2M

Fundamental Immune Mechanisms

Session Moderator: Alanna Ruddell, Comparative Medicine
MGH 287
3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Antibody Binding Affinity and Self-Aggregation in Serum

Isabel Yannatos, Recent Graduate, Chemistry, University of Washington
UW Post-Baccalaureate Research Education Program
Mentor: Abhinav Nath, Medicinal Chemistry

Protein therapeutics such as monoclonal antibodies (mAbs) are gaining importance and number in the drug development pipeline and in the clinic. Most standard techniques used to characterize these drugs require dilution into aqueous buffers, which do not accurately resemble biological conditions. We hypothesize that the heterogeneous and crowded environment in serum affects the binding and self-aggregation behaviors of mAbs. We directly measured binding affinities and self-aggregation of mAbs in fetal bovine serum and phosphate-buffered saline using fluorescence correlation spectroscopy (FCS). FCS detects individual fluorescent particles diffusing across a small (˜1fL) confocal volume. The diffusion time is directly proportional to hydrodynamic radius. We determined binding affinity between Alexa488-labeled streptavidin and anti-streptavidin mAbs based on the difference in diffusion time between the free antigen and the larger antigen-mAb complex. We found that binding with an anti-streptavidin IgG2 was ˜3-fold tighter in neat serum versus buffer, while affinity of anti-streptavidin IgG1 did not change. We tested the effects of viscosity, excluded volume, and specific interactions with serum components through experiments in solutions of sucrose, polymeric crowders, and serum components such as albumin and γ- globulins, respectively. Viscosity, crowding, and specific serum components may contribute differently to the difference in IgG2 binding affinity. We also used FCS to measure self-aggregation of a labeled IgG1 in serum and buffer and found that aggregation occurred faster and formed larger complexes in serum at 45C. These results demonstrate that antibody behavior is different in serum than in buffer conditions. Advancing our understanding of how mAbs and related molecules behave in physiological conditions such as serum will accelerate the development of safe, effective protein therapeutics and thereby improve our ability to treat diseases such as cancer and autoimmune disorders.

Inhibition of *Staphylococcus aureus* Protein A by Nitric Oxide

Helen Ivory (Helen) Warheit Niemi, Senior, Microbiology
UW Honors Program
Mentor: Ferric Fang, Laboratory Medicine & Microbiology
Mentor: Rodolfo Urbano, Microbiology

Nitric oxide (NO) is a reactive radical produced by cells of the innate immune system. NO reversibly binds to protein cysteine residues through a process called S-nitrosylation, often resulting in reduced protein function. This antimicrobial molecule serves as an important defense mechanism used by the immune system to fight off infections by many different pathogens, including the Gram positive bacterium *Staphylococcus aureus*. *S. aureus* is a common opportunistic pathogen and a major cause of wound infections, food poisoning, pneumonia, and invasive disease. Its ability to cause acute and chronic infections throughout the body is the result of numerous virulence factors. While many of these virulence factors have been extensively studied, little is known about how *S. aureus* pathogenesis is affected by its interaction with NO. We show that NO inhibits the expression of protein A, an extracellular IgG binding protein that is a key immune evasion factor in Staphylococcal disease. Reverse transcriptase quantitative PCR (RT-qPCR) shows that *S. aureus* cultures treated with NO exhibit dose-dependent inhibition of protein A transcription with increasing concentrations of NO. Western blots further confirm that transcription inhibition results in reduced protein A levels. Using allelic exchange mutagenesis, we determined that inhibition is likely due to NO targeting XdrA, a transcription activator of the *spa* gene encoding protein A. Using the Biotin-switch method, we have shown that XdrA is directly modified by NO and structural analysis of the protein suggests that NO modifications could detrimentally affect its ability to bind the *spa* promoter as it does when exogenous NO is not present. These findings introduce a novel mecha-

nism by which the innate immune system could compromise the ability of *S. aureus* to evade immune detection during infection through NO inhibition of protein A.

Analysis of Mast Cell Surface Proteome

Victoria Sabina Kasprzak, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Adrian Piliponsky, Pediatrics, Seattle Children's Research Institute
Mentor: Nicholas Shubin, Immunity and Immunotherapies

Mast cells are bone marrow-derived, granule-containing immune cells that are widely distributed in connective and mucosal tissues. Although mast cells are known to store and release histamine and other inflammatory agents in IgE-mediated allergic reactions, the specifics of their extracellular signaling and regulatory mechanisms are not fully understood. Therefore, this study aimed to further characterize the mast cell surface proteome to better understand the signals that promote mast cell activation. Murine bone marrow-derived mast cells were cultured and their membrane proteins were purified using hydrazide chemistry to isolate galactose and sialic-acid moiety-containing glycopeptides; these peptides are involved in post-translational modifications and anchorage of proteins to cell membranes. Post-cell lysis and purification, both liquid chromatography and mass spectrometry were used to classify and quantify detectable proteins. Use of online databases identified characteristics of these proteins, such as the presence of transmembrane domains, glycosylphosphatidylinositol (GPI) linkage, or target sites. As the cell surface proteome of bone marrow-derived macrophages is well characterized, they were subjected to the same treatments as mast cells, serving as a control. By comparing the mast cell and macrophage cell surface proteomes, we distinguished which proteins are mast cell-specific. Results show the presence of 69 transmembrane proteins including proteins known to be expressed by mast cells such as receptors for stem cell factor (c-kit) and interleukin-33 (ST2). Additionally, we detected and confirmed the expression of a potential cell surface histidine transporter, solute carrier family 15A4 (SLC15A4), with quantitative polymerase chain reaction (qPCR). The identification of mast cell-specific proteins can help us further understand their effects on mast cell activation and subsequent degranulation.

A Novel Protein Regulates Lymph Node Lymphangiogenesis

Aubrey D (Aubrey) Brown, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar
Mentor: Alanna Ruddell, Comparative Medicine

This abstract is no longer available.

Identification of Tumor-Draining Lymph Node Immunosuppressive Signaling Pathways

Ernie Tao, Senior, Political Science, Biochemistry
Mary Gates Scholar, UW Honors Program
Mentor: Alanna Ruddell, Comparative Medicine

This abstract is no longer available.

Stability of Remodeling-Associated and Immunomodulatory Gene Expression by Primary Bronchial Epithelial Cells from Asthmatic and Healthy Children over Increasing Cell Passage

Maryam Naushab, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Jason Debley, Pediatrics

Studies using primary airway epithelial cells from asthmatic and healthy donors have reported differences in gene expression of a variety of genes between cells from asthmatic and healthy donors. However, few studies have investigated the stability of gene expression by primary cells with increasing cell passage number, or how viral infection might affect the stability of gene expression with increasing cell passage number. This research investigated: 1) whether expression by primary bronchial epithelial cells (BEC) of genes IFIH1, which codes for a viral sensor protein with an important role in the innate immune response, and TGFβ2, a pro-remodeling cytokine, from asthmatic and healthy children, is stable with increasing cell passage number, and 2) if BEC infection with respiratory syncytial virus (RSV) alters the stability of IFIH1 and TGFβ2 expression with increasing cell passage number. To answer these questions, we differentiated passage 1, 2, 3, 4, and 5 BECs from asthmatic and healthy children at an air-liquid interface for 3 weeks. RNA was then harvested from BECs and RT-PCR was performed for TGFβ2 and IFIH1. To assess the expression stability across increasing cell passage number for each gene, we compared expression at passages 2-5 to expression at passage 1. Preliminary data collected shows that expression of TGFβ2 and IFIH1 from asthmatic and healthy children was stable with no significant differences between passages 1, 2 and 3. However, expression at cell passages 4 and 5 was significantly greater and more variable than by passage 1 BECs. We anticipate gene expression to be even more variable for RSV-infected passage 4 and 5 BECs. These observations illustrate the importance of using BECs from passage ≤ 3 when comparing gene expression between asthmatic and healthy primary BECs, and of characterizing the expression pattern across increasing cell passage number for each gene studied.

The New Pattern Recognition Receptor RECON Acts as Part of the Intestinal Immune System

Alexie Anne Carletti, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar, Innovations in Pain Research Scholar
Mentor: Joshua Woodward, Microbiology
Mentor: Adelle McFarland, Molecular and Cellular Biology/Microbiology

We have recently identified a new cytosolic sensor for bacterial cyclic dinucleotides, the aldo-keto reductase RECON. RECON plays a critical role in surveying the host cytosol for intracellular bacterial pathogens, orchestrating the innate immune response to those pathogens and aiding in bacterial clearance. In the host, RECON is most highly expressed in the small intestinal epithelium, enterocytes and M cells. Its high expression at these important mucosal immune sites raises the question as to whether RECON is part of the intestinal immune system. We have recently made a RECON knockout (KO) mouse and found significant alterations in intestinal inflammation and commensal abundance, particularly an increase in segmented filamentous bacteria (SFB). Using quantitative real-time PCR copy number analyses of SFB 16S gRNA, we detected high SFB burden in the RECON KO mice while SFB was undetectable in wild-type mice. SFB have been shown to impact the development of the immune system, particularly T cell populations, in rodents by colonizing and attaching to the follicle-associated epithelium of the small intestines. We predict that the high abundance of SFB in RECON KO mice may either be the result of immunosuppression due to the presence of too many T regulatory cells or will associate with the increased presence of pro-inflammatory T helper type 17 cells. Current work is aimed at establishing whether there are changes in these T helper cell types in the intestine, as well as looking at the broader dysbiosis by 16S deep sequencing. This project will establish which immune axes are dysregulated in the absence of RECON and will direct future mechanistic investigations into how loss of RECON enzymatic activity is driving immune dysregulation.