated mice completely ablated neutrophil accumulation in allograft arteries. Also, treatment of graft recipients with magnesium acetate similarly prevented neutrophil accumulation in grafts placed into antibiotic-treated mice.
Conclusion: In transplant recipients, neutrophil dysregulation caused by antibiotic treatment early in life is a result of differences in the composition of the gut microbiota and may involve immunoregulatory processes controlled by acetate.

Poster # 28

Identification of novel Adenosine Deaminase 2 gene variants and varied clinical phenotype in pediatric vasculitis.

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Objectives: Individuals with deficiency of adenosine deaminase 2 (DADA2), a recently recognized autosomal recessive disease, present with various systemic vascular, inflammatory, and hematological manifestations, often with early-onset age, or recurrent strokes. Their clinical features and histological findings overlap with those of childhood onset polyarteritis nodosa (PAN), a primary "idiopathic" systemic vasculitis. Despite similar clinical presentation, individuals with DADA2 may respond better to biologic therapy than traditional immunosuppression. We aimed to screen an international registry of children with systemic primary vasculitis for variants in adenosine deaminase 2 (*ADA2*).

Methods: We sequenced the coding exons of *ADA2* in 60 children and adolescents with a diagnosis of PAN, cutaneous PAN, or unclassifiable vasculitis (UCV), any chronic vasculitis with onset 5 years of age or younger, or history of stroke. The functional consequence of identified variants was assessed by ADA2 enzyme activity assay and immunoblotting.

Results: We identified nine children with DADA2; one with no rare variants in the coding region of *ADA2* and eight with biallelic, rare (MAF < 0.01) variants of known (p.Gly47Arg, p.Gly47Ala) and novel (p.Arg9Trp, p.Leu351Gln, and p.Ala357Thr) association with DADA2. Clinical phenotype varied widely. All individuals showed loss of ADA2 enzyme activity.

Conclusion: Our findings support previous reports that DADA2 has extensive genotypic and phenotypic variability. We suggest that screening *ADA2* among children with vasculitic rash, UCV, PAN, or unexplained early-onset CNS disease with systemic inflammation may enable an earlier diagnosis of DADA2.

Poster # 29

Antibiotics suppress intestinal antiviral responses in a microbiota-independent manner

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BACKGROUND: Oral antibiotics are commonly administered to mice, and effects seen following these treatments are typically ascribed to the depletion of the intestinal microbiota; however, these effects may also be due to direct effects of the antibiotics on the host. In vitro studies have shown that various antibiotics can suppress immune functions, but despite the frequent use of antibiotics in the clinic and research, potential microbiota-independent effects of antibiotics in vivo have been understudied. Here, we use the model intestinal pathogen murine norovirus strain CR6 (MNV-CR6) to investigate the effects of oral antibiotics on intestinal immune responses.
AIMS: We investigated the effects of an oral antibiotics on antiviral immune responses to MNV-CR6, including whether antibiotic-mediated effects were microbiota-dependent.
METHODS: Conventional and Germ-Free (GF) mice drank a cocktail of ampicillin, gentamicin, metronidazole, neomycin, and vancomycin, before infection with MNV-CR6. After 11 days, colonic lymphocytes were tetramer stained to identify MNV-specific CD8+ T cells. Colonic viral loads were assessed by qPCR.
RESULTS: GF status and antibiotic treatments did not significantly impact viral load. However, antibiotic treatment prevented the generation of MNV-specific CD8+ T cells in both conventional and GF animals. Ampicillin alone was sufficient to mediate this effect.
CONCLUSIONS: Our results indicate that antibiotic treatment can profoundly suppress antiviral immune responses in a microbiota-independent manner. These results may have implications for the use of antibiotics clinically and in microbiota research.

Poster # 30

The Role of Monocyte Derived Macrophages in Inflammation Induced Metastasis.

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To become metastatic, circulating tumor cells must be able to survive hematogenous transit, extravasate into the parenchyma of distant sites and proliferate into micrometastatic lesions. To determine if inflammation facilitates this process, the model of bleomycin-induced sterile lung injury coupled with the introduction of B16F0 melanoma cells into the microvasculature of the lungs was used. Strikingly, inflammation increased overt metastasis of B16F0 cells without increasing their extravasation into the lung. Interestingly, this phenotype was also observed early in metastases with an increase in the number and size of micrometastases at 96hr post B16F0 injection. Bleomycin induced inflammation led to the increased survival of B16F0 tumor cells and the recruitment of monocyte derived macrophages (MoDM) to the lungs. These MoDMs are located near the micrometastatic niche and their presence correlates with increased metastatic tumor cell burden. Furthermore, co-adoptive transfer of MoDM with B16F0 cells resulted in an enhanced number of melanoma lung metastases. Additionally, MoDM conditioned media generated from inflamed lungs rescued stressed or drying B16F0 cells *in vitro*. Therefore, we hypothesized that MoDM produce a soluble factor that promotes the survival of metastasizing B16F0 cells in the inflamed lung. Differential gene expression analysis of RNAseq data from sorted MoDM and alveolar macrophages suggested that growth factors like the hepatocytic growth factor (HGF) may be promoting the survival of B16F0 cells in the lung. *In vitro*, HGF promoted the survival of dying B16F0 cells and B16F0 cells express the HGF receptor c-Met. Finally, we show that inflammation induced both HGF production by MoDM and the activation or phosphorylation of c-Met in the lung. Thus, MoDM may be producing factors to prime the premetastatic niche and play a key role in increasing the metastatic potential of circulating cancer cells. Taken together these findings illustrate the importance of the microenvironment in metastasis to distant sites and they highlight MoDMs and inflammation as an important modifier of this microenvironment.

Poster # 31

Investigating interorgan trafficking of lung group 2 innate lymphoid cells after activation

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Group 2 innate lymphoid cells (ILC2s) reside in mucosal and non-mucosal tissues including the lung and the liver. In the lung, they respond to epithelial-derived cytokines IL-33, IL-25 and TSLP and produce IL-5 and IL-13, which induce eosinophilia and mucus hyperproduction, respectively, leading to type 2 inflammation. They have also been identified in humans and implicated in various diseases such as asthma, liver hepatitis and fibrosis. Very few ILC2s are found in peripheral blood (PB) or spleen of naive mice. However, we have now found that activation of lung ILC2s by intranasal administration of IL-33 results in increased numbers of ILC2s in PB and spleen up to 1 month, and in the liver up to 2 months after treatment. Using parabiotic mice, ILC2s that were detected in the liver after intranasal IL-33 stimulation were found to be lung ILC2s that migrate to the liver, rather than liver resident ILC2s that proliferate. In vitro stimulation of liver ILC2s demonstrated that they produce greater amounts of IL-5 and IL-13, but also IL-6 compared to lung ILC2s. Flow cytometric analyses of chemokine receptors and integrin molecules suggest that CXCR6 and CD103 may be involved in regulating migration of lung ILC2s to the liver and their retention in the lung, respectively. These results show that activated lung ILC2s emigrate from the lung, circulate, settle in the liver and persist. Re-activation of those ILC2s may result in type-2 immune-mediated diseases such as hepatitis or liver fibrosis, which may lead to the development of liver cancer.

Poster # 32

The Role of MALT1 in Macrophages

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At BC Children's Hospital, a patient with severe combined immunodeficiency accompanied by dramatic inflammation along the gastrointestinal tract was diagnosed with a homozygous mutation in the intracellular signalling molecule, MALT1. Malt1 acts both as a scaffolding molecule and an enzyme with proteolytic activity. The consequences of MALT1 immunodeficiency have largely been attributed to its role in T and B cells, in which MALT1 acts downstream of the T or B cell receptor to drive lymphocyte proliferation and activation. MALT1 is also activated in macrophages downstream of the C-type lectin receptors, dectin-1 and toll-like receptor 4 (TLR4). However, the role of MALT1 in macrophage-mediated inflammation has not been explored.

Intestinal inflammation is largely driven by macrophages, suggesting that macrophage Malt1 may contribute to this inflammation. Based on this, we hypothesized that Malt1 deficiency in macrophages causes intestinal inflammation by increasing inflammatory cytokine production. We found that in vivo Malt1 deficiency exacerbated DSS-induced colitis in mice. Macrophage stimulation increases Malt1 protein expression but decreases its activity. We found that Malt1 deficient murine macrophages had higher level of IL-1β production than wild type macrophages and pharmacological inhibition of Malt1 protease activity increased some pro-inflammatory cytokine production in response to innate immune stimuli

Taken together, our studies suggest that Malt1 deficiency causes inflammation in vivo by increasing macrophages IL-1β production. In future studies, we will investigate the cell-specific contribution of MALT1 deficient macrophages to inflammatory disease using mice with myeloid-specific MALT1 deficiency. These studies will provide critical information about the cell specific role of MALT1 and possible side effects of MALT1 inhibitors currently used for lymphoma treatment.

Poster # 33

Are eyes more than just windows to the soul? Exploring the link between Alzheimer's and retinal degeneration

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We propose an early "biomarker" of Alzheimer's Disease (AD) may be pathological changes in the eyes, as retinal pathologies commonly seen in various age-associated eye conditions have been found in the eyes of AD patient. We found that Tg2576, our animal model of AD, eyes showed increased disruption in retinal blood barrier cells and expression of amyloid, the causative agent of AD. Surprisingly, when mice without the amyloid precursor protein gene were reconstituted with Tg2576 bone marrow, those mice had lower amounts of amyloid in their eyes compared to those reconstituted with wild-type bone marrow.

Poster # 34

Role of Cellular Autophagy ("Self-eating") in the Activity of Novel Site-Specific Immunomodulators

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Immune cell dysfunction and chronic inflammation are etiologically-associated with the pathology of inflammatory bowel disorders, as well as certain cancers and persisting infections (eg. HIV). Recently, a series of novel, microbe-derived, Site-Specific Immunomodulators (SSIs) (Qu Biologics) have been described to strategically repurpose the innate immune response to exert therapeutic effects at specific sites of pathology. Such therapeutic effects have been observed in animal models and patients with ulcerative colitis and lung cancer for eg. The mechanism for these effects and their organ/site-specific nature are poorly understood. We hypothesized that cellular autophagy, a critical immune defense and inflammatory regulator is involved. Results have suggested that SSIs (QBECO, QBKPN) differentially activate autophagy in monocyte and colon epithelium lineage cells. Subsequent experiments will focus on establishing what cellular signal transduction pathways are implicated in SSI-induced autophagy and whether autophagy is required for SSI functional activity (eg. cytokine production).

Poster #35

Single cell analysis of RORα lineage tracer mice revealed functional heterogeneity of lung ILC2s

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Lung group 2 innate lymphoid cells (ILC2s) drive allergic inflammation and promote tissue repair. To investigate whether distinct ILC2 subsets mediate those different functions and elucidate their developmental relationship, we generated RORα lineage tracer mice and analyzed them by single cell RNA sequencing, flow cytometry and functional assays. Adult murine lung ILC2s were divided into IL-18Rα^{+}ST2^{-} and IL-18Rα^{-}ST2^{+} subsets. The former had an immature ILC phenotype, produced little cytokines and contained ILC progenitor like cells expressing Tcf7, whereas the latter was conventional ILC2s. Neonatal murine lung ILC2s were divided into two distinct effector subsets and an IL-18Rα^{+}ST2^{-}Tcf7^{+} progenitor-like subset. The two effector subsets were defined by the expression of ICOS and KLRG1, and they differentially produced the growth-factor amphiregulin and type 2 cytokines. Therefore, effector ILC2s diverge into tissue-repairing and pro-inflammatory subsets, which differ in transcriptional and phenotypic properties. The IL-18Rα^{+}ST2^{-}Tcf7^{+} cells are likely IL-18 responsive lung ILC progenitors, which may contribute to ILC-poiesis in neonatal and inflamed lungs.

Poster # 36

Differential Expression of Granzymes in Polarized Macrophages

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Granzymes (Gzm) are a group of serine proteases widely recognized as the apoptotic agents of cytotoxic T-lymphocytes (CTL) and natural killer (NK) cells. In addition to CTL and NK cells, other immune cells, such as mast cells and macrophages, have been shown to express Gzms. These cells typically do not form immunological synapses and/or express the pore forming protein perforin which facilitates Gzm entry into target cells, suggesting that Gzms expressed from these cells may be secreted directly into the extracellular environment where they can influence the wound environment. Macrophages are of particular interest, given that differential polarization results in a phenotype that either promotes (M1) or resolves (M2) inflammation. Although macrophages are known producers of Gzms, it is unclear as to whether all macrophages are able to express Gzms or whether their expression is limited to a particular polarization phenotype. Using THP-1 derived macrophages, we found that GzmK was exclusively expressed by M1 macrophages whereas GzmB was expressed by all polarized states (M0/1/2) but that M2a macrophages expressed significantly more GzmB than M0 or M1. Additionally, M1 cells specifically secreted GzmK into the extracellular milieu and correlated with activation of PAR1 and induction of IL6 from fibroblasts and keratinocytes. Although all polarized cells expressed GzmB, only M2a cells secreted GzmB. Differential expression and secretion of Gzms from macrophages may contribute to sustained inflammation and matrix remodeling during the wound healing process.

Poster # 37

Lyn tyrosine kinase is a key regulator of monocyte development, steady-state signaling and life-span, and modulates tumor metastasis and atherosclerosis.

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Monocytes are key effectors of the mononuclear phagocyte system, playing critical roles in regulating tissue homeostasis and coordinating inflammatory responses, including those involved in cancer and chronic inflammatory diseases, such as atherosclerosis. Monocytes have traditionally been divided into two major subsets termed conventional (cMos) and non-conventional/patrolling (pMos) monocytes. Patrolling monocytes were identified relatively recently, and the genes and signaling pathways controlling their development and function are still poorly defined. CyTOF and phospho-CyTOF analysis of monocyte subsets and signalling pathways in cMos and pMos revealed distinct baseline signalling profiles and far greater heterogeneity than previously described. *Lyn* deficiency led to a selective expansion of pMos, an increase in pMo life-span, and alterations in specific signalling pathways within these cells. Additionally, *Lyn* deficient mice exhibited reduced disease severity in high-fat diet induced atherosclerosis and diminished melanoma metastatic burden, which correlated with significant increases in aorta- and lung-associated pMos, respectively. Together, our data identify LYN as a key regulator of pMo numbers and as a potential therapeutic target in diseases regulated by pMos

Poster # 38

Bioinformatic design of mini-promoters for T-cell subset specific chimeric antigen receptor T-cell therapy

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Though cancer accounts for 60% of gene therapy clinical trials, the existence of cell-type specific promoters to allow for restricted expression of transgenes in targeted immunotherapy is lacking. Chimeric antigen receptor T-cell (CAR-T) therapy has proven successful in the treatment of various haematological malignancies, however the current manufacturing process is cumbersome and expensive. Currently, CAR-T production requires extraction of patient T-cells, followed by lentiviral transduction, expansion, and infusion back into the patient. Although effective, CAR-T therapy may be achieved with the use of systemic gene therapy through direct infusion of the lentivirus. However the current design of the viral construct for CAR-T therapy presents risk due to the non-specific VSV-G coat protein resulting in transduction of all cells, and the CAR transgene is ubiquitously expressed due to the constitutively active EF1a promoter.

Here, we propose a bioinformatic method to design mini-promoters to enable CAR transgene expression in various T-cell subtypes of interest. For this, we are integrating publicly available expression data from 10,500 tissue samples from the Genotype-Tissue Expression (GTEx) project, 1,500 immune cells, and multi-omics data from over 300 cell/tissue types. We will leverage this large dataset to identify candidate T-cell specific regulatory regions to design synthetic promoters comprised of the minimal promoter elements. Candidate mini-promoters will then be validated in mixed cell populations to confirm their ability to drive transgene expression in restricted cell types. The development of our computational pipeline, will enable cell-type specific transgene expression with the design of mini-promoters, and in turn targeted immunotherapies may be developed.

Poster # 39

Topical immunomodulation for the treatment of experimental autoimmune encephalomyelitis (EAE)

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Multiple sclerosis (MS) is an autoimmune disease characterized by T cell-mediated destruction of myelinated axon sheaths in the central nervous system. Topical application of the vitamin D analogue, calcipotriol, can abrogate skin hypersensitivity responses in a T cell-dependent manner. We aim to test whether topical calcipotriol or tretinoin (vitamin A), a similarly immunomodulatory compound, can modulate the disease course of experimental autoimmune encephalitis (EAE), a mouse model of MS. We hypothesize that topical treatment with calcipotriol or tretinoin reduces EAE disease severity by reducing the generation of encephalitogenic T cells. Mice were pre-treated with tretinoin, calcipotriol, or vehicle for 2 days once daily before EAE induction. Mice were monitored for up to 28 days, scored and weighed daily. Lymphoid organs were harvested for flow cytometric analysis of T cell subsets and cytokine expression. Pre-treatment with tretinoin, but not calcipotriol, reduced EAE severity as reflected by lower clinical disease scores and sustained maintenance of body weight. Immune cell phenotype analysis showed reduced interferon-gamma expression in CD4 and CD8 T cell compartments of animals pre-treated with tretinoin. The nature of topical immunomodulation as a non-invasive therapeutic intervention makes it an appealing treatment option. Further studies are warranted to verify the efficacy and mechanisms of topical retinoids on modulation of systemic immunity.

Poster # 40

Interleukin-5 drives the expansion of “innate-like” B-1 cells and restricts pulmonary metastasis

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Lung cancer is the leading cause of cancer deaths in Canada. The use of immunotherapy has resulted in long-lasting responses in lung cancer patients, highlighting the critical role of the immune system in the lung. Despite this, a large proportion of patients do not respond to immunotherapy. B cells are an immune population that secrete antibodies, and despite the presence of intratumoral B cells, the role of B cells in lung cancer is largely undefined. We have found that IL5Tg mice, which over-express the cytokine IL-5, have both an expansion of lung B cells and a decrease in lung metastasis. Therefore, we hypothesize that B cells may decrease lung tumor growth. Lewis Lung carcinoma (LLC) cells were injected intravenously to seed the lungs of WT and IL5Tg mice. We found that the increase in lung B cells in IL5Tg mice was due to an increase in B-1 cells which co-expressed the antibody class IgM. Importantly, IL5Tg mice had a decrease in lung metastasis compared to WT mice, as well as an increase in the total number of lung-infiltrating B cells compared to naive mice, suggesting that IL-5 may promote anti-tumorigenic immune cell activities in the lungs. Circulating IgM was elevated in IL5Tg mice compared to WT, and we found that these IgM antibodies were able to bind to LLC cells. We are currently investigating whether binding of IgM to tumor cells can induce tumor cell cytotoxicity. Tumors that don’t respond to immunotherapy often lack expression of peptide neoantigens, but these tumors may be vulnerable to IgM antibodies which have been shown to also target carbohydrate- and lipid-rich antigens. Illuminating the specific role of B-1 cells and IgM in lung cancer may reveal new clinical intervention points to improve responses to immunotherapy, as well as deconvolute the complex interplay between host immune cells and malignant cells.

Poster # 41

Granzyme B: A Novel Target for Pemphigoid Diseases

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Pemphigoid diseases are a subgroup of autoimmune skin blistering diseases characterized by widespread tense skin blisters. The standard treatment is systemic corticosteroid, which often causes fatal adverse effects. Several proteases play important pathological roles in the pemphigoid diseases, however, clinical trials targeting proteases have been limited, because these systemic inhibitors often cause severe adverse effects. In this study, we tested the hypothesis that the serine protease, granzyme B (GzmB), contributes to the diseases through anchoring protein cleavage and inflammatory regulation. In addition, the efficacy of our newly developed topical GzmB inhibitor was assessed in murine models.

Skin biopsies and blister fluids obtained from pemphigoid disease patients were assessed for GzmB and anchoring proteins. Abundant GzmB and loss of anchoring proteins (collagen XVII and $\alpha 6\beta 4$ integrin) were identified. GzmB-mediated anchoring protein cleavage and cell attachment strength were assessed in cultured keratinocytes. GzmB cleaved anchoring proteins to reduce attachment strength. To test its function in the blistering of the pemphigoid diseases, a passive transfer model of pemphigoid disease on GzmB-deficient mice was analyzed. GzmB-deficient mice with the disease exhibited a reduction in affected-body surface area, histological blisters, neutrophil infiltration, MIP-2 level, and anchoring-protein loss, compared to the wild-type mice with the disease. Finally, the topical GzmB inhibitor was applied on the ears of pemphigoid disease model mice. Application of topical GzmB inhibitor reduced affected surface area and ear thickness.

We conclude that GzmB contributes to pemphigoid disease pathology through the anchoring protein cleavage and inflammatory regulation, and topical GzmB inhibitor application is effective for the treatment on the murine model.

Poster # 42

Identification of Intratumoral Immunomodulatory Signatures in High Grade Serous Ovarian Cancer

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High grade serous ovarian cancer (HGSC) displays a high level of heterogeneity in terms of TIL marker expression between tumour sites within an individual patient, with tumours falling into three categories: high TIL present in tumour epithelium (TE) and stroma (hot); TIL present in stroma only (warm) and low TIL presence (cold). Here, we analyse the microregional TIL gene expression patterns of HGSC in order to elucidate the immunomodulatory mechanisms occurring across multiple tumour sites of HGSC patients. Three patients with two HGSC tumour sites falling into different TIL categories were chosen. FFPE tumours were sectioned onto PEN membranes and stained for CD3/CD8. Laser capture microdissection was used to extract six regions representing hot, warm or cold TE or stroma from each tumour. Transcriptome sequencing was performed for each region. Bioinformatics analyses were used to measure gene expression and GO pathway enrichment was performed using GSVA in R. TIL-related gene expression confirmed the presence of the three immunophenotypes across the cohort, and the expression patterns correlated with immunophenotypes as classified by IHC. Five distinct groups of co-regulated pathways were identified corresponding to 1) upregulation and 2) downregulation of cell proliferation, 3) epithelial to mesenchymal transition and 4) immune response activation and 5) immune regulation. These results demonstrate that while TIL presence may typically indicate better prognosis, the immunomodulatory processes differ within a tumour site and may provide targeting mechanisms for more robust treatment of HGSC.

Poster # 43

Cellular stressors alter cytokine responses in human IBD and healthy control-derived colonoids, thereby driving DC maturation

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Intestinal epithelial cells (IECs) sense nearby commensal bacteria through their pattern recognition receptors (PRRs), with such recognition ultimately promoting homeostatic T cell responses, such as tolerance, to resident gut bacteria. IECs also respond to stress signals such as ATP, H_2O_2, and endoplasmic reticulum (ER) stress as a means to coordinate their cellular and inflammatory responses to potential noxious stimuli. However, in patients with inflammatory bowel disease (IBD) these stress pathways are altered, leading to abnormal anti-commensal immune responses. Whether these abnormal responses to stress subsequently drive the dysfunctional T-cell immune responses seen in IBD patients remains elusive. We previously showed that two important stress/danger signals, namely H_2O_2 and extracellular ATP, alter IEC innate immune responses to TLR agonists. Moreover, mouse IECs stressed with ATP and then stimulated with a TLR1/2 agonist released factors that conditioned bone marrow-derived dendritic cells (DCs) to become pro-inflammatory, increasing their cytokine production, CD80 expression and T cell activation capacity. Based on these results, we tested whether ATP and ER stress would affect primary human IECs, grown as colonoid-derived monolayers, in a similar fashion, helping to explain the link between stress in IECs and chronic inflammation in IBD. Here we show that human-derived ascending colon organoids, when treated with thapsigargin to induce the UPR, produce significantly more IL-8 and TNFα and less CCL20 after TLR5 stimulation with flagellin. In addition, colon organoids from IBD subjects produce more baseline IL-8 than healthy control colon organoids yet have a blunted ER stress IL-8 response, and a reduced ability to produce TNF after TLR5 activation. These findings suggest a permanent epigenetic change in intestinal stem cells in IBD. In addition to an altered innate immune response due to ER stress, colon organoids secrete factors that condition monocyte derived dendritic cells (moDC) to become pro-inflammatory by increasing CD80, CD86, and MHCII and decreasing CD103, a tolerogenic marker. Correlation analysis between IEC ER stress-induced IL-8 production and DC receptor expression demonstrates that the amount of stress IECs undergo determines the maturation status of moDCs. Together these findings suggest a link between the UPR in IECs and the damaging T cell responses that characterize inflammatory bowel disease.

Poster # 44

Virus Infection Induces Gut Dysbiosis to Promote Type 1 Diabetes Onset

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In combination with genetic determinants, susceptibility to autoimmune diseases such as Type 1 Diabetes (T1D) is established by various environmental factors including microbial dysbiosis, exposure to dietary antigens, antibiotic use, vitamin D deficiency, and infection. Clinical and epidemiological studies have implicated infection with certain viruses such as coxsackievirus B (CVB) to be a risk factor associated with diabetes onset. However, little is known about how this virus affects the intestinal microbiome despite compelling evidence indicating commensal bacteria and viruses are important cofactors in T1D development and pathogenesis. Recently, mucosa-associated invariant T cells (MAIT) cells were shown to be altered leading up to diabetes onset in patients. These cells sense microbial products derived from riboflavin biosynthesis in the gut and are normally activated to promote intestinal integrity, but they can also take on a more inflammatory phenotype and participate in autoimmune responses in the pancreas. Ultimately, there exists a significant potential for cross-talk between CVB infection, the microbiome, and gut-resident immune cells impacting T1D susceptibility. To address this, we used a non-obese diabetic (NOD) mouse model and found MAIT cells respond to CVB and that infection results in intestinal dysbiosis which may be promoting the development or progression of T1D.

Poster # 45

STAT3 transfection restores lack of Th17 differentiation in neonatal CD4 T cells

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Candida commonly causes bad oral thrush and "diaper rashes" in newborns whereas it rarely becomes invasive in adults. T helper 17 effector CD4 T cells (Th17) play an important role in controlling *Candida* infection at mucosal surfaces. Neonatal T cells intrinsically lack the capacity to differentiate into Th17 cells. In this research, we show reduced expression of Signal transducer and activator of transcription 3 (STAT3) in neonatal naïve CD4 T cells compared to adult counterparts. STAT3 is required for signaling leading to Th17 cell differentiation. We hypothesize that the lack of neonatal Th17 differentiation is in part modulated through reduced STAT3.To confirm this, neonatal naïve CD4 T cells were isolated from umbilical cord blood and adult peripheral blood via magnetic bead separation. The STAT3 gene was cloned into a pIRES2-eGFP plasmid, which was then transfected into neonatal naïve CD4+ T cells. Transfection efficiency was confirmed by flow cytometry and transfected cells were stimulated for 3 days (anti-CD3/CD28) in the presence of Th17 polarizing cytokines (IL-1β, IL-6, IL-23 ± TGF-β). Secretion of IL-17 and IL-22 were measured by ELISA.

Our results essentially show that exogenous STAT3 can restore Th17 differentiation in neonatal T cells. However, without STAT3, only IL-22 is produced in Th17 conditions. We conclude that, in the absence of sufficient STAT3, neonatal T cells are skewed towards non-stereotypic Th17 differentiation results in a predominant production of IL-22 rather than IL-17. Preferential IL-22 in neonates may help protect mucosal surfaces from pathogenic effects of IL-17, but also increase their vulnerability to *Candida* infections.

Poster # 46

Mixed Phenotype in a Case of an Undefined Autoinflammatory Syndrome

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Introduction: Autoinflammatory diseases (AInD) result from defects in innate immunity and basic cellular processes. Children with AInDs suffer from recurrent episodes of systemic inflammation in the absence of infection, injury, or autoimmunity. Despite recent advances in diagnosis and understanding of many AInDs diseases, there are still a great number of patients with phenotypes that do not fit any clinically- and/or genetically-defined disorders. To achieve best care for those patients, a detailed understanding of their disease's etiology and mechanism is required.

Objective: To investigate the pathogenesis of an undefined autoinflammatory syndrome in a 14 yr old boy with severe episodes of autoinflammation (i.e. fever, rash, arthralgia) since the age of 2.5 years and only partial symptom control with IL-1® inhibition therapy.

Methods: Biosamples collected from the patient and healthy family members were analyzed for evidence of genetic (whole exome sequencing) and cellular (inflammasome activation and cytokine production) abnormalities that could explain the child's symptoms.

Results: Whole exome trio sequencing identified known and novel variants in the patient in several genes known to cause autoinflammatory disease; notably T260M and T320M variants in the NLR family pyrin domain containing 12 (*NLRP12*) gene. This correlated with persistent elevation of multiple circulating pro-inflammatory cytokines most notably Type I and II Interferons (IFN).

Conclusions: The patient's disease appears to be mediated by both IL-1® and IFN. The operation of more than one underlying mechanism could explain why complete blockade of IL-1 resulted in incomplete control of inflammation and symptoms.

Support provided by Andrew Nord Fellowship in Rheumatology, UBC (IN) and MSFHR Scholar Award (KB).

Poster # 47

Rapid, automated lymphocyte isolation directly from whole blood with EasySep™ Direct

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Automation is an important requirement for streamlining and standardizing technical procedures, such as cell isolation, in a routine laboratory. We have previously developed RoboSep™, the first instrument to fully automate the cell labeling and cell isolation steps of an immunomagnetic cell isolation procedure. RoboSep™ can isolate up to four individual samples at one time and single-use disposable filter tips eliminate the possibility of sample cross contamination.

We have recently developed EasySep™ Direct, a rapid method to isolate highly purified lymphocytes directly from whole blood without the need for any pre- or post-cell processing steps. This study describes automation of the EasySep™ Direct procedure using RoboSep™ to isolate total lymphocytes, T cells and B cells. In brief, whole blood is loaded onto the machine and unwanted cells are targeted for depletion by addition of an antibody cocktail and magnetic particles. After cell labeling, the sample is moved to the separation tube in the magnet by the robotic arm of the instrument. Cells of interest are then transferred to a collection tube, while unwanted cells are retained in the magnet.

Following RoboSep™ isolation, purities of 97.8 ± 0.9% (n=8), 93.1 ± 2.4% (n=4) and 96.8 ± 2.2% (n=12) were obtained using the EasySep™ Direct Total Lymphocyte isolation kit, T cell isolation kit or B cell isolation kit, respectively (mean ± SD). From 1mL of whole blood, 6.6 ± 1.5 x 10e5 total lymphocytes, 6.6 ± 1.3 x 10e5 T cells or 5.8 ± 4.4 x 10e4 B cells were obtained using each of the three EasySep™ Direct kits. Similar values were obtained in head-to-head experiments using the manual silver magnet EasySep™ Direct procedure for each isolation kit (not shown).

Automation of the EasySep™ Direct procedure with RoboSep™ enables standardization of the cell isolation procedure thus freeing up valuable technologist time. The highly purified lymphocytes can immediately be used in crossmatch assays or other laboratory techniques.

Poster # 48

IL12 and IL15 cooperate for the co-expression of L-Selectin and PSGL-1 P-Selectin ligand on activated CD8 T cells

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Background: The recruitment of effector leukocytes from circulating blood into inflamed tissue is a critical step in immunological defence. Recruitment begins with tethering and rolling of circulating leukocytes on vessel walls. This dynamic, low-affinity, interaction is mediated in part by endothelial P-Selectin tethering to its canonical ligand O-glycan-modified PSGL-1 (PSGL1*) expressed by myeloid cells and induced on T cells after activation. We reported the discovery of a second P-Selectin ligand displayed on the surface of murine $CD8^+$ T cells activated in lymph nodes and designated PSL2 *(Carlow, Tra, and Ziltener. 2018, PLoS One)*. PSL2 is CD8 T cell-extrinsic, acquired by CD8+ cells after activation by docking onto L-Selectin. L-Selectin expression on activated CD8+ T cells is necessary for PSL2 display. The discovery of PSL2 suggests that recruitment of activated CD8 T cells into inflamed tissue may involve P-Selectin engagement of both PSGL1* and the L-Selectin/PSL2 complex. The persistent expression of L-Selectin after T cell activation is characteristic of central memory T cells considered to be most effective in adoptive transfer of immunity and seeding of resident memory T cells. Consequently, we are investigating if/how the L-Selectin/PSL2 complex contributes to recruitment of activated CD8+ T cells into inflamed tissue.

Approach and observations: Our approach is to define conditions for generating ovalbumin-activated OTI T cells in vitro that co-express PSGL1* and L-Selectin, or each alone, and to then compare recruitment efficiencies of these cell types in competitive recruitment assays *in vivo*. As a first step we explored how to maximize expression of PSGL1* and/or L-Selectin. Our data suggest that the dual expression of both PSGL1* and L-Selectin arises as an independent phenotype requiring cytokine signals (IL12+IL15) distinct from those driving high expression of PSGL1* (IL2) or L-Selectin (IL15). Conditions to maximize PSGL1* expression (IL2) induced loss of L-Selectin expression, while conditions promoting L-Selectin expression (IL15) poorly supported PSGL1* formation.

Conclusions: These results highlight the distinctive signals required after T cell activation to generate the PSGL1*/L-Selectin+ phenotype and provide tools to assess the function of the PSL2/L-Selectin complex during recruitment of activated CD8+ T cells.

Poster # 49

The Effect of Obesity and Smoking on Chronic Inflammation, Innate and Adaptive Immune Responses and Blood Cell Composition

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While obesity and smoking are two lifestyle factors that are clearly associated with an increased risk of cancer only a small proportion of obese individuals and smokers go on to develop cancer. To identify those most at risk we evaluated the levels of chronic inflammation (CI), since CI is a well established risk factor for many cancers, in obese subjects (BMI>35) and heavy smokers (current smokers with >30 years of 1 pack/day or more). In addition, we explored their innate immune responses to bacterial and viral challenges, ex-vivo, using whole blood samples. We found that while smoking increased endogenous C-reactive protein (CRP) and IL-6 levels, compared to age-matched controls, being obese elevated not only CRP and IL-6 but IL-1RA, IL-17, IL-6, VEGF and PGE2 levels as well. On the other hand, smoking increased hemoglobin levels, MCV, MCH and MCHC values, all of which were not observed in obese volunteers. However, both obesity and smoking led to significantly higher white blood cell counts, because of increased monocytes and granulocytes. Both groups also had lower levels of natural killer cells and increased regulatory T cells compared to controls, which likely reduce their ability to kill nascent tumour cells. Despite these similarities, whole blood samples from obese subjects produced a comparable or greater level of cytokines/chemokines in response to *Escherichia coli* (*E. coli*)/HSV-1 stimulation, than non-obese controls, while whole blood from smokers dampened down *E. coli*-stimulated pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, and MIP1α). Consistent with an exaggerated innate immune response, when stimulated with anti-CD3/CD28, the PBMCs from these obese subjects produced significantly greater IFNγ than non-obese subjects. Smoking, on the other hand, produced a comparable level of IFNγ to non-smokers. Taken together, while both smoking and obesity increase the risk of cancer, they affect immune markers differently and may promote cancer through distinct mechanisms of action.

Poster # 50

Discovery and Mechanistic Studies of Novel Suppressors of Tat-mediated HIV Expression

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Background: Latent reservoirs harbouring dormant, replication-competent proviruses are the main obstacle to HIV eradication. A deep-latency or "block-and-lock"-based approach, which uses Pro-Latency Agents (PLAs) to inhibit viral reactivation even after PLA withdrawal or subsequent proviral stimuli, could theoretically lead to a drug-free HIV remission. The HIV Tat transactivator protein is a key enhancer of latency reversal and viral transcription. However, few PLAs that target Tat-mediated pathways are reported to additionally induce HIV deep-latency, indicating a likely need to discover and develop additional PLAs.

Methods: The Jurkat-derived 'JurTat' cell line, which contains an inducible Tat-Dendra protein that in turn drives HIV LTR-mCherry expression, was used to screen 97,152 compounds from the Rockefeller University HTSRC chemical library at 10 μM by high-throughput microscopy. Compounds that selectively inhibited Tat-driven mCherry expression were confirmed for activity by flow cytometry. Compounds of interest were then assessed for potential PLA properties in J-Lat cell lines containing HIV-GFP provirus, infected CEM-GXR T cells, and infected primary cells from HIV-positive donors with long-term cART-suppressed viremia.

Results: 2 compounds (C11 and A7) inhibited both ≥ 50% of Tat-driven mCherry but not Dendra expression by high-throughput microscopy and latency reversal in J-Lat cells at low micromolar concentrations. C11 further inhibited *in vitro* multi-cycle viral replication with EC50s of ~ 3.0 μM without concomitant cytotoxicity. Both 10 μM C11 and A7 further suppressed up to 33% of PMA + ionomycin-induced infectious virus production from PBMCs isolated from 4 HIV-positive donors. Mechanistic studies suggest that C11, but not A7, inhibits CDK9, a component of the host p-TEFb complex required for Tat-mediated transcription.

Conclusion: We report 2 novel inhibitors of Tat-mediated HIV expression which can be used to probe mechanisms of HIV transcription and inform ongoing "block-and-lock"-based therapeutic strategies.

Poster # 51

Biomarker harmonization to measure immunological effects of ustekinumab in type 1 diabetes.

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Type 1 diabetes (T1D) is caused by T-cell-mediated destruction of pancreatic beta cells. It is likely that blockade of pathogenic T-cells in individuals with recent-onset T1D would halt the destruction of beta cells and may allow restoration of endogenous insulin secretion. Ustekinumab inhibits IL-12/23 p40 and thereby limits the function of IL-17 and/or IFN-γ secreting T-cells, both of which have been implicated in the pathogenesis of T1D.

In 2016-2017, a phase IIa trial was undertaken to test the safety of ustekinumab administration to 20 young adults (18-25yrs) with recent-onset T1D. Biomarker assays were used to measure immune cell populations before and after treatment revealing a dose-dependent increase in the frequency of memory regulatory T-cells (Tregs; $p<0.01$), changes in the Treg signature, and a reduction in the frequency of IL-17^{+}IFN-γ^{+} Th17.1-cells ($p<0.05$). Moreover, patients treated with 90mg of ustekinumab had a higher clinical response compared to those treated with 45mg. Results from this trial indicated that changes in immune cell populations may predict a clinical response to ustekinumab therapy.

Two randomized, placebo-controlled clinical trials to test the efficacy of ustekinumab in new-onset T1D are planned in Canada and the UK. We have harmonized sample collection timing, processing and storage conditions, and undertook a cross-lab training process to standardize a series of bioassays to measure the effects of ustekinumab on different T-cell populations. By using standardized assays we will increase the statistical power of these independent trials and provide a platform for adoption of harmonized biomarkers in future immunotherapy trials in T1D.

Poster # 52

Rapid isolation of untouched PBMCs from whole blood without RBC lysis or density gradient separation in less than 20 minutes

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Peripheral blood mononuclear cells (PBMCs) consisting of monocytes and lymphocytes are commonly prepared from whole blood (WB) using density gradient centrifugation (DGC) and hypotonic lysis to remove granulocytes, platelets (PLTs), and red blood cells (RBCs). However, there are circumstances where it is desirable to avoid RBC lysis or DGC, or where an easily automated procedure is desired. We have developed a rapid immunomagnetic method for isolating untouched PBMCs without DGC or RBC lysis that can be fully automated with RoboSep™-S.

The EasySep™ Direct PBMC isolation kit labels RBCs, PLTs, and granulocytes with antibodies and magnetic particles. Following magnetic separation, the labelled cells are retained within the EasySep™ magnet and untouched PBMCs are simply poured off into a new tube, in as little as 20 minutes. Starting with WB, 1.55 ± 0.11 and 1.88 ± 0.46 million PBMCs were recovered per mL of WB using EasySep™ Direct and DGC, respectively (mean ± SEM, n > 13). Although the recovery of PBMC was similar between the two approaches, EasySep™ Direct PBMC Isolation gave significantly reduced granulocyte (1.2 ± 0.3% vs. 6.8 ± 1.0%), PLT (2.1 ± 0.6% vs. 56.2 ± 6.0%), and RBC (2.2 ± 0.3% vs. 11.4 ± 2.9%) contamination compared to DGC (mean ± SEM, n > 13, $P<0.05$ in all cases). Importantly, the relative frequency of monocytes and lymphocytes was maintained using both approaches. Additionally, cells isolated using the EasySep™ Direct PBMC isolation kit maintained high RNA integrity and are responsive to antigen and mitogen stimulation. Although EasySep™ Direct PBMC isolation was developed for use with WB, it can be modified to isolate PBMC from umbilical cord blood and leukopheresis samples. Furthermore, we have developed a fully automated RoboSep™-S protocol to process 4 samples simultaneously in <20 min.
In summary, our rapid, fully immunomagnetic EasySep™ Direct PBMC isolation kit can isolate PBMCs with significantly lower levels of granulocyte, PLTs, and RBC contamination than RBC lysis and density gradient separation.

Poster # 53

Reference intervals of adenosine deaminase 2 activity in childhood for improved clinical utility

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Loss of adenosine deaminase (ADA) 2 (ADA2) enzyme activity leads to deficiency of ADA2 (DADA2), which often presents in children and is associated with systemic inflammation and stroke. To use ADA2 activity as a diagnostic tool we optimized an ADA activity assay for rapid detection of ADA2 activity in blood. With samples from healthy children and adults we establish normative ranges of ADA2 activity and demonstrate that increasing age negatively correlates with ADA2 activity. This will aid in identifying abnormal ADA2 activity in DADA2 and other pediatric inflammatory diseases.

Poster # 54

Optimization of human T cell activation and expansion protocols improves efficiency of genetic modification and overall cell yield

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Cancer immunotherapy using CAR T-cells is a rapidly progressing field and manufacturing these cells is a complex process that requires multiple optimization steps. We have developed reagents for the isolation, activation and expansion of human T cells that will be available for clinical cell therapy manufacturing. Soluble ImmunoCult™ Human T Cell Activators induce T cell activation via cross-linking CD3 and co-stimulatory molecules on the surface of cells. Activated T cells then can be genetically modified and subsequently expanded in ImmunoCult™-XF, a serum- and xeno-free T cell expansion medium. Here, we present several optimization strategies with ImmunoCult™ products in order to obtain high transfection efficiency and maximum cell yield. By evaluating activation dynamics of T cells and determining the optimal transfection time points, the transfection efficiency can be substantially improved in both CRISPR/Cas9- and lentiviral-mediated gene-modification methods. Our study also suggests that maintaining T cells at lower cell density after the third day following activation greatly improves cell viability and cumulative cell growth, resulting in an >1000-fold expansion of total human T cells with >85% viability over 10-12 days of culture. Expanded T cells co-express CD45RO+CD62L+ with low expression of PD-1. As an example, we applied the workflow described here to generate TCRαβ KO T cells from healthy donors with up to 90% knockout efficiency. The purity of TCRαβ KO T cells can be further increased with the use of an EasySep™ Human TCRαβ depletion kit. Taken together, the processes outlined in this study can be easily and rapidly implemented to improve T cell manufacturing efficacy.

Poster # 55

Title: Modeling antibody affinity design goal by a site of action pk model

Authors: Tina Wang, Heather Sweet, John Harrold, Ian Foltz

It is important to establish success criteria for antibody selection, such as affinity requirements, for therapeutic antibodies early within a program. The optimal antibody affinity is dependent on target expression and turnover at the Site of Action (SoA). However, these data are often unavailable until in vivo experimental studies are conducted *after* a set of candidates has been identified. The SoA model was developed to mechanistically evaluate the impact of target affinity on coverage. The model was built on normal non-targeted monoclonal antibody PK from literature and Amgen internal data, and further extended to the tissue of interest–site of action–with large molecule biodistribution data. The SoA model enables us to create affinity design goals to reduce affinity maturation work later. If few or no antibodies with sufficient affinity are identified, early initiation of additional immunization campaigns or affinity maturation work can be taken strategically to shorten the development timeline. This model also gives an early insight of possible dosing limitations or other PK issues. The SoA model is a valuable tool to enable early setup of the affinity design goal for candidate selection, to potentially increase the success rate of therapeutic antibody drug development.

Poster # 56

Linking IgG Heavy and Light Chains to Preserve Native Sequence Pairing

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Antibody production involves immortalization of B-cells by fusion with myeloma cells and generation of hybridomas. This is a very inefficient process: 99.9% of B cells are lost. Bypassing hybridoma generation has many advantages when looking for rare clones. Our ongoing project aims at isolating single B-cells and, through RT-PCR, construct a linked heavy (HC) and light (λC/ κC) chain. The construct is then cloned into an expression vector to produce antibodies. So far, we have recovered HC- and λC/ κC-cDNA from both mRNA and cell sources and generated combined sequences from purified mRNA.

Poster # 57

Fast and easy immunomagnetic isolation of functional and particle-free CD8+ T cells in less than 30 minutes

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The immune function of CD8+ T cells in research fields such as infectious disease, autoimmunity and cancer continues to be a highly relevant and exciting area of study, with many immunotherapies targeting this cell population. To meet the demand for fast and particle-free CD8+ T cells isolation, we have recently developed a new EasySep™ Release Human CD8 Positive Selection Kit. This kit utilizes the Releasable RapidSpheres™ particle technology, which allows for the gentle removal of particles to mitigate potential interference in downstream assays. The 29-minute protocol involves first incubating target cells with antibody complexes followed by the Releasable RapidSpheres™. Labelled cells are purified following three incubations in a magnetic field. Particles are then removed after an incubation with a mild dissociation reagent and a final magnetic separation step. Using this EasySep™ method, CD8+CD3+ T cells can be isolated with high purities of 96.7% ± 3.4% and recoveries of 58.6 ± 17.4% (mean ± S.D., n = 28) and with minimal contamination of NK cells (CD8+CD3-). Additionally, when isolated cells are stimulated in culture with ImmunoCult™ Human CD3/CD28 T Cell Activator, CD25 and CD69 expression levels increase, and cells undergo robust proliferation and retain high cell viability. In summary, we have developed a fast and easy kit for the isolation of particle-free human CD8+CD3+ T cells that are ready for many downstream applications.

Poster # 58

Efficacy of a novel immune stimulating oncolytic virus: VG161 in gastrointestinal cancers

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Oncolytic HSV-1 (oHSV-1) are among the most promising therapies for cancer. Besides the oncolytic activity delivered by the virus against tumor cells, this treatment can also induce a potent immune response against tumor antigens (whether classic TAA, or neoantigens specific to the tumor being targeted).

We have constructed an oncolytic HSV-1 virus (VG161) to deliver 2 immunomodulator cytokines, IL12 and IL15, to the tumor micro-environment. These two cytokines can work synergistically to trigger an efficient anti-tumor immune response. A PD-L1 mimic peptide capable of blocking PD-1/PD-L1 interaction is also delivered as a Fc fusion peptide (TF-Fc) to help in maintaining an effective immune response against the tumor. For the construction of the recombinant virus, the exogenous components were cloned under CMV promoter control. The ICP34.5 gene was deleted as a safety measure for the recombinant virus.

The expression of Il12, IL15 and TF-Fc were confirmed, in vitro and in vivo, by qRT-PCR, ELISA and Western blot. Upon intra-tumoral injection of VG161, expressed IL12, IL15 and TF-Fc were detected only in the injected tumor mass, but not in the animals' blood or other organs. The bio-distribution of the virus was tested by qPCR after injecting VG161 intratumorally, and most of the injected virus were concentrated in the tumor mass. Minimal amounts of the virus were detected in the liver and spleen for short periods after virus injection.

We have demonstrated the superior activity of the VG161 virus, compared to the back-bone virus (with no payloads) using immune-competent mouse models (CT26) and nude mice implanted with human tumor cells lines (LNCaP and U87). VG161 induced tumor oncolysis resulting in complete tumor regression in nude mice. In the CT26 model, the tumor regressed to undetectable limits upon intra-tumoral injection with VG161. When the treated mice were challenged with the same tumor, the tumor cells did not grow. The added efficacy in CT26 tumor model can be attributed to the immune response generated by the modified virus, as demonstrated by a higher number of tumor-infiltrating CD8 T cells. The specific activity of T cells against the tumor cells was also demonstrated by ELISpot assay. Finally, memory T-cells were evident in the treated animals demonstrated by multiple assays.

Conclusions: This work has demonstrated the safety and efficacy of VG161, a novel oncolytic virus which can induce a strong anti-tumor immunity and oncolytic activity. We have also demonstrated that the Intra-tumoral expression of multiple immune regulatory factors may significantly change the tumor immune microenvironment to enhance the efficacy of the oncolytic virus.

Poster # 59

Twenty gene signature demarcates subtypes of paediatric small-to-medium sized vessel vasculitis

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Anti-neutrophil-cytoplasmic antibody (ANCA) associated vasculitis (AAV) describes a group life threatening diseases characterized by inflammation of small-to-medium blood vessels. Overlapping clinical features between AAV subtypes microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA) in the absence of mutually exclusive classification criteria make them hard to distinguish. The purpose of this study is to validate a putative 20 gene signature to aid classification of patients into clinically homogenous groups. RNA sequencing (Illumina HiSeq 2500) was performed on whole blood from 30 pediatric patients with an initial diagnosis of GPA, MPA or other small-to-medium vessel vasculitis. Sequenced data (fastq) reads mapped to human genome (STAR software) and analyzed for differential expression (DESeq2) divided samples into two groups resembling GPA and MPA. Further analysis identified a signature of 20 genes with maximal relative differential expression between the two groups. Relative quantification of real-time PCR data was used to investigate the expression of these 20 genes in samples from the discovery cohort, in a replication cohort of patients, and in healthy pediatrics. Significant difference in expression between GPA-like and MPA-like samples from the discovery cohort and between GPA-like samples and healthy pediatrics was observed for the genes ARL4C, IL21R, TGFA, SRGN, ARL4C, ETS2, FLOT2, TP53I11 and NFE2. The 20 gene signature for differentiating GPA and MPA is validated in the discovery cohort of patients. Ongoing studies are validating the gene signature in a replication cohort of patients. These studies will provide insight into disease etiology of AAV and enable improved characterization of disease course and outcomes.

Poster # 60

Discovery of Novel HIV Inhibitors from Australian Natural Products

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Background: HIV inhibitors that act by mechanisms that are distinct from existing antiretrovirals can provide novel insights into viral replication and potentially inform development of new therapeutics.

Methods: We tested 512 pure natural product compounds and analogs from the Davis Open Access Library of Compounds Australia for their ability to inhibit HIV replication in a multi-cycle replication assay using CEM-GXR cells. Compounds of interest were then assessed for dose-dependent effects on cell viability and ability to suppress induced virus expression in J-Lat, a Jurkat T cell line harbouring an HIV proviral genome, and JurTat cells, which contain an indcubile viral Tat protein that drives mCherry reporter expression of a viral LTR.

Results: We identified 4 compounds that were able to suppress 75% of HIV replication at 10 μM, for a hit rate of 0.8%. A chemical series exemplified by hopealphenol, a resveratrol tetramer, blocked HIV expression with an EC50 < 1μM, and at sub-micromolar concentrations, was able to suppress HIV expression induced by PMA or Panobinostat in J-Latcells and Tat-drived mCherry expression.

Poster # 61

Generation of NK Cells from Hematopoietic Stem and Progenitor Cells in a Stroma-Free, Serum-Free Culture System

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Natural killer (NK) cells play an important role in innate immunity by secreting proinflammatory cytokines and killing tumor cells and virus-infected cells. Human NK cells can be generated by culturing $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) with stromal cells and cytokines. The use of stroma (and serum) is, however, not desirable in clinical applications. We developed a culture method for generating large numbers of NK cells from purified $CD34^+$ cord blood (CB) HSPCs in the absence of serum and stromal cells. $CD34^+$ CB cells were isolated by EasySep™ magnetic separation, seeded into culture plates coated with a Notch ligand and cultured for two weeks in StemSpan™ Serum-Free Expansion Medium II (SFEM II) with StemSpan™ Lymphoid Progenitor Expansion Supplement. These conditions promote expansion of HSPCs and their differentiation into $CD7^+CD5^+$ lymphoid progenitor cells. Cells were then transferred to non-coated plates and cultured for two additional weeks in StemSpan™ SFEM II supplemented with StemSpan™ NK Cell Differentiation Supplement (containing IL-15) and the small molecule UM729 to promote differentiation into NK cells. After culture, on average 77% (range: 42 - 94%, n = 45) of cells expressed the NK cell marker CD56 and 58% (range: 25 - 79%, n = 10) co-expressed CD56 and NKp46 (an NK cell-activating receptor). These NK cells expressed additional NK cell markers such as NKG2D, CD94, and CD16. The average yield of $CD56^+$ NK cells was ~9,000 (151 - 41,000) per initial $CD34^+$ cell. Functionality of NK cells generated in culture was demonstrated by their killing activity against target K562 cells, which was comparable to NK cells isolated from normal peripheral blood. Additionally, cultured NK cells secreted IFN-γ when stimulated with phorbol 12-myristate 13-acetate (PMA)/ionomycin. These findings show that HSPCs can be expanded and differentiated into NK cells in culture under stroma- and serum-free conditions. This novel system will be a valuable tool in the development of cancer immunotherapies for which large numbers of NK cells are needed.