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Online Proceedings

2B

Enhancing Immune Responses Targeting Infection, Injury and Cancer

Session Moderator: Kristin Anderson, Immunology

MGH 228

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

ELISA Detection of Antibodies Against *Klebsiella pneumoniae* Capsule in Rabbit Immune Sera

Kathryn Shea Willebrand, ,
Mary Gates Scholar
Mentor: Frank DeLeo, Laboratory of Bacteriology
Mentor: Scott Kobayashi, Laboratory of Bacteriology

Klebsiella pneumoniae is a gram-negative bacterium that asymptomatically colonizes the intestinal and upper respiratory tract of healthy humans. It is also an opportunistic pathogen that causes bacteremia, pneumonia, urinary tract infections, and other severe infections in individuals with significant comorbidities. *Klebsiella* infections are often healthcare-associated and antibiotic-resistance is a major problem for treatment. A *K. pneumoniae* lineage known as multilocus sequence type 258 (ST258) is resistant to virtually all beta-lactam antibiotics, including the carbapenems, and many clinical isolates are also resistant to fluoroquinolones and aminoglycosides. Additionally, the capsule of ST258 confers resistance to phagocytosis by neutrophils and killing by the complement system. However, it is readily killed if phagocytosed. One possible therapeutic approach is to develop an antibody-based immunotherapy that targets the capsular polysaccharide (CPS) of ST258. As a step toward developing the tools needed for such an approach, we developed an enzyme-linked immunosorbent assay (ELISA) that can be used to detect CPS and titer CPS-specific IgG antibodies. Polystyrene ELISA plates were coated with purified ST258 CPS and then incubated with rabbit antiserum containing antibodies specific for CPS. Anti-CPS IgG was then detected with goat anti-rabbit IgG antibodies and a detection reagent. We anticipate the ELISA will be an important quantitative tool in our efforts to develop an immunotherapy that targets the CPS of *K. pneumoniae.*

Increased Immune Response in Male Rats with Acute Spinal Cord Injury

Seph Williams, Senior, Neuroscience, Seattle Central College
Mentor: David Baisch, Biology, North Seattle College

Statistics have revealed that males are more susceptible to significant spinal cord injuries than females. Carlos Ayala, MD-PhD student from the Keck Center of Collaborative Neuroscience at Rutgers University, has found preliminary evidence that males have a heightened immune response comparison to females following injury. This immune response involves an increase in inflammation surrounding the injury site, and the release of damaging cytokines that suppress neuronal growth and promote axon demyelination following injury. At the injury site, there is also an aggregation of M1 macrophages, which are known to have inflammatory properties and are involved in the promotion of cell death in the surrounding tissues. Another type of macrophage present are M2 macrophages, which are known to be anti-inflammatory and promote tissue regeneration, but these macrophages appear to be suppressed at the site of injury due to myelin debris and other chemical signals. Recent studies suggest that human umbilical cord blood (hUCB) has anti-inflammatory properties which could be beneficial in decreasing the inflammatory response that occurs in males. In this study, we propose to investigate the use of hUCB derived monocytes to treat spinal cord injury in male rats to determine whether it causes the polarization of monocytes into M1 or M2 macrophages *in vivo*. Using the classification of monocytes standard of myself, Carlos Ayala, Cameron Wolf, and Johvany Plaisime of Rutgers Univerity, the next step is to determine which monocytes are precursor cells to M1 (inflammatory) and M2 (anti-infammatory) macrophages. Monocytes are a heterogeneous population with cells found at various stages of life. Some of the critera include size of the cell, amount of and form of cytoplasm (smooth vs jagged), density of the cytoplasm, how indented the nucleus is, number of nuclei the cells have, and cell markers that are present for monocyte/macrophage

specific cell membrane receptors. If this study is successful, hUCB could be further studied for possible therapeutic applications for the treatment of spinal cord injury.

T-Bet Mediated Control of Effector T Cell Phenotypes

Leonard Daniel Chen, ,
Mary Gates Scholar
Mentor: Hao Yuan Kueh, Bioengineering
Mentor: Matthew Wither, Bioengineering

T cells of the immune system protect humans from most threats because they can recognize and eliminate foreign targets, as well as protect from reinfections. However, the persistence of an infection in the body leads to chronic stimulation of T cells, causing them to lose their effector function and enter a state known as "exhaustion". Exhausted T cells are defined by increased expression of inhibitory receptors, loss of immune cell regulation, and most importantly, loss of cytotoxicity and effector function. Studies have shown that the transcription factors, T-bet and Eomes, play crucial roles in regulating T cell differentiation, with T-bet being highly associated with effector T cell differentiation. T-bet expression is dampened in exhausted T cells, and therefore, I hypothesize that controlled induction of T-bet expression can reverse the exhausted phenotype in antigen-experienced T cells. I constructed a T-bet overexpression vector containing T-bet cDNA fused to a fluorescent protein and destabilizing domain. The destabilizing domain, or degron, facilitates degradation of the constitutively expressed T-bet transgene in the absence of the ligand, Shield-1, which when added at varying concentrations allows for a range of protein stability. This method of overexpression confers faster control kinetics compared to commonly used transcriptional approaches, such as inducible promoters. I have characterized the range of the T-bet transgene in Jurkat cells by titrating Shield-1 to provide a working range of 5-60% overexpression of T-bet compared to endogenous levels. Validating these parameters in primary T cells will allow me to apply this T-bet overexpression vector in a mouse model of T cell exhaustion. This tool has significant implications for improving immunotherapy strategies, such as TIL and CAR-T therapies, where exhaustion of the therapeutic T cells has led to reduced efficacy of the treatment.

Cell-Molded Silica Microparticles as Artificial T Cell Activation Platform

Caleb Ricardo Perez, ,
Mary Gates Scholar, Washington Research Foundation Fellow
Mentor: Suzie Pun, Bioengineering
Mentor: Brynn Olden, Bioengineering

T cell-based immunotherapy has shown immense therapeutic potential for the late-stage treatment of cancer. The synthetic *ex vivo* activation of T cells is a key step in manufacturing these therapies, inducing proliferation and cytotoxic functionalization of the cells before reinfusion into the patient. This is generally done with the use of artificial antigen presenting cells (aAPCs) consisting of antibodies triggering the receptor signaling necessary for activation directly conjugated to a spherical polystyrene bead. Although intended to mimic the function of antigen presenting cells (APCs) in the body, currently used aAPCs do not fully recapitulate natural size, morphology, or membrane fluidity, all of which have been demonstrated to be important determinants of activation properties. Therefore, there is a need for an activation platform that more closely mimics APCs *in vivo*, which could significantly improve the efficiency of T cell activation for use in cancer immunotherapies. To achieve this, we utilize cell-molded silica microparticles that retain the size and morphology of their cellular templates. The fusion of an antibody-loaded lipid bilayer to these silica microparticles results in a closer recreation of natural APCs. We have fabricated these cell-molded silica aAPCs with various cell template morphologies and lipid compositions. Using a variety of cell-based assays, we are characterizing the capacity of these aAPCs to induce T cell proliferation, cytokine release, and surface marker expression, all of which are metrics indicative of activation. If these results indicate that this platform successfully improves the efficiency of T cell activation as compared to current alternatives, this has the potential to improve the cost and scalability of these promising cancer treatments.

Immunotherapy for Ovarian Cancer: Combining Engineered T Cell Therapy with Immune Checkpoint Blockade

Maddie Grace Burnett, ,
Mentor: Kristin Anderson, Immunology, Fred Hutchinson Cancer Research Center
Mentor: Philip Greenberg, Medicine and Immunology

Ovarian cancer is the deadliest of gynecological malignancies, with approximately 70% of patients diagnosed at an advanced stage of disease. With the current standard of care, cytoreductive surgery plus chemotherapy, the relapse rate is about 70% and the 5-year survival rate is less than 50%. Innovative therapeutic interventions are greatly needed, and one promising new strategy mimics the body's endogenous anticancer immune response. This immunotherapy approach uses donor T cells engineered to express a T cell receptor (TCR) that is specific for a tumor-expressed antigen. Mesothelin (MSLN) is a validated antigen target, frequently overexpressed in cancer cells compared to healthy tissues. Using the mouse ID8 ovarian tumor model, we found that T cells engineered to express a high-affinity MSLN-specific TCR can kill ovarian cancer cells. However, the T cells lose effector function over time, which correlates with increased expression of the T cell inhibitory receptors PD-1, Tim-3 and Lag-3, markers of T cell exhaustion. Signaling through inhibitory

receptors is stimulated by respective ligands that we showed are present in the tumor microenvironment (TME). Blocking these "immune checkpoint" signals can improve T cell proliferation and anti-tumor function within various TMEs. By applying checkpoint blockade, we hypothesized that both donor and endogenous T cells will have prolonged longevity and increased effector function in our ovarian cancer model. In ongoing studies, ID8 ovarian tumor-bearing mice received MSLN-specific T cells plus antibodies that block PD-1, Tim-3 and Lag-3 checkpoints; T cell phenotype and function were characterized at early and late time-points. We will discuss our findings and the potential relevance of this combination immunotherapy as a clinical treatment of ovarian cancer.