2J

INFECTIOUS DISEASES

Session Moderator: James Mullins, Microbiology
254 MGH
3:45 PM to 5:15 PM

* Note: Titles in order of presentation.

Determining Reservoirs of HIV-1

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Initiative for Maximizing Student Development Scholar
Mentor: James Mullins, Microbiology
Mentor: Richard Fox, Microbiology

Administration of Anti-Retroviral Therapy (ART) slows disease progression and reduces HIV-1 viremia to near undetectable levels. Poor adherence or withdrawal of therapy often results in rapid rebound of viremia with the potential for the establishment of drug resistant variants. HIV is known to establish latency in long-lived central memory T-cells (CM) and is often referred to as “the reservoir”. However, we now understand that there is both latent and actively replicating HIV-1 that comprises reservoir sites. Using sequence information and computational tools we can define reservoirs as cells or tissues harboring HIV-1 with genotypic features of reduced temporal structure, high diversity within the population, and low divergence from their most recent common ancestor (MRCA). This is because reservoirs are populated with virus seeded at both early and late periods of infection. If a reservoir is established in a site with reduced drug penetrance, “drug-sanctuaries”, continued viral replication and diversification could occur. This may result in the accumulation of drug resistant variants that lead to therapy failure. Here we hypothesize that tissue and cellular sites with phenotypes reflective of reservoirs may also be drug restricted providing a boundary for unrestricted viral replication. To test this hypothesis we sequenced single genome template derived amplicons (SGA) of env and pol regions of HIV-1. Presented is data from the autopsy of subject S104. S104 presented with multi-drug resistant genotypes in all tissues sampled. Bioinformatical analysis measuring viral genetic diversity within and divergence from the MRCA of the viral population of S104 demonstrated reservoir phenotypes in tissue virus isolated from lung. We continue to expand our characterization of reservoirs and compartments to clearly define sites that must be targeted to eradicate HIV-1 in the host.

Intravaginal Recruitment of Dendritic Cells Using DC Chemokines

Hunter R. (Hunter) Bennett, Senior, Bioengineering
Amgen Scholar, Levinson Emerging Scholar, Mary Gates Scholar
Mentor: Kim A. Woodrow, Bioengineering
Mentor: Renuka Ramanathan, Bioengineering

A significant challenge in intravaginal vaccination against mucosal pathogens concerns the immune privileged environment in the genital tract, which prevents migration of immune cells in the absence of inflammation. Dendritic cells (DC), the major antigen-presenting cell to T cells in lymph nodes, play a particularly important role in generating an effective immune response to vaccination. Our goal is to explore a new intravaginal vaccination strategy utilizing chemokines - short biological peptides that activate cells - to recruit DCs. We intravaginally administered CpG, MIP-3 Alpha and Beta-Defensin 2 to female C57BI/6J mice (Jackson Laboratories). Mice were euthanized after 24 hours and cells were isolated from both the spleen and reproductive tract. DCs were quantified using fluorescent activated cell sorting (FACS). We have demonstrated that DCs are actively recruited to vaginal mucosal tissues following intravaginal administration of the chemotactic peptides MIP-3 Alpha and Beta-Defensin 2. Future work will involve time course studies of DC recruitment and the delivery of nanoparticle encapsulated chemokines.

Molecular Mechanism of Human CD1A-Deficiency

Meera Kerki (Meera) Shenoy, Senior, Microbiology
Mary Gates Scholar
Mentor: Thomas Hawn, Medicine
Mentor: Chetan Seshadri, Medicine

Mycobacterium tuberculosis infection is a leading cause of death worldwide, but the full details of how the human immune system responds to these bacteria are not known. Our lab studies CD1 proteins, which allow T-cells to recognize and respond to lipids which are the major constituents of the

mycobacterial cell wall. We recently discovered that low expression of CD1A on dendritic cells is present in ˜10% of the population and is associated with increased susceptibility to tuberculosis in Vietnam. In addition to low surface protein expression, CD1A transcription is also impaired in CD1A-deficient individuals. The molecular mechanism of CD1A-deficiency is unknown. I hypothesized that CD1A expression is regulated by a single nucleotide polymorphism (SNP) that alters transcription factor binding. I cloned and analyzed the CD1A 5' untranslated region (UTR) from four CD1A-deficient and five control individuals. There was one SNP, rs366316, that showed an association with CD1A-deficiency. I genotyped 163 individuals and examined the association of the rs366316 genotypes with CD1A expression. I found that the C/C genotype (N=9) was associated with CD1A-deficiency in comparison to the C/T and T/T genotypes (N=154, $p<0.01$). Based on this association, I examined whether the C/C genotype regulated transcriptional activity. I isolated 5'UTR sequences in normal and deficient individuals that were identical except for rs366316. I used site-directed mutagenesis to generate C to T mutants and T to C mutants. I made separate luciferase expression vectors for each of the four 5'UTR constructs and found that the normal and mutated 5'UTRs with a C allele showed 44% less expression than the normal and mutated 5'UTRs with a T allele ($p<0.001$). These data show that a SNP in the 5'UTR of CD1a is causally associated with human CD1A-deficiency. Together, these data suggest that CD1A-deficiency is common, regulated by a SNP, and important in MTB pathogenesis.

In Vitro Expansion of Factor VIII-Specific T Regulatory Cells

Bryn Smith, Senior, Microbiology
Washington Research Foundation Fellow
Mentor: Carol H. Miao, Pediatrics
Mentor: Chao Lien Liu, Immunology and Immunotherapies, Seattle Childrens Research Institute

Hemophilia A is characterized by a deficiency of factor VIII, a protein necessary for the formation of blood clots. Treatment involves infusions of replacement clotting factor but about one-third of patients develop inhibitors to the clotting factor, resulting in reduced efficacy of the infusion and increased cost of treatment. Immunosuppressant drugs have been found to reduce an inhibitor response, but their off-target effects can complicate treatment further. Alternatively, T regulatory cells have a naturally suppressive function and their proliferation in vivo in response to treatment with IL-2 has been studied previously in the Hemophilia A mouse model. This project aims to expand a population of factor-VIII specific Treg cells in vitro by co-stimulation with anti-CD3 and anti-Crry, in addition to IL-2 treatment. Treg cells are harvested from the spleen, and then isolated by magnetic separation and FACS Aria sorting for CD25high cells. Analysis by flow cytometry has indicated that the expanded cells maintain Foxp3 expression – an intracellular marker for Treg cells – and further experiments are being done to analyze the suppressive functionality of the expanded cells compared to fresh Treg cells. Future expansions will be focused on factor VIII-specific Tregs using the Hemophilia A mouse model and the techniques mentioned previously. The goal is to generate an expanded population of these suppressive cells that show specificity to only factor VIII antigen. This method of expansion has translational potential for suppressing the inhibitory immune response to treatment in human Hemophilia patients. Treating patients with their own expanded cells can reduce possible rejection of the treatment and provide promising long-term results.

Analysis of Global Cellular Metabolic Alterations During Vaccinia Virus Infection: Glutamine Metabolism is Required for Successful Viral Replication

Roman Daniel (Roman) Camarda, Senior, Biochemistry, Art (Photography)
Mary Gates Scholar
Mentor: Michael Lagunoff, Microbiology

Viruses require host cell metabolism to provide the necessary energy and biosynthetic precursors for viral replication. Recently, our lab conducted a global metabolic study to determine how vaccinia virus (VACV), a large double-stranded DNA virus, alters host cell metabolism. VACV is a model system to study poxvirus infections and was used as the live vaccine against smallpox. In our study, dual mass spectrometry platforms were used to analyze a broad range of metabolites from mock and VACV-infected fibroblast cells harvested at multiple time points within 24 hours of infection. One of the most notable metabolic alterations observed during VACV infection was increased glutamine utilization, or glutaminolysis. We have since shown that under conditions of glutamine starvation there is an approximate 90% decrease in VACV production in fibroblasts, thus revealing an essential role for glutamine during infection. Furthermore, this decrease in virus production could be rescued by the addition of tri-carboxylic acid (TCA) cycle intermediates, suggesting glutamine's use as an anaplerotic, or replacement, substrate for the TCA cycle during infection. To determine at what stage(s) in the VACV life cycle glutamine is required, quantitative real-time RT-PCR and western blot analyses were performed to examine early and late viral transcript and protein levels. We have found that glutamine deprivation does not inhibit viral gene expression, but does significantly impact viral protein synthesis. Importantly, the addition of TCA cycle intermediates recovered viral protein levels, highlighting the need for glutamine carbon to maintain the TCA cycle at this stage in the VACV life cycle. This study reveals the robust metabolic program implemented by vaccinia virus during the course of infection and provides insight into the role

metabolic reprogramming plays during viral replication. Ultimately, studies such as these may help to identify novel therapeutic methods to specifically target infected cells and inhibit viral replication.

The Evolution of Interactions between the Primate Antiviral Protein SAMHD1 and the Lentiviral Antagonist Vpr

Theresa Santos, Senior, Biology (Molecular, Cellular & Developmental), Sociology
Howard Hughes Scholar
Mentor: Michael Emerman, Microbiology, Fred Hutchinson Cancer Center

The primate immune system has evolved to counter viral attacks. Yet, after several cross species transmission events of SIV, HIV has infected about 60 million people since the beginning of its epidemic. This is in part due to the ongoing evolutionary arms race between antiviral proteins and viral antagonists. SAMHD1 is an antiviral protein that is degraded by the Vpr protein expressed in HIV and SIV. In this evolutionary arms race, SAMHD1 evolves to escape degradation, while Vpr evolves to bind to these new variants of SAMHD1. African Green Monkeys (AGM) are divided into phenotypically distinct and geographically isolated subspecies, each infected with their own species-specific SIVagm, making them ideal to study. A cloned SAMHD1 gene, Tan4, from AGM has been found to be resistant to Vpr from a number of different viruses, except for one called the tan1 Vpr. Sequencing Tan4 revealed that one residue of its nuclear localization signal was mutated. Since nuclear localization of SAMHD1 is required for degradation by Vpr, we hypothesized Tan4 evolved to be cytoplasmic in order to resist degradation. Using immunoflouresence, our results confirmed Tan4 to be in the cytoplasm. Moreover, we found that tan1 Vpr and Vpr from another AGM subspecies, vervet, share similar gene sequences, but only tan1 can degrade Tan4. Currently, I am constructing chimeras between the two Vpr proteins to find the residues in tan1 responsible for degradation of Tan4. Likewise, two Vpr proteins of the sabeus monkey share similar gene sequences yet cannot degrade the same SAMHD1. My ongoing work explores the mechanisms used by the tan1 and sabeus Vpr proteins to evolve in order to successfully degrade SAMHD1 despite its attempts at escape. This will elucidate a better understanding of the ongoing evolutionary arms race for both primate host and virus survival.

Drug Screening for Malaria Yields Securinine-Related Compounds that Activate *Plasmodium falciparum* dUTPase

Jack Mo, Senior, Biochemistry
Mentor: Gregory Crowther, Medicine
Mentor: Wes Van Voorhis, School of Medicine

Malaria is a prominent parasitic disease that afflicts 300 to 500 million people and results in 1 to 2 million deaths each year, approximately 90% of which are young children under the age of 5 and pregnant women in sub-Saharan Africa. New therapeutic compounds for treating malaria are urgently needed due to the emergence of multidrug-resistant parasites including *Plasmodium falciparum*, the most fatal Malaria causing species that infects humans. A mass spectrometry-based screening of a natural product library yielded seven securinine-based compounds that were determined to bind to *Plasmodium falciparum* 2'-deoxyuridine 5'-triphosphate nucleotidohydrolase (*Pf*dUTPase). This enzyme catalyzes the formation of deoxyuridine monophosphate (dUMP) from deoxyuridine triphosphate (dUTP) which has important implications on DNA replication and thus makes dUTPase a good target enzyme for drug discovery. These seven compounds were tested using a standard biochemical assay and enzyme activity was measured through the detection of pyrophosphate, a product of the catalyzed reaction. Detection was done with two independent detection kits: indirectly through the detection of inorganic phosphate (pyrophosphatase and Malachite Green Kit) or directly through the detection of pyrophosphate (PPiLight Kit). In most drug screens, the goal is to discover possible drug-like molecules that inhibit the target enzyme, however, these securinine compounds were actually found to increase *Pf*dUTPase activity. While it is unusual to pursue compounds that do not inhibit target enzymes, these compounds might still prove to be possible drug candidates by over activating *Pf*dUTPase and killing the parasite in this manner. Whether this is a viable method of utilizing these compounds remains to be tested.