Undergraduate Research Symposium May 17, 2013 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

Commons East, Easel 70

11:00 AM to 12:30 PM

Construction of an Adenovirus Vector Expressing the Latent Locus of Kaposi's Sarcoma-Associated Herpesvirus

Breanna L (Breezy) Wentz, Non-Matriculated,
Mentor: Michael Lagunoff, Microbiology

Kaposi's sarcoma-associated herpesvirus (KSHV) is a human pathogenic γ-herpes virus and is the infectious cause of Kaposi's sarcoma (KS), primary effusion lymphoma, and plasmablastic multicentric Castleman's disease. The KS tumor is highly vascularized and is characterized by latently infected spindle cells of endothelial origin. While in the KS tumor 1-5% of the cells are lytic and producing more virus, greater than 90% are latently infected and not producing virus. The goal of this project is to investigate whether the latent genes expressed by KSHV are sufficient to alter endothelial cells to induce angiogenesis, metabolism, and oncogenic cell signaling. We will determine if latent genes alone induce these effects or if the small percentage of lytic genes play a paracrine role. In order to do this we are cloning the entire KSHV latent locus into a gutted adenovirus expression vector that eliminates the contamination from expression of adenovirus genes. The latent locus includes the 4 genes expressed during latency and the 12 virally encoded microRNAs. The adenovirus vector expressing the latent locus will be used to infect endothelial cells and we will examine the cells expressing the KSHV latent locus for changes in factors involved in angiogenesis, metabolic pathways and oncogenic cell signaling. If the latent genes recapitulate the pathways of a KS infection, this will indicate that latent gene expression alone, without the low percentage of lytic genes in the culture, are sufficient to induce many cellular pathways induced by KSHV and are relevant for the pathogenesis of KSHV in KS tumors.

POSTER SESSION 2

Commons East, Easel 85

12:45 PM to 2:15 PM

Epitopes of Viral Sensitivity to Broadly Neutralizing Antibodies in HIV

Beth Bingjie (Bingjie) Wang, Senior, Biology (Molecular, Cellular & Developmental), Public Health-Global Health
Mary Gates Scholar
Mentor: Julie Overbaugh, Microbiology
Mentor: Leslie Goo, Global Health

Neutralizing antibodies, which prevent entry of the HIV virus into a host cell by binding to the HIV envelope and neutralizing the biological effects of the virus, is an important focus of HIV vaccine design. It is hypothesized that these neutralizing antibodies may protect against initial infection from HIV if they are elicited by vaccination and are thus present at the time of virus exposure. The high rate of HIV mutation results in an enormous number of HIV variants circulating in the human population. Thus, an effective neutralizing antibody-based vaccine must be broad and potent. In order to understand how to create factors that elicit broadly neutralizing antibodies (bNAbs), it is important to identify and map regions of the viral envelope important for recognition by bNAbs. The best way to map the molecular basis is to identify two closely related variants that are or are not susceptible to neutralization by a certain virus and identify the regions that differ between the two variants. PGT128- is a HIV-1 monoclonal antibody that has been identified as having a broad neutralizing activity that would be desirable for a vaccine to elicit. Our lab previously found that longitudinal viruses from a HIV-1 infected patient, QA255, across 560 days post infection (dpi) display increasing ability to be neutralized by PGT128. PGT128 recognition has recently been found to depend on residue N332 of the V3 envelope region of HIV. As this residue is intact in QA255 viruses over time, there are additional determinants of sensitivity to PGT128. We found that chimeric sequences containing 560 dpi DNA in the V1-C3 region of the HIV envelope sequence show more sensitivity to PGT128 compared to chimeric sequences from earlier time points. This result suggests that amino acids in the V1-C3 region are important for PGT128 sensitivity.

POSTER SESSION 2

Commons East, Easel 57

12:45 PM to 2:15 PM

HIV Reservoirs, Compartments, and Sanctuaries: Where Does Virus Persist Despite Antiretroviral Therapy?

Jennifer Mackenzie (Jennifer) Whitehead, Senior, Biology (General)
Matthew Eugene Pouw, Senior, Microbiology
Mary Gates Scholar
Natalie Chen, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: James Mullins, Microbiology

To be infected with human immunodeficiency virus (HIV) is to face a lifelong battle. Even with the combined force of multiple antiretroviral drugs to target different phases of the HIV life cycle, one cannot eliminate human immunodeficiency virus (HIV) from the body. This is due to persistence of virus reservoirs, compartments, or sanctuaries. Reservoirs are tissue sites characterized by collection of virus over time: viral populations show low divergence from the founder virus and high diversity between each other. Compartments are tissues with limited gene flow: they are pockets of virus with limited entry and exit. Sanctuaries are tissues that resist penetration of antiretroviral drugs: they can set up the conditions for reservoirs or compartments. To understand how and where these sites of persistent infection endure in the body even during antiretroviral therapy (ART), we are conducting a systematic characterization of possible sites in the infected host. We are studying viral populations present in biopsy-derived gut-associated lymphoid tissues (GALT) and other autopsy-derived tissues of patients who died while on antiretroviral therapy (ART). Viral populations are sampled from tissues using molecular lab techniques (including polymerase chain reaction and Sanger sequencing) and analyzed using phylogenetic trees and computational programs that measure diversity, divergence, and clustering. Our study addresses two main aims: (1) to find the tissue sites in the host critical in maintaining latent HIV infection during suppressive ART and (2) to find tissue sites critical in supporting continued viral replication and the emergence of pathogenic phenotypes during ART by characterizing sites as reservoirs, compartments, or drug sanctuaries. Hopefully our research will help to direct future methods of HIV treatment by allowing researchers and clinicians to better understand how and where virus persists in the body.

POSTER SESSION 2

Commons East, Easel 56

12:45 PM to 2:15 PM

Exploiting the Antimicrobial Properties of a Type VI Effector-Immunity Pair

Taylor Eliza Gardiner, Senior, Microbiology
Howard Hughes Scholar, Mary Gates Scholar
Mentor: Joseph Mougous, Microbiology
Mentor: Seemay Chou, Microbiology

The export of signals is a vital aspect of bacterial communication with its environment and it was recently found that Gram-negative bacteria can send signals directly to neighboring bacteria via the type VI secretion system (T6SS). The bacterial T6SS translocates antibacterial toxins into neighboring bacterial cells and provides a means for bacteria to compete in their environment. Bacteria can also target their own cells with the T6SS but are protected against self-intoxication by cognate immunity proteins that inactivate their partner toxins. For example, one T6-exported toxin found in Pseudomonas aeruginosa, Tse1, degrades the cell wall of the recipient cell. The cell wall provides structural support for bacteria, and degradation of this protective layer by Tse1 causes the target cell to lyse. P. aeruginosa is immune to this toxicity, however, because an immunity protein, Tsi1, in the cell wall layer of P. aeruginosa binds and inhibits T6-exported Tse1. Our lab has found that Tse1 and Tsi1 are members of a large superfamily of type VI amidase effector-immunity widely found in Gram-negative bacteria, suggesting that these T6 toxin-immunity pairs may play a major role in influencing the structure of polymicrobial communities in general. Because of the large influence the T6SS can have on polymicrobial environments, I predict the T6-toxin-immunity system could also be utilized as an antimicrobial strategy. My aim is to engineer a novel Tse1–Tsi1 toxin–immunity pair that escapes rescue by immunity proteins in naturally occurring bacteria, thus giving the engineered P. aeruginosa strain the ability to outcompete the natural wild-type strain. To do this I will use a structure-based approach in conjunction with a genetic directed evolution strategy to identify and mutate Tse1 and Tsi1 residues that play an important role in Tsi1–Tse1 recognition and inhibition.

POSTER SESSION 2

Commons East, Easel 53

12:45 PM to 2:15 PM

Role of Translesion Synthesis Polymerases in Repair of DNA Damage and Replication Conflicts in *B. subtilis*

Ariana Nakta (Ariana) Samadpour, Senior, Art (Painting and Drawing), Microbiology
Mary Gates Scholar
Mentor: Houra Merrikh, Microbiology

DNA replication faces many obstacles as the genome is duplicated in all organisms. These obstacles include breaks in the DNA, tightly bound proteins as well as transcription. The encounters between replication and roadblocks can lead to repli-

cation stalling and genomic instability. To deal with replication conflicts, cells have mechanisms that act at regions of conflicts and help resolve them. Our lab is interested in these factors and in understanding how replication conflicts are dealt with in our model organism, *Bacillus subtilis*. Because Translesion Synthesis (TLS) polymerases are thought to act at certain stalled replication forks, in the context of DNA damage, we hypothesize that they play a more generalized role in resolving many different replication conflicts including those that are due to breaks. To determine if this is the case, we generated a deletion mutant of the *yqjH* gene. We have characterized this strain by specifically looking at its phenotypes under DNA damaging conditions. We are now looking to see if YqjH has any role in replication conflicts, both in terms of the severity of stalling and replisome stability, in *B. subtilis*.

SESSION 2J

INFECTIOUS DISEASES

Session Moderator: James Mullins, Microbiology

254 MGH

3:45 PM to 5:15 PM

* Note: Titles in order of presentation.

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.

SESSION 2J

INFECTIOUS DISEASES

Session Moderator: James Mullins, Microbiology

254 MGH

3:45 PM to 5:15 PM

* Note: Titles in order of presentation.

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.

SESSION 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
Cameron Riley (Cameron) Adams, Senior, Biochemistry
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.

SESSION 2L

CANCER MECHANISMS

Session Moderator: Michael Lagunoff, Microbiology
271 MGH
3:45 PM to 5:15 PM

* Note: Titles in order of presentation.

Delineating Viral Mechanism of KSHV-induced Angiogenesis
Sreetha Sidharthan, Senior, Biochemistry
Howard Hughes Scholar, Mary Gates Scholar
Mentor: Michael Lagunoff, Microbiology

Angiogenesis is the development of new blood vessels from pre-existing vasculature and is a hallmark of Kaposi's sarcoma (KS), a highly vascularized neoplasm prevalent among immunosuppressed individuals. The etiological agent of KS, Kaposi's sarcoma-associated herpesvirus (KSHV), induces angiogenic phenotypes in endothelial cells during latent infection. Interestingly, KSHV infection leads to down-regulation of transforming growth factor-beta 2 (TGF-β2), an anti-angiogenic cytokine. In addition to six viral genes, KSHV also encodes 17 recently discovered microRNAs (miRNAs) during latent infection. These endogenously encoded RNAs of 19-23 nucleotides post-transcriptionally regulate gene expression through basepairing interactions with the 3' untranslated region (UTR) of target messenger RNAs (mRNAs) and either mark them for degradation or prevent ribosomal binding. These viral miRNAs appear to directly contribute to pathogenesis and angiogenesis by targeting host gene expression. We screened the miRNAs of KSHV to determine if any are involved in the down-regulation of TGF-β2 during KSHV infection. We found that two KSHV miRNAs, miR-K3 and miR-K8, are independently sufficient to down-regulate TGF-β2. To determine if these miRNAs directly target the 3'UTR of TGF-β2 as opposed to altering genes that affect TGF-β2 expression, we used a TGF-β2 3'UTR luciferase reporter construct. We also used competitive inhibitors of miR-K3 and miR-K8 to determine if these miRNAs are necessary for the down-regulation of TGF-β2. By identifying specific targets of KSHV miRNAs such as TGF-β2, we can improve our understanding of how KSHV is able to maintain latent infection and target host gene expression to alter endothelial cells.

POSTER SESSION 3

Commons East, Easel 44

2:30 PM to 4:00 PM

Site Directed Mutagenesis as a Strategy to Understand Viral Protein: Cell Protein Interactions

Alyssa Sandner, Junior, Biological Sciences with teaching , Montana State University

McNair Scholar

Mentor: Michele Hardy, Immunology and Infectious Diseases, Montana State Unversity

Rotavirus is particularly important to viral research because it is known to cause diarrhea in small children, usually leading to death. Viruses that are able to infect mammals have created mechanisms to evade host response by antagonizing cellular antiviral responses such as interferon response. Interferon Regulatory Factors initiate the immune response cascade by detecting the viral presence. Rotavirus NSP1 protein causes the dismantling of certain antiviral Interferon Regulatory factors depending on the particular strain. Antiviral Interferon Regulatory Factors 3 and 7 are important to initiate interferon creation, and post infection NSP1 induces degradation. We compared three strains from bovine, Human and porcine sources and used *in vitro* Site Directed Mutagenesis to try and investigate what about the protein causes such specificity in its selection of which antiviral Interferon Regulatory factor to break down. The overall test was to determine if mutagenizing NSP1 from one strain to another would change the substrate the NSP1 selects to degrade. This leaves the question, what does the transition from OSU to W161 mean? Site directed Mutagenesis changes nucleotides to create a mutation. These amino acids they code for are thought to be important to protein function. With such a detrimental virus, more research is necessary to discover more about the virus' actions.

POSTER SESSION 3

Commons East, Easel 75

2:30 PM to 4:00 PM

Cloning a Library of Envelope Variants from HIV-1 Superinfected Individuals to Test Immune Response

Willimark Manulat (Willimark) Obenza, Non-Matriculated, Initiative for Maximizing Student Development Scholar

Mentor: Julie Overbaugh, Microbiology

Mentor: Keshet Ronen, Global Health

Despite considerable effort, immune correlates of protection against HIV have not been identified, therefore making a successful vaccine challenging. In other words, since we have no examples of a successful immune response against HIV, we are unable to mimic that in a vaccine. One aim of the Overbaugh Lab is to identify immune correlates of protection by studying individuals who have mounted an immune response against HIV and who are at risk of superinfection, which is a second infection of HIV after the initial infection. My project is to clone a library of autologous HIV envelope genes from superinfected individuals whose immune responses we're interested in studying, isolating both initial and superinfecting variants. These clones I generate will be tested for functionality (infectivity), used to produce target viruses in neutralization assays that measure the neutralizing antibody response in patient plasma, and used in assays measuring non-neutralizing antibody functions. Cloning patient envelope sequences involves isolating viral RNA from patient plasma and reverse transcribing it into DNA. After a polymerase chain reaction (PCR) to amplify the target envelope DNA sequence, I then ligate it into a mammalian expression vector. Sequencing analysis of the clones we obtain from each sample enables us to ensure that viral variants from the initial and second infection events are represented so that we can measure immune responses against both. After working with three patients, I have successfully obtained 11 initial and superinfecting variants from one patient, which produced 10 replicating viruses. From the next two patients, I cloned nine variants from each. Performing the functionality assay is in progress for these patients, as well as the neutralization assay. Currently, the assay to measure non-neutralizing antibody response is under optimization.

POSTER SESSION 4

MGH 241, Easel 148

4:15 PM to 5:45 PM

Investigation of Biofilm Production in *P. acnes*

Huy Q. (Huy) Pham, Senior, Microbiology

Amgen Scholar

Mentor: Roger Bumgarner, Microbiology

Standard orthopedic surgical procedures fail for reasons that are currently not understood. Joint replacements become unexpectedly stiff, well-fixed implants become loose and fusions fail to heal. These failures are costly to the patient and the healthcare system, especially if they result in substantial number of these 'aseptic' failures may be due to pernicious infection with relatively low virulence organisms, such as Propionibacteria, that do not stimulate the body's usual immune response or usual clinical evidence of infection. *P. acnes* is aerotolerant anaerobic, slow-growing, and found in the skin's sebaceous gland as commensal microbial flora. The Bumgarner group has sequenced a large number of *P. acnes* isolated from prosthetic implants. There is a strong interest in understanding the genes responsible for variation in biofilm forming capacity. My project within the Bumgarner group involves measuring biofilm production capacity across several strains of *P. acnes* and a related species, *P. humerusii*. The goal is to catalog and associate the phenotypes with the

genotypes associated with pathogenic strains. This will contribute to the understanding of surgical pathology, and lead to improved aseptic practice in future operations.