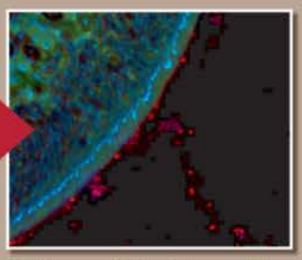

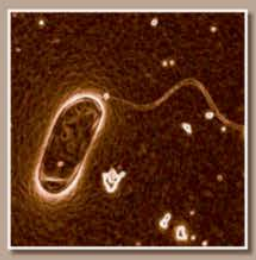

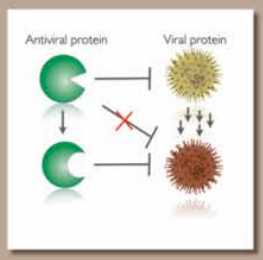

College of Medicine – Tucson
Department of Immunology

Fourteenth Annual: Frontiers in Immunobiology & Immunopathogenesis

Plenary Speakers

Russell Vance – Immunology
University of California, Berkeley
HHMI Investigator
Strategies for Cytosolic Detection of Bacterial Pathogens

Jeffrey Weiser – Bacteriology
New York University, School of Medicine
Targeting Microbial Transmission

Harmit Malik – Virology
Fred Hutchinson Cancer Research Center
HHMI Investigator
Rules of Engagement: Molecular Arms Races between Host and Viral Genomes

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Agenda- Frontiers in Immunobiology & Immunopathogenesis Symposium

Morning Activities
Health Sciences Innovation Building (HSIB), 1st Floor Lobby and Forum

7:45 - 8:15 AM **Registration & Continental Breakfast**

8:20 - 8:40 AM **Welcome Announcements**
Janko Nikolich-Žugich, MD, PhD
Professor and Department Head, Immunobiology
Co-Director, Arizona Center on Aging

Anne Cress, PhD
Deputy Dean, Research and Academic Affairs,
College of Medicine
Professor, Cellular and Molecular Medicine, Radiation and Oncology

Session I: Virology
Moderator: Josh Kochanowsky

8:40 - 8:50 AM *Brittany L. Uhlorn*
"Human Papillomavirus Exploits Mitotic Golgi Vesiculation to Evade Innate Immune Detection"

8:55 - 9:15 AM *Lynn Enquist, PhD*
Department of Molecular Biology and Princeton Neuroscience Institute, Princeton University
"Silenced or Productive Infection? Engagement of an Alphaherpesvirus with Peripheral Nervous System Neurons"

9:20 - 10:10 AM **Plenary Lecture**, ***Harmit Malik, PhD***
Fred Hutchinson Cancer Research Center
HHMI Investigator
"Rules of Engagement: Molecular Arms Races Between Host and Viral Genomes"

10:15 - 10:35 AM **BREAK**, *coffee available in Lobby*
Please visit our sponsors for a chance to win an iPad.

Session II: Immunology

Moderator: Jacob Zbesko

10:35 - 10:45 AM *Katrina Lichauco*
"CD4 Function is Regulated by Evolutionarily Layered Motifs"

10:50 - 11:00 AM *Nico A. Contreras*
"Life-long Control of Cytomegalovirus (CMV) by T Resident Memory Cells in the Adipose Tissue Results in Inflammation and Metabolic Disturbances"

11:05 - 11:55 AM **Plenary Lecture**, ***Russell Vance, PhD***
Immunology Division, Department of Molecular and Cell Biology, University of California, Berkeley
HHMI Investigator
"Strategies for Cytosolic Detection of Bacterial Pathogens"

12:00 - 1:00 PM **Lunch Sessions with Plenary Speakers**
Immunology Hot Topics
HSIB, Room 306
Jacob Zbesko, student moderator

Virology Hot Topics
HSIB, Room 322-323
Josh Kochanowsky, student moderator

Bacteriology Hot Topics
HSIB, Room 320-321
Emily Merritt, student moderator

1:05 - 1:15 PM *Megan S. Molina*
"Flt3 Signaling Pathway in Graft-versus-Host Disease"

1:20 - 1:40 PM *Justin Wilson, PhD*
Department of Immunobiology, University of Arizona
"Suppression of Intestinal Cancer by the Innate Immune Sensor AIM2"

Session III: Bacteriology

Moderator: Emily Merritt

1:45 - 1:55 PM *Zhera Esra Ilhan, PhD*
"Vaginal Microbiota, Genital Inflammation, and HPV Infection Modulate Cervicovaginal Metabolomes in Cervical Carcinogenesis"

2:00 - 2:20 PM **BREAK**, *coffee available in Lobby*
Please visit our sponsors for a chance to win an iPad.

2:20 - 2:30 PM *Katherine Rhodes, PhD*
"Type IV Pilus Retraction is Essential for *Neisseria musculi* Persistent Colonization *in vivo*"

2:35 - 3:25 PM **Plenary Lecture**, ***Jeffrey Weiser, MD***
Department of Microbiology, NYU School of Medicine
"Targeting Microbial Transmission"

3:30 - 3:35 PM **Closing Remarks/Acknowledgements**

3:35 - 3:40 PM **BREAK & Poster Setup**
Please visit our sponsors.

Evening Activities

Health Sciences Innovation Building (HSIB), Room 306

3:40 - 5:00 PM **Poster Session and Reception**

5:10 PM **Poster Awards & Drawing for iPad**

Plenary Lecture

Rules of Engagement: Molecular Arms Races Between Host and Viral Genomes

Harmit Malik, PhD

Fred Hutchinson Cancer Research Center. Howard Hughes Medical Institute Investigator, Seattle, Washington

In the past decade, virologists have revealed a novel arm of intracellular, cell-autonomous immunity that mammalian cells mount against a variety of viral infections. In collaboration with colleagues Michael Emerman and Adam Geballe, we have used an evolutionary approach that identifies potential antiviral genes based on evolutionary signatures: higher than expected rates of amino acid changes that are fixed by selection (positive selection). We have used the signature of positive selection to identify the amino acid residues in antiviral proteins that are responsible for specific recognition of viral components. For example, we recently showed that such an evolution-guided approach could also reveal the interaction interface of the broadly acting MxA antiviral protein, which had eluded investigations that used more traditional virology and biochemical approaches. We postulate that viruses much older than those in the present day have driven selection for our current antiviral specificities. This has led us to propose an alternate approach of "indirect paleovirology," i.e., inferring the presence and action of ancient viruses by virtue of the evolutionary episodes of selection they drive in host antiviral genes. Together with the identification of fossilized imprints of ancient viruses in animal genomes, these reveal an ancient tapestry of viral infections throughout animal evolution. Occasionally, some genes of these ancient viruses are usurped by genomes for host function. We are interested in identifying such cases and understanding the host biology they participate in. Thus, evolution can provide a means to identify potential antiviral genes, to reveal functional sites of host-virus antagonism and ancient viruses themselves, and to understanding differences in susceptibility to infectious diseases.

Plenary Lecture

Strategies for Cytosolic Detection of Bacterial Pathogens

Russell Vance, PhD

University of California, Berkeley. Howard Hughes Medical Institute Investigator, Berkeley, California

Innate detection of pathogens is the first critical step in any protective immune responses. There are two fundamental strategies used by the innate immune system to detect pathogens. In the first and best-characterized strategy, hosts use germ-line encoded receptors to bind directly to pathogen-encoded ligands. The Toll-like receptors, which bind bacterial flagellin and other pathogen-derived molecules, represent canonical examples of this first strategy. The second fundamental strategy of innate immune surveillance is for hosts to recognize pathogens via detection of their virulence-associated activities. This latter strategy is well-described in the plant immunity literature, but until recently, was not believed to be widely employed in vertebrates. In my talk, I will describe some of our recent work describing a new mechanism by which vertebrates initiate immunity via detection of pathogen-encoded virulence activities. In particular, I will discuss the idea that hosts may employ 'decoy sensors' to detect pathogens. I will attempt to place our work within the broader context of the innate immune response to infection.

Plenary Lecture

Targeting Microbial Transmission

Jeffrey Weiser, MD

Department of Microbiology, NYU School of Medicine, New York, New York

A key step in the life cycle of pathogens is transmission from one host to another. In general, this process involves colonization of host surfaces, exit ("shedding"), and acquisition and establishment of the organism by a new, susceptible host. An example of this paradigm is bacterial pathogen *Streptococcus pneumoniae* (Spn), which colonizes the mucosal surfaces of the human nasopharynx and is shed in secretions. Pneumococcal transmission among the human population occurs through close contact with upper respiratory tract (URT) secretions of colonized individuals. Numerous epidemiological studies on the impact of pneumococcal conjugate vaccines (PCVs) have indicated that their major contribution to public health is due to the indirect effect of vaccination (herd immunity), by which reduced carriage of Spn in vaccinated children decreases pneumococcal transmission to vulnerable unvaccinated groups. Although pneumococcal disease and colonization have been extensively studied in animal models, there is still little known about transmission of this common pathogen. This presentation will summarize our efforts to understand the biology of pneumococcal transmission with a view towards interrupting this key step in the infectious process.

Human Papillomavirus Exploits Mitotic Golgi Vesiculation to Evade Innate Immune Detection

***Brittany L. Uhlorn*[1,5], *Shauna M. Bratton*[4,5], *Eduardo R. Gamez*[4,5], *and Samuel K. Campos*[1,2,3,5]**

[1]Cancer Biology GIDP, [2]Department of Immunobiology, [3]Department of Molecular and Cellular Biology, [4]Department of Physiology, College of Medicine-Tucson, [5]BIO5 Institute, University of Arizona, Tucson, AZ

Oncogenic human papillomaviruses (HPVs) replicate in differentiating epithelium, causing 5% of cancers worldwide. During infection, HPV traffics viral genome (vDNA) to keratinocyte nuclei. Minor capsid protein L2 transports vDNA to the Golgi; upon mitosis, the L2-vDNA complex penetrates limiting membranes and localizes to mitotic chromosomes.

DNA sensor cGAS recognizes cytosolic dsDNA and produces cGAMP, activating ER-resident STING. STING relocalizes to the Golgi and recruits TBK1 to phosphorylate IRF3, initiating a type-I IFN response. The cGAS/STING pathway is assumed to be inactive during mitosis to avoid detecting self-DNA. Since the Golgi is a platform for STING/TBK1/IRF3 activation, we hypothesize mitotic Golgi dispersal deactivates cGAS/STING activity. Further, we hypothesize HPV evolved to traffic to and translocate from the mitotic Golgi as an immunoevasive tactic to avoid detection by cGAS/STING.

HaCaTs were transfected with DNA or infected with HPV pseudovirions and analyzed for cGAS/STING activation. DNA transfection resulted in STING and IRF3 activation. Chemical disruption of the Golgi blocked DNA-mediated IRF3 activation, suggesting Golgi morphology may modulate cGAS/STING. Accordingly, DNA-dependent IRF3 phosphorylation was transiently reduced in mitotic cells, but chemical impairment of mitotic Golgi vesiculation enabled cGAS/STING activation, without foreign DNA transfection. Furthermore, HPV infection resulted in minimal IRF3 phosphorylation, indicating HPV evades detection during initial infection. To determine if HPV's unique trafficking enables evasion, cationic liposomes were used to force premature virion translocation. Such treatment renders a non-infectious, translocation-defective mutant HPV infectious, yet susceptible to cGAS/STING sensing. Overall, cGAS/STING may be inactivated by mitotic Golgi dispersal, allowing HPV to evade detection.

Silenced or Productive Infection? Engagement of an Alphaherpesvirus with Peripheral Nervous System Neurons

Lynn Enquist, PhD

Department of Molecular Biology and Princeton Neuroscience Institute, Princeton University, Princeton, New Jersey

Alpha herpesvirus infections stay life-long in infected human and animal hosts` nervous systems in a silent state ready to reactivate upon various stress signals. Remarkably, infection of epithelial cells with these viruses results in productive infection whereas infection of peripheral nervous system neurons results in non-productive silent infection (i.e. latency) in the natural hosts. More interestingly, infection of dissociated peripheral neurons in culture also results in productive infection unless DNA replication inhibitors are used. To study the molecular mechanisms of escape from latency, we used primary neurons cultured in compartmented tri-chambers. By this way, we recapitulated the natural route of infection by infecting axons with low dose of virus which resulted in a silent infection in a small number of neuronal cell bodies without the use of any inhibitors. Using these cultures, we developed a new complementation assay to investigate the molecular signals leading to escape from latency and establishment of productive infection. We found two different mechanisms to escape from silencing: a cellular stress-mediated slow route and viral tegument protein mediated-fast route. Furthermore, we showed that the stress-mediated pathway requires protein kinase A and c-Jun N-terminal kinase activity while the viral tegument-mediated fast escape does not require these host cell kinase activities. We also concluded that a general response to DNA virus infection or presence of excess herpesviral genomes in the nucleus to saturate silencing complexes is not enough to escape from silencing. Induction of a productive infection in neurons even at low MOI, requires either the presence of tegument proteins or the activation of the PKA and JNK pathway.

CD4 Function is Regulated by Evolutionarily Layered Motifs

***Katrina Lichauco*[1], *Mark S. Lee*[1], *Heather L. Parrish*[1], *Koenraad Van Doorslaer*[2], *and Michael S. Kuhns*[1]**

[1]Department of Immunobiology, College of Medicine-Tucson, [2]Department of Animal & Comparative Medical Sciences, College of Agriculture & Life Sciences, University of Arizona, Tucson, AZ

CD4 plays a critical role in the development and differentiation of CD4+ T cells. Dogma has it that CD4 contributes to T cell receptor (TCR) signaling by (1) interacting with the Src kinase Lck via a CxC clasp and (2) recruiting Lck to the T cell receptor (TCR)-CD3 complex when both the TCR and CD4 engage the same peptide:MHC (pMHC). In support of this idea, we have found that mutating the CxC clasp to SxS prevents CD4 association with Lck and coreceptor function. But, we and others have previously reported that a truncated version of CD4 (CD4T) that lacks the CxC clasp still enhances TCR-CD3 signaling. We are then left with a question of how to reconcile these findings. Here we asked if CD4 is regulated by motifs layered throughout the entire protein and turned to evolutionary analysis to determine if we can identify motifs under selective pressure that would suggest functional significance. Our analysis points to multiple motifs within CD4 that are under significant selective pressure. For example, the CxC clasp arose with the earliest vertebrates and has remained unchanged. Much later a GGxxG motif in the transmembrane domain, and a CVRC palmitoylation motif located at the junction of the TMD and ICD, co-arose in mammals. Palmitoylation has been posited to localize both CD4 and Lck into lipid rafts. Since the GGxxG and palmitoylation motifs co-arose we hypothesized that they might work together to target CD4 to lipid rafts, thereby contributing to CD4's signaling capacity through increased local concentration of Lck. We tested this hypothesis by investigating how each of these motifs influences lipid raft localization and function in full length CD4. Our data indicate that the GGxxG, palmitoylation, and CxC clasp motifs all contribute to CD4 membrane domain localization and function. Our data also strongly suggest the existence of a yet-to-be-characterized 4th motif in CD4's ICD that plays an important regulatory function that explains the disparate results between CD4T and full-length CD4 lacking the CxC clasp. Through this work we provide evidence for evolutionarily layered motifs that regulate mammalian CD4's contribution to T cell activation by regulating membrane domain localization, association with Lck, and function.

Life-long control of cytomegalovirus (CMV) by T resident memory cells in the adipose tissue results in inflammation and metabolic disturbances

***Nico A. Contreras*[1], *Ilija Jeftic*[1,2], *and Janko Nikolich-Žugich*[1,*]**

[1]Department of Immunobiology and the University of Arizona Center on Aging, College of Medicine – Tucson, University of Arizona, Tucson, AZ; [2]Department of Pathophysiology, Faculty of Medical Sciences, University of Kragujevac, Serbia

Cytomegalovirus (CMV) is a ubiquitous herpesvirus infecting most of the world's population. CMV has been rigorously investigated for its effects on lifelong immunity and potential complications arising from lifelong infection. A rigorous adaptive immune response mounts during progression of CMV infection from acute to latent states. CD8 T cells, in large part, drive this response and have very clearly been demonstrated to take up residence in the salivary gland and lungs of infected mice during latency, however the extent to which the adaptive immune system utilizes tissue resident CD8 T cells as an ongoing defense mechanism against CMV has yet to be fully revealed and has been restricted to these anatomical locations. Therefore, we sought to identify additional locations of anti-CMV T cell residency and the physiological consequences of such a response. Through RT-qPCR we found that mouse CMV (mCMV) infected the visceral adipose tissue and resulted in an expansion of leukocytes in situ. We further found, through flow cytometry, that adipose tissue became enriched in cytotoxic CD8 T cells that are specific for mCMV antigens from day 7 post infection through the lifespan of an infected animal (> 450 days post infection). Furthermore, we found that inflammatory cytokines are elevated alongside the expansion of CD8 T cells. Finally, we show a correlation between the inflammatory state of adipose tissue in response to mCMV infection and the development of hyperglycemia in mice. Overall, this study identifies adipose tissue as a location of viral infection leading to a sustained and lifelong adaptive immune response mediated by CD8 T cells that correlates with hyperglycemia. These data potentially provide a mechanistic link between metabolic syndrome and chronic infection.

Flt3 Signaling Pathway in Graft-versus-Host Disease

Megan S. Molina, Jessica Stokes, Emely Hoffman, Richard J. Simpson, Emmanuel Katsanis

Departments of Immunobiology, Pediatrics, Nutritional Sciences, Medicine and Pathology University of Arizona, Tucson, AZ

Fms-like tyrosine kinase 3 (Flt3) is a surface receptor for the cytokine Flt3 Ligand (Flt3L) and regulates differentiation, proliferation and survival of hematopoietic progenitor cells. Flt3 signaling is particularly important for dendritic cell (DC) development and homeostasis, and requires STAT3 for the transition from common lymphoid/myeloid progenitor to common DC precursors. Flt3L-driven DCs have been documented to closely resemble steady-state DCs, displaying an "immature" phenotype and an enhanced ability to traffic to lymph nodes. Immature DCs are critically important for defining the immunologic self and inducing tolerance, and as such have a notable beneficial role in transplantation tolerance.

Our laboratory has previously demonstrated that bendamustine with total body irradiation (BEN+TBI) is a safer alternative to cyclophosphamide (CY+TBI) as conditioning in an MHC-mismatched murine BMT model of GvHD (Stokes). Here we sought to investigate the role of Flt3L administration using our BEN/CY +TBI conditioning regimen. BALB/c recipient mice were given daily injections of Flt3L from days -10 to -3, comparable doses of bendamustine (40 mg/kg BEN i.v.) or cyclophosphamide (200 mg/kg CY i.p.) on day -2, and 400 cGy of TBI on day -1. On day 0, recipient mice were transplanted with T-cell depleted bone marrow (TCD-BM) and total T-cells (tT) from C57BL/6 donors. Survival, engraftment, and clinical GvHD score were monitored for 150 days.

BEN is a hybrid molecule, containing alkylating groups and a purine analog, which confers unknown anti-metabolite functions. BEN is known to bind directly to the SH2 domain of STAT3 and inhibit its canonical signaling. We hypothesize that by transiently inhibiting STAT3, BEN manipulates the Flt3 signaling pathway and influences the DC compartment. To test this hypothesis *in vitro*, BALB/c bone marrow was cultured in the presence of Flt3L to generate bone marrow-derived DCs (BMDCs) in the presence of BEN. Flow cytometry was performed to identify DC subsets and measure Flt3 and CCR7 expression.

We report here that Flt3L administration prior to BEN/CY +TBI conditioning significantly improves GvHD severity and mortality while shortening the time to engraftment. Furthermore, *in vitro* studies generating Flt3L-driven BMDCs in the presence of BEN resulted in significant, dose-dependent increases of Flt3 and CCR7 expression, providing support of our hypothesis that BEN manipulates the Flt3 signaling pathway. Together, these findings suggest a potential therapeutic advantage to using BEN+TBI conditioning rather than CY+TBI for the reduction of GvHD.

Suppression of Intestinal Cancer by the Innate Immune Sensor AIM2

***Justin E. Wilson*[1], *A. Alicia Koblansky*[2] *and Jenny P.Y. Tin*[2]**

[1]Department of Immunobiology, College of Medicine-Tucson, University of Arizona Tucson, AZ; [2]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC

The cytosolic double-stranded DNA sensor Absent in Melanoma 2 (AIM2) plays an important function during the innate immune response to double stranded DNA viruses and intracellular bacteria. Upon binding to dsDNA, AIM2 forms a multi-protein complex with ASC and Caspase-1 (known as the AIM2 inflammasome), resulting in production of the pro-inflammatory cytokines IL-1β and IL-18. My previous work identified a non-canonical, inflammasome-independent function for AIM2 in suppressing the PI3K/Akt pathway of cellular survival. Through this mechanism, AIM2 provides a protective role during the AOM/DSS experimental model of inflammatory bowel disease-associated colon cancer and the $APC^{min/+}$ model of sporadic colon cancer. Unlike traditional innate immune cells predominantly known for inflammasome expression (e.g., macrophages and dendritic cells), AIM2 suppresses Akt activity in colonic epithelial cells. My recent work indicates that AIM2 also suppressed spontaneous tumorigenesis in the small intestine. Mechanistically, AIM2 limited aberrant Wnt/β-catenin signaling in intestinal epithelial cells in an Akt-dependent fashion. Furthermore, loss of Aim2 resulted in increased c-Myc oncoprotein expression in vivo and in vitro, which was reversed in vitro following treatment of epithelial organoids with a beta-catenin inhibitor. These findings implicate a role for AIM2 in suppressing key tumor-promoting factors in the intestine. Future studies will focus on determining which physiological ligands are responsible for regulating the tumor suppressive capacity of AIM2, with a focus on intestinal microbiota-derived double-stranded DNA.

Vaginal Microbiota, Genital Inflammation, and HPV Infection Modulate Cervicovaginal Metabolomes in Cervical Carcinogenesis

***Zehra Esra Ilhan*[1], *Paweł Łaniewski*[2], *Natalie Thomas*[2], *Denise J. Roe*[3], *Dana M. Chase*[4], *and Melissa Herbst-Kravoletz*[1,2]**

[1]Department of Obstetrics and Gynecology, College of Medicine-Phoenix, University of Arizona, Phoenix, AZ; [2]Department of Basic Medical Sciences, College of Medicine-Phoenix, University of Arizona, Phoenix, AZ; [3]UA Cancer Center, University of Arizona, Tucson/Phoenix, AZ; [4]US Oncology, Phoenix, AZ

Dysbiotic vaginal microbiota have been implicated as contributors to persistent HPV-mediated cervical carcinogenesis and genital inflammation. However, the biological mechanisms driving persistence and carcinogenesis have not been elucidated. Metabolic profiling provides a unique understanding of the complex interactions between the host and microbes in carcinogenesis. Our objective was to perform metabolic profiling of the cervicovaginal microenvironment to identify interactions between virus, host and microbes in the context of genital inflammation, dysplasia, and cancer.

In a multicenter study, metabolic profiles of 78 premenopausal, non-pregnant women with low-grade (LSIL) and high-grade squamous intraepithelial lesions (HSIL), invasive cervical cancer (ICC), or healthy controls (HPV-positive and -negative Ctrl) were analyzed using gas chromatography-mass spectrometry. Vaginal microbiota profiles and genital inflammation were characterized using 16S rRNA gene sequencing and Bioplex Immunoassay system, respectively. Metabolome and vaginal microbiome datasets were integrated using state-of-the-art bioinformatic tools (PICRUSt, AMON, and MIMOSA). Hierarchical clustering (HCA) and principal component analysis (PCA) were employed to reveal the influence of genital inflammation, patient groups, and microbiota on metabolic profiles. Receiver Operating Characteristics (ROC) analysis was used to discriminate metabolites for each patient group. Statistical differences were tested using ANOVA or Mann-Whitney U test.

Features of the cervicovaginal microenvironment, genital inflammation, vaginal pH, and vaginal microbiota composition explained differences in the metabolic profiles of the patients across cervical carcinogenesis. Metabolomes of ICC patients (n=468 metabolites) formed a distinct cluster on PCA and HCA plots, due to enrichment of membrane lipids. Amino acid and nucleotide metabolites were depleted in HPV-positive Ctrl, LSIL and HSIL groups ($P<0.05$). Microbial communities were predicted to alter amino acid and nucleotide metabolisms. Eicosenoate, 3-hydroxybutyrate, and oleate/vaccenate (AUC > 0.9, $P<0.01$) discriminated ICC from healthy patients. Sphingolipids and plasmalogens positively correlated with genital inflammation (Spearman's rho > 0.7). Anti-inflammatory nucleotides, adenosine and cytosine positively correlated with *Lactobacillus* abundance (Spearman's rho>0.5) and negatively correlated with genital inflammation (Spearman's rho<-0.3). HCA of metabolites demonstrated that metabolic profiles were driven by cancer, genital inflammation and *Lactobacillus* dominance. The complex virus-host-microbe interplay within the cervicovaginal microenvironment lead to unique metabolic fingerprints that could be exploited for future development of diagnostics, preventatives or treatments that positively impact women's health outcomes.

Type IV Pilus Retraction is Essential for *Neisseria musculi* Persistent Colonization *in vivo*.

***Katherine Rhodes*[1], *Mancheong Ma*[1], *Daniel Powell*[1], *Magdalene So*[1,2]**

[1]Department of Immunobiology, College of Medicine-Tucson, Tucson, AZ , and [2]BIO5 Institute, University of Arizona, Tucson, AZ

The Type IV pilus (Tfp) controls many *Neisseria*-host interactions. In cultured cells, Tfp retraction triggers mechanosensitive host pathways, culminating in the formation of a cytoprotective environment that promotes bacterial survival. Tfp retraction is also important for biofilm formation *in vitro*. The importance of Tfp retraction has never been studied *in vivo*. Using our natural mouse model for persistent colonization, we showed that PilE, the pilus fiber structural subunit, is an essential colonization factor of commensal *Neisseria musculi* (Nmus). Here, we used the model to examine the role of Tfp retraction in Nmus colonization and persistence.

Nmus mutants deleted of the Tfp retraction motor gene (Δ*pilT*) or expressing an attenuated retraction motor ($pilT_{L201C}$) were constructed and validated for growth and for Tfp functions using DNA transformation, adhesion, invasion, and biofilm formation assays. Wild-type (wt), Δ*pilT* and $pilT_{L201C}$ were inoculated into CAST/EiJ mice, and CFUs in the oral cavity (OC) and fecal pellet (FP) were determined weekly for 16 weeks. CFUs along the alimentary tract were also determined at Week 16.

Like *N. gonorrhoeae* Δ*pilT* and $pilT_{L201C}$, Nmus Δ*pilT* is non-transformable and $pilT_{L201C}$ is genetically competent. Δ*pilT* and $pilT_{L201C}$ adhered to cultured mouse epithelial cells as well as wt but had invasion and biofilm formation defects.

Wt Nmus stably colonized the OC and gut of mice, while Δ*pilT* did not colonize these sites at any time. As Δ*pilE* has an identical defect, this indicates colonization requires not only the Tfp fiber but also the ability of the fiber to retract.

$pilT_{L201C}$ had yet a different phenotype. $pilT_{L201C}$ colonized the mouse OC and gut, but CFUs from these sites varied from week to week. Taking into account its biofilm defect, the cyclic recovery of $pilT_{L201C}$ CFUs is likely caused by the inability of the mutant to maintain a stable niche on the mucosa.

We have, for the first time, tested the role of Tfp retraction in *Neisseria* colonization and persistence *in vivo*. Our findings strongly suggest that niche establishment requires the presence of a retractable Tfp fiber, and niche maintenance requires a fully functional PilT ATPase. Our current efforts are on identifying the molecular link between Tfp retraction and persistent colonization.

Poster Titles

Poster	Author	Abstract Title
1	Xianxian Wu	Fungi Induced Autophagy and Cell Death in Airway Epithelium but not in Macrophages
2	Yuchen Liu	Gene Profile Identifies Predominate Genes about Immunity by Fungal Exposure
3	Christopher P. Coplen	Reactivation of Latent mCMV Shapes the Immune Response to Unrelated 3rd Party Infections
4	Mark S. Lee	Reciprocal TCR-CD3 and CD3 Engagement of a Nucleating pMHCII Stabilizes a Functional Receptor Macrocomplex
5	Richa Jain	Role of Age-related Intrahepatic CD8+T cell Accumulation in Nonalcoholic Steatohepatitis Progression
6	Nicole E. Behrens-Bradley	Evaluation of HIV-Specific T-cell Response in HIV-Infected Aging Patients with Controlled Viremia on Long-term Antiretroviral Therapy
7	Yuecheng Xi	Human Cytomegalovirus Remodeling of Host Phospholipid Metabolism Requires PERK, a Member of the ER Stress Response
8	Vikas Vikram Singh	Impact of Immune Aging and Ethnic Disparities in People Living with HIV
9	Faith M. Warner	Age-related Changes in Organization and Function of Lymph Node Microenvironment
10	Natalie Thomas	Interaction between *Fusobacterium* Species and Female Reproductive Tract Epithelial Cells
11	Sebastian Zeltzer	HCMV's pUL138 Suppresses Fanconi Anemia Signaling
12	Danielle A. Becktel	Cyclodextrin Treatment Substantially Reduces the Chronic Inflammatory Response Following Ischemic Stroke in Adult and Aged Mice
13	Partha Samadder	Antigen Induced Lysine Deacetylation Regulates CD8+ T cell Asymmetry Mediated Functional Fate
14	Shuaizhi-Li	L2 Amphipathic Helix is Essential for HPV Infection by Facilitating Post-Endosomal Trafficking of Viral Genome
15	Thessaly Alexander	Impact of IL-36γ Treatment on HSV-2 Replication in the Female Reproductive Tract
16	Kimberley Owen	Bacterial Screen of Under-Investigated Vaginally Isolated Bacteria on Vaginal Cell Cytotoxicity and Proinflammatory Cytokine Induction
17	Geetanjali Gupta	Resident Memory CD8+ T ($CD8^+$ T_{RM}) Cells for Adoptive Cell Therapy
18	Jameson Gardner	IL-36γ is Crucial for HSV-2-induced Neutrophil Recruitment and Protects Against Neuroinvasion in Genital HSV-2 Pathogenesis
19	Kevin Ferro	The Cell Adhesion Molecule Hemomucin is a Key Determinant of Host-Parasitoid Interactions
20	Vivian / Thuy-Vi Nguyen	Alzheimer's Disease Pathology is a Chronic Sequela of Ischemic Stroke in Two Mouse Models of Mixed Dementia

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