EMERGING PATHOGENS INSTITUTE

RESEARCH DAY 2009

Letter from the Director

February 5, 2009

Welcome to the second annual EPI Research Day! My hope is that today's sessions will give you a feel for the wide range of emerging pathogens-related research conducted on the University of Florida's campus, and that the diverse ideas presented may catalyze new collaborations. I especially welcome investigators from the Florida Department of Health, the USDA/ARS Center for Medical, Agricultural, and Veterinary Entomology, and UF investigators from outside of Gainesville, including the Florida Medical Entomology Laboratory at Vero Beach, and the Whitney Laboratory at St. Augustine.

We are honored to have two outstanding speakers for our afternoon session: Dr. Rita Colwell, a former Director of the National Science Foundation and currently a Professor at the University of Maryland in College Park, and Dr. Balakrish Nair, Director of the National Institute of Cholera and Enteric Diseases, in Kolkata, India. Both serve on the EPI External Advisory Committee (Dr. Colwell as Committee Chair), and I greatly appreciate their contribution to today's activities, and to the ongoing guidance and development of EPI.

EPI itself continues to grow: new faculty are being recruited, and we anticipate moving into our new building in early fall of 2009. It's an exciting time, and I appreciate your being here today to be part of our research day activities. Visit our website, www.epi.ufl.edu, to join our list-serves, and to keep up with our news, events and seminars throughout the year.

- J. Glenn Morris, Jr., M.D., M.P.H. & T. M.
EPI Director, and Professor of Medicine

EMERGING PATHOGENS INSTITUTE: UNIVERSITY OF FLORIDA

EPI

Research Day 2009: Schedule of Events

EPI is pleased to present keynote speeches by:

Dr. Rita Colwell:
Former National Science Foundation director, and Professor at Univ. of Maryland, College Park

Dr. Balakrish Nair:
Director, National Institute for Cholera and Enteric Diseases in Kolkata, India

---

Coffee.....9:30 a.m. - 10:30 a.m.

Poster Session.....10:00 a.m. - 1:00 p.m.

Lunch.....12:00 p.m. - 1:00 p.m.

Keynote Speakers.....1:30 p.m. - 3:30 p.m.

(1) SOCS-1 Mimetics Protect Mice against Lethal Poxvirus Infection: Identification of a Novel Endogenous Antiviral System

Chulbul M. I. Ahmed, Ph.D., Rea Dabelic, Lindsey D. Jager, and Howard M. Johnson,
Department of Microbiology and Cell Science, U.F., P.O. Box 110700, Gainesville, FL 32611-0700

Suppressor of cytokine signaling-1 (SOCS-1) protein modulates cytokine signaling by binding to and inhibiting the function of JAKs, ErbB, and other tyrosine kinases. We have developed a small tyrosine kinase inhibitor peptide (Tkip) that binds to the autophosphorylation site of tyrosine kinases and inhibits activation of STAT transcription factors. We have also shown that a peptide corresponding to the kinase inhibitory region of SOCS-1, SOCS1-KIR, similarly interacts with the activation loop of JAK2 and blocks STAT activation. Poxviruses activate cellular tyrosine kinases such as ErbB-1 and JAK2 in infection of cells. We used the pathogenesis of vaccinia virus in C57BL/6 mice to determine the ability of the SOCS-1 mimetics to protect mice against lethal vaccinia virus infection. Injection of mice i.p. with Tkip or SOCS1-KIR containing a palmitate for cell penetration, before and at the time of intranasal challenge with 2 x 106 pfu of vaccinia virus, resulted in complete protection at 100 µg. Initiation of treatment 1 day post-infection resulted in 80% survival. Administration of SOCS-1 mimetics by the oral route also protected mice against lethal effects of the virus. Both SOCS1-KIR and Tkip inhibited vaccinia virus transcription and replication at early and possibly later stages of infection. Vaccinia virus induced phosphorylation of ErbB-1 and JAK2 was inhibited by the mimetics. Protected mice mounted a strong humoral and cellular response to vaccinia virus. The use of SOCS-1 mimetics in treatment of poxvirus infections reveals an endogenous regulatory system that previously was not known to have an antiviral function.

(2) Effects of West Nile Virus Dose on Temporal Infection of the Culex pipiens quinquefasciatus Say (Diptera: Culicidae) Midgut

Sheri L. Anderson, Stephanie L. Richards, Chelsea T. Smartt, and Walter J. Tabachnick
University of Florida/IFAS, Florida Medical Entomology Laboratory, 200 9th St. S.E., Vero Beach, FL

Culex pipiens quinquefasciatus were fed blood meals containing either 6.7 logs plaque-forming units (pfu)/mL or 4.8 logs pfu/mL of West Nile virus. Midguts and legs from five mosquitoes per dose were collected every other day from 4-12 days post-infection (dpi) to investigate temporal midgut infection and dissemination patterns. Midgut infection was assessed with immunofluorescent staining and virus dissemination to the legs was determined with qRT-PCR. Dissemination was calculated as the percentage of midgut infected mosquitoes with infected legs. At 6 dpi, the high dose group showed 100% infection and 80% had disseminated infections. For the low dose group at 6 dpi, only 20% of mosquitoes had infected midguts and none showed disseminated infections. The low dose group showed 80% disseminated infections by 10 dpi. The midgut infection rate at 6 dpi was significantly different between the high (100%) and low dose (20%) groups ($p=0.048$). Both virus dose groups showed 100% infection and 80% dissemination by 10 and 12 dpi, respectively. This suggests the presence of a midgut escape barrier, regardless of initial virus dose. Our results indicate that virus dose had the greatest effect on infection at 6 dpi. We demonstrate temporal variation patterns of infection and dissemination between doses. This may help explain variation in vector competence seen at different points of the extrinsic incubation period due to differences in environmental factors such as host viremia.

(3) Use of molecular docking to identify inhibitors of FadR of Vibrio vulnificus

*J.M. Asencio[1], D.A. Ostrov[2], R.N. Brown[1], and P.A. Gulig[1]**
[1]Department of Molecular Genetics and Microbiology, U.F., Gainesville, FL
[2]Department of Pathology, Immunology, and Laboratory Medicine, U.F., Gainesville, FL

Molecular docking is a computational tool that examines the ability of libraries of small molecules whose physical-chemical properties are known to fit into chosen sites of protein targets whose crystal structures have been solved. We recently showed that the FadR protein of Vibrio vulnificus, an opportunistic pathogen that contaminates raw seafood, is essential for disease in a mouse model. We used molecular docking to identify small molecules that could inhibit the function of FadR in V. vulnificus. Because the crystal structure of the E. coli FadR protein, which is 52% homologous to the V. vulnificus protein, has been solved, we modeled the structure of the V. vulnificus FadR protein using the Phyre program. We identified amino acids in the DNA binding pocket of FadR to develop coordinates for docking with the small molecules. The DOCK6 program was used on the University of Florida High Performance Computing Facility to examine >130,000 small molecules from the National Cancer Institute Developmental Therapeutics Program library for fit in the FadR DNA-binding pocket in 5,000 orientations each. The top 40 hits were obtained from the NCI and were screened for their ability to inhibit growth of V. vulnificus in a 96 well culture assay, read as OD600 in a plate reader. Six compounds inhibited the growth of V. vulnificus at concentrations ranging from 0.025 mM to 0.1 mM. In follow-up culture tube experiments, most of these compounds not only stopped growth of V. vulnificus but also killed the bacteria. This was unexpected since a fadR deletion mutation only slows growth in vitro. We are now examining the efficacy of these compounds to inhibit disease in our mouse model. These results demonstrate the usefulness of molecular docking to rapidly identify small molecules that bind to and inhibit bacterial targets.

4 Soft Tissue Penetration of Ceftobiprole Following Single Dose Administration in Healthy Adults

A. Barbour[1], B. Murthy[2], S. Schmidt[1], S. Sabarinath[1], D. Skee[2], H. Tian[2], D. Desai-Kreiger[2], D. Balis[2], M. Grant[1], C. Seubert[1], H. Derendorf[1]
[1]University of Florida, Gainesville, FL
[2]Johnson & Johnson Pharmaceutical Research and Development, Raritan, N.J.

Background: Ceftobiprole is a broad-spectrum cephalosporin with activity against methicillin-resistant staphylococci and has demonstrated clinical efficacy in the treatment of complicated skin and structure infections (cSSSI). The purpose of this study was to evaluate the penetration of ceftobiprole into the subcutaneous (sc) adipose and skeletal muscle tissues. *Methods*: This was a single-center, open-label study in healthy subjects. Microdialysis probes were inserted into the sc adipose and muscle tissues. Twelve subjects received a single 2-hour infusion of ceftobiprole 500 mg. Serial plasma (13 samples in 24 hours) and tissue samples (every 20 minutes for the first 12 hours) were collected and analyzed for ceftobiprole concentrations using validated LC-MS/MS and LC-UV methods, respectively. Pharmacokinetic parameters for each tissue were determined using noncompartmental analysis. Penetration was measured by the AUC ratio of the tissue to the unbound plasma. Results: Ceftobiprole exposure in plasma exceeds that of skeletal muscle, which exceeds that of sc adipose tissue. Overall penetration of ceftobiprole into skeletal muscle and sc adipose tissue was 69% and 49%, respectively, and was within the range reported for other cephalosporins (12% to 79%). Estimates of plasma CL, V, and t1/2 were comparable to that observed in previous studies. Assuming an MIC of 4 µg/mL, the %T>MIC exceeded 30% in all tissues studied. Ceftobiprole was well tolerated and no subject was discontinued for a serious adverse event. *Conclusion*: Ceftobiprole penetrates into the skeletal muscle and sc adipose tissue to a similar extent as other cephalosporins.

5 Development of a tumor registry informatics system for the Florida Center for Brain Tumor Research

Christopher Barnes, June Nogle, Ph.D., Keith Muller, Ph.D., and Elizabeth Shenkman, Ph.D.
Institute for Child Health Policy, Clinical and Translational Research Informatics Program, U.F., Gainesville, FL

The Florida Center for Brain Tumor Research was established at the University of Florida in 2006. This new center works with hospitals statewide to collect and analyze tumor specimens, and then disseminate data on the characteristics of brain tumors. A web-based computer database system integrated with Bar-coding Label Printers and Scanners was created to collect associated clinical information and to facilitate the submission of tissue, specimen samples, and clinical, radiological and pathologic data from multiple sites with the ability to add n sites to the consortium. The database collects basic demographic, radiological, and pathological information on each patient. Additionally, it prompts sites to update information on follow-up survival, MRI, and treatment information every 3 months. Tissue submissions are tracked by detailed information on each specimen, assignment of a unique bar code to each specimen, and automatically printing mailing labels and shipping manifests. This poster will summarize the design of the database, software and hardware systems that support the Florida Brain Tumor Research Consortium.

6 Rift Valley Fever: A Multi-Agency Test Of Florida's Response To An Hypothetical Introduction To The State

Stasia Bembenek[1], Greg Christy[2], Thomas Holt[2], Jocelyn Mullins[1], Tineke Kramer[1] and Paul Gibbs[1]
[1]College of Veterinary Medicine, U.F., Gainesville, FL
[2]Department of Agriculture and Consumer Services, Tallahassee, FL

Rift Valley fever (RVF) is a zoonotic viral disease affecting ruminants and people. It was first recognized, as the name suggests, in the Rift Valley of East Africa, but it now recognized to be an endemic disease affecting most of Sub-Saharan Africa and Madagascar. Since 1970, on occasion, it has shown an ability to spread northwards to cause epidemics in Egypt, Yemen, and Saudi Arabia. It is considered an emerging pathogen. The disease in most humans is characterized by fever and malaise, but a small percentage of patients develop either fatal encephalitis and/or generalized hemorrhage. In ruminants, the disease is particularly severe in lambs and calves, which die of generalized hemorrhage; pregnant animals commonly abort. RVF virus is transmitted by several species of mosquito, but human infection is often associated with the slaughter of infected animals for food. Experimental studies have established that US species of mosquito can transmit the virus, and the RVF virus is classified as a select agent. It is feared that RVF virus, if introduced accidentally or through bioterrorism, could have an even greater impact than West Nile virus on the animal and human populations of North America.In partnership with the State's Emergency Operations Center, a multi-agency exercise (State and Federal) was organized through SART to test the Florida's response to a simulated outbreak of RVF in both ruminants and humans. The exercise involved approximately 100 professionals November 18-20, 2008. The outbreak was characterized by increased calf mortality and mild human cases on a large ranch in Southern Florida. A case of hemorrhagic fever in West Palm Beach was connected to the slaughter of goats, and a case of retinitis in Gainesville, FL was connected to the initial introduction of the virus. The introduction of virus to Florida was linked to a bioterrorism event. The poster will describe the scenario and discuss the difficulties met by the different agencies in combating the spread of the virus and determining its origin.

7 Myosin-X Facilitates Shigella Filopodia Formation and Cell-to-Cell Spread

E. Bishai, G.S. Sidhu, A.B. Bohil, R.E.Cheney, and F.S. Southwick
Division of Infectious Diseases, College of Medicine, U.F., Gainesville, FL

The intracellular pathogen Shigella flexneri forms filopodia in order to spread from cell to cell. We have found that as Shigella form filopodia, myosin-X (Myo10) concentrates along the sides of the bacteria. Listeria monocytogenes, another intracellular pathogen that induces filopodia formation in host cells does not attract Myo10. To assess the functional significance of this finding scanning electron micrographs of Shigella-infected HeLa cells were subjected to RNAi knockdown of Myo10 and compared to cells treated with control RNAi. Knockdown of Myo10 was associated with the formation of broad-based filopodia, as compared to control cells in which filopodia were attached by thin stalks. Myo10 knockdown also significantly reduced the length of Shigella-induced filopodia (mean length 8.6 ± 0.5 um vs. control 11.1 ± 0.5 um), and resulting in a 26% decrease in plaque formation. Live video of GFP-full-length Myo10 and a head-coil-coil GFP-construct revealed that both proteins cycle backward and forward along the sides of the bacteria within filopodia. Transfection of Cos7 (contain low levels of Myo10) with full-length Myo10, but not the head construct, increased filopodia length (mean length 14.6 ± 0.5 um vs. 11.6 ± 0.4 um). Enhanced filopodia formation required the PH domain of the tail, but not the FERM domain. GFP-Myo10-PH localized to the cell membranes including filopodia, while GFP-Myo10-MyTh4-FERM did not. These findings suggest Myo10 generates the force to enhance filopodia formation by binding its head region to Shigella-associated actin filaments, and its PH tail domain to the host cell membrane. This same mechanism is likely to contribute to Myo10-induced filopodia formation in uninfected cells.

8 Transient Virulence of Emerging Pathogens

Benjamin M. Bolker[1], Arjun Nanda[2], Dharmini Shah[2]
[1]EPI, Department of Botany & Zoology, U.F., Gainesville, FL
[2]Food Science & Human Nutrition Department, U.F., Gainesville, FL

Why should emerging pathogens be unusually virulent? Existing theories of virulence evolution that are based on tradeoffs between rapid transmission and high virulence imply that epidemic growth conditions will select for higher virulence, leading to a transient peak in virulence near the beginning of an epidemic. This transient selection could explain the high virulence of emerging pathogens. Using a simple model of the epidemiological and evolutionary dynamics of emerging pathogens, along with estimated parameters for pathogens such as SARS, West Nile virus (WNV), and myxomatosis, we estimated the potential magnitude and timing of such transient virulence peaks. Pathogens that are moderately evolvable, highly transmissible, and highly virulent at equilibrium could temporarily double their virulence during an epidemic; thus, epidemic-phase selection could contribute significantly to the virulence of emerging pathogens. We bring together evidence for tradeoffs and evolvability for other pathogens such as HIV, and discuss the need for better data on tradeoff curves and genetic variability. Despite the recent focus on within-host dynamics of pathogens, the genetic and phenotypic properties of virulence tradeoffs deserve continued attention.

9 Plasmodium Falciparum Signal Peptide Peptidase (Pfspp) As A Novel Target For Malarial Chemotherapy

J. Alfredo Bonilla, Tonya D. Bonilla, Charles A. Yowell, John B. Dame
Department of Infectious Diseases and Pathology, University of Florida, Gainesville, FL

The *P. falciparum* signal peptide peptidase (PfSPP) is an intramembrane-cleaving aspartate protease and a viable therapeutic drug target. The intramembrane-cleaving proteases are unique in that they catalyze peptide bond cleavage in the hydrophobic lipid bilayer of a membrane. In *Plasmodium* spp. all three major classes of intramembrane- cleaving proteases, metallo-, serine- and aspartate proteases, have been identified. Modeling of PfSPP indicates it is an integral membrane protein that spans the membrane nine times by α-helical transmembrane segments and retains the C-terminus in the cytosol and the N-terminus in the lumen. Quantitative RT-PCR and western blot analysis demonstrates that PfSPP is highly transcribed and translated throughout the asexual cycle and most abundant in the trophozoite and schizont stages. Immuno-fluorescence microscopy localizes PfSPP primarily to the endoplasmic reticulum of the parasite in the trophozoite stage and at the plasma membrane of the daughter merozoites within a late-stage schizont. Inhibitor studies demonstrate that inhibition of PfSPP during all stages of development impairs parasite viability, and inhibitors specific to γ-secretase activity do not inhibit the growth of the parasite. This important distinction demonstrates PfSPP is a bona-fide signal peptide peptidase and suggests that the design of specific inhibitors to the parasite SPP versus human SPP and other presenilin-like homologs is feasible. Moreover, SPP is refractory to disruption in *P. berghei*, highly suggestive of a critical role in the parasite.

10 In Situ Visualization of the Apicoplast and Apicoplast Nucleoid in Asexual Stages of Plasmodium falciparum

Tonya D. Bonilla, J. Alfredo Bonilla, and John B. Dame
Department of Infectious Diseases and Pathology, U.F., Gainesville, FL

Among the most striking sub-cellular morphological features of the Plasmodium falciparum parasite are

the elaborate configurations taken on by the developing apicoplast. However, little is known regarding the mechanisms in which this organelle is replicated and divided in Plasmodium. At the culmination of schizogeny, a segment of the apicoplast is partitioned into each newly formed daughter cell. This process is expected to be directed in part by components of the eukaryotic division apparatus, and may also be linked to the structural characteristics and positioning of the nucleiod, to ensure proper segregation of the apicoplast genome. In this study, combined immunofluoresence and fluorescent in situ hybridization were used to follow both organellar morphology and nucleoid distribution in asexual stages of P. falciparum. The DNA gyrase inhibitor ciprofloxacin was used to probe the effects of apicoplast DNA loss on organellar morphology. In untreated parasites, the apicoplast DNA was predominately organized as single discrete nucleoid structures in non-replicating rings and trophozoites. In maturing trophozoite stages when the apicoplast began to elongate, the nucleoid was often visualized as semi-discrete foci at both ends of the elongating apicoplast. As the apicoplast elaborated and began to branch, multiple nucleoids were observed within the apicoplast that varied in shape, size and hybridization intensity, and often appeared to encompass the entire stromal compartment. In ciprofloxacin treated parasites, the nucleoid signal was significantly reduced in size, intensity and number. Furthermore, in the ciprofloxacin treated parasites the apicoplast signal was highly fragmented. Also, in many instances the apicoplast DNA did not fully co-localize with the apicoplast. These data indicate that the apicoplast nucleoid is required for proper development and segregation of the apicoplast.

(11) Toward the Analysis of the Transcriptome Response to West Nile Virus Infection in the Equine Host

M. A. Bourgeois[1], M. T. Long[1], K. K. Seino[2], D. S. Barber[1], and N. D. Denslow[1]
[1]University of Florida, College of Veterinary Medicine, Gainesville FL
[2]Washington State University, College of Veterinary Medicine, Pullman, WA

West Nile virus is one of the leading causes of arboviral encephalitis in the United States. Despite the impact of WNV, little is known about disease pathogenesis and host response to central nervous system infections. The overall goal of this project is to gather and use host expression data from tissues of horses experimentally infected with WNV as a model to develop interventional strategies for viral encephalitis. It is hypothesized that there are gene pathways whose expression changes in a consistent manner during WNV infection, disease, and recovery. This will be investigated by creating a tissue specific expression library from CNS tissues and spleen from naïve and non-naïve horses experimentally infected with WNV and from normal horses. The cDNA library has been sequenced and assembled, resulting in the identification of 41,040 sequences. BLAST analysis of these sequences identified 31,357 good sequence hits (e<10-4), including neurologically specific genes, and genes involved with transcription, the immune response, signal transduction, RNA modification, and translation. 73.7% of these sequences had been previously identified by equine genome databases. 9,504 (23.1%) of the sequences were missed by the equine predicted databases, while 1,280 (3.1%) of the sequences were missed by all of the equine databases, including the equine genome project. Of the completely novel sequences, 709 (55.4%) of the sequences were grouped under GO categories. An equine specific microarray will be created from this library to analyze changes in gene expression which will be subjected to pathway analysis. Relationships between gene expression and WNV survival will be identified, and biomarkers that can lead to rapid identification of neurological disease established. This information will be used to develop new interventional strategies, predict survival, and create better diagnostic tests for WNV and other viral encephalitides.

(12) Crystallographic Studies of Potential HIV-1 Protease Inhibitors

Edith Bracho-Sanchez, Sandra Koch, Melissa R. Marzahn, Roxana M. Coman, David Ostrov, Ben M. Dunn
Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

The Human Immunodeficiency Virus type 1 (HIV-1) is known for its high genetic variability and has been divided into types, groups, subtypes, and recombinant forms (1). Subtype A of the HIV-1 virus is mostly found in Sub-Saharan Africa and accounts for 23% of the current 33.2 million infections in the world (2). Current treatment for HIV infections involves the use of protease inhibitors. The HIV protease is crucial in the formation of an infectious virus. We have concerned ourselves with conducting crystallographic studies of the HIV-1 subtype A protease complexed with potential inhibitors suggested by a molecular docking program. We successfully purified the protease and obtained crystal growth two days following crystal trials.

(13) The Risk of Arbovirus Transmission Increases as Mosquito Infection Rates Increase: Using Simple Models to Evaluate This Assumption

Dulce M. Bustamante, Cynthia C. Lord
Florida Medical Entomology Laboratory, Vero Beach, FL

The "infection rate" is an estimate of the arbovirus infection prevalence in a mosquito population. It is used in surveillance to assess the risk of virus transmission to humans and domestic animals. It is assumed that as the "infection rate" increases the risk arbovirus transmission also increases. However, not all infected mosquitoes are involved in transmission. Taking that into consideration, we assessed the assumption of linearity between "infection rate" and risk by investigating (a) how the infection prevalence in the mosquito population relates to the proportion of those mosquitoes that are capable of virus transmission (infectious), and (b) how the infection prevalence relates to "infection rates" obtained after sampling, pooling, and testing mosquitoes for virus. A model was used to approximate the proportion of infected mosquitoes in a cohort that become infectious as a function of temperature, mosquito and virus species. Results suggested that the relationship between the

proportions of infected and infectious mosquitoes is not linear, and that similar "infection rates" estimated at different times, under different temperatures, or for different mosquito-virus systems may not indicate the same level of risk. Numerical simulations were used to study the "infection rate" as an estimate of the prevalence of infection in the mosquito population. These simulations suggested that "infection rates" usually underestimate prevalence and that they only show true changes in prevalence under certain sampling and testing conditions. In conclusion, the "Infection rates" appear not directly related to mosquito infectiousness and risk of transmission, and those rates must be interpreted together with other biological and environmental factors in order to provide a better assessment of the risk of transmission.

(14) **Analysis of capsule/O-antigen genes in** *Vibrio parahaemolyticus*

Yuansha Chen, J. Glenn Morris, Judith A. Johnson
Emerging Pathogens Institute, Department of Pathology, College of Medicine, U.F., Gainesville, FL

Vibrio parahaemolyticus is a marine bacterium that commonly causes infections in human through contaminated seafood. A recent pandemic of *V. parahaemolyticus* has caused tens of thousands of cases of disease worldwide. Molecular studies indicated that the strains causing the pandemic were closely related or clonal. However, the pandemic strains change their serotypes rapidly and have evolved into more than 20 serovariants in the last decade. This rapid diversification of LPS (O-antigen) and CPS (K-antigen) provides an outstanding model to study the genesis and evolution of polysaccharide antigens and suggests that specific mechanisms may exist to promote recombination in these genes. Thorough understanding of the nature of serotype conversion in *V. parahaemolyticus* will have strong implications in bacterial vaccine development. We hypothesized that genes for the synthesis of O-antigen and capsule polysaccharide share the same genomic region in *V. parahaemolyticus.* This region is subject to frequent lateral gene transfer, resulting in the changes of both antigen structures it defines and the rapid emergence of new pandemic serotypes of *V. parahaemolyticus* from the original virulent strain. We have identified and analyzed the putative LOS/capsule region on chromosome I in the published genome of *V. parahaemolyticus*. Our data suggests that the capsule exportation is wza dependent in the pandemic strain. We successfully deleted a wza gene in *V. parahaemolyticus* and generated a translucent mutant (acapsular) using the chitin based transformation method, confirming the function of the putative capsule region.

(15) **Differentiation of Mycobacterial Species by the Intergenic Region Between rpoB and rpoC**

Jianli Dai, Judith A. Johnson, J. Glenn Morris
Emerging Pathogens Institute, U.F., Gainesville, FL

Keywords: *Mycobacterium*, genomics, multilocus sequence typing, bioinformatics
Mycobacteria are Gram-positive, aerobic bacteria characterized by a thick hydrophobic, waxy cell wall. Infections by mycobacteria are difficult to treat and post great threat to the public health, including notorious *Mycobacterium tuberculosis* (MTB) and *M. leprae*. Nontuberculous mycobacteria (NTM) are ubiquitous environmental organisms posing great risks to human health by causing severe respiratory diseases as well as other infection in human, especially those with immunodeficiency. In contrast to TB, NTM infection rates in the U.S. have been rising steadily over the last several decades and have already surpassed that of MTB. However, our understanding of NTM infections as well as diagnostics and treatment methods are limited due to our lack of understanding of the population structure and genetic variability of NTM. With the number of NTM species continuously increases, current typing methods have difficulty to identify them accurately. Bioinformatic studies of sequenced mycobacterial genomes revealed several informative loci for typing. Among them, one locus between rpoB and rpoC, flanking by two highly homologous regions is an excellent candidate for differentiation of mycobacterial species. This locus was sequenced from a collection of mycobacterial strains. As expected, there are dramatic differences among sequences from different species while sequences come from same species are the same. The sequences from this locus are so divergent that they cannot be aligned in a multiple sequence alignment program. This locus can be used alone or combined with other loci in a multilocus sequence typing system for accurate identification of Mycobacterium and will greatly improve the typing method for mycobacteria.

(16) **Insecticide Resistance of Mosquito Strains in Manatee County, FL**

Shainnel Eans, Chelsea T. Smartt, & Robert L. Frommer
University of Florida/IFAS, FMEL, 200 9th St. S.E., Vero Beach, FL

Mosquitoes play an important role serving as vectors for a wide variety of pathogenic diseases, including malaria, encephalitis, West Nile, dengue, and dengue hemorrhagic fever (Greenwood, 2002). The more temperate and tropical regions of the world find themselves facing constant outbreaks of these diseases because mosquito populations thrive under these conditions. In an attempt to control mosquito-borne disease outbreaks around the world, the use of insecticides has risen. However, this has also resulted in selection for mosquitoes that possess high tolerance/resistance to many insecticides (Levy 2007). It is important to understand the mechanisms involved with the development of mosquito resistance to insecticides to be able to predict where targeted control measures may be needed and how mosquitoes will react to new insecticides. We may even be able to completely negate or minimize mosquito defenses towards insecticides by directly manipulating genes that control the resistance phenotype or reduce the development of resistance through changes in how insecticides are applied. Thus far we have isolated a two distinct PCR fragments from Culex nigripalpus using esterase primers. A BLAST and VectorBase search revealed that the one of the Cx. nigripalpus translation products shares a high homology (82%) to a protein of unknown function from Culex pipiens quinquefasciatus. Additional sequence

analyses are presently being performed to determine its identity. Using the same search engines for the second isolated translation product, we found that it shares a high homology with the Culex pipiens Esterase-3 gene (86%). We are currently using gene expression studies to determine if it plays a role in the formation of insecticide resistance in Cx. nigripalpus.

(17) Using transgenic NPR1 to enhance systemic acquired resistance (SAR) in citrus

Vicente J. Febres and Gloria A. Moore
Horticultural Sciences Dept and PMCB program, U.F., Gainesville, FL

SAR is an inducible defense mechanism in which infection by a pathogen leads to an enhanced defense state that is long lasting and provides resistance or tolerance to a wide range of pathogens in subsequent challenges. Manifestation of this enhanced defense state is the increased expression of pathogenesis related (PR) genes both at the initial site of infection and systemically. Some PR proteins have demonstrated antimicrobial properties while others have unknown functions and it is thought that their combined action leads to the demise of the invading pathogen(s). The activation of PR genes is preceded by the accumulation of salicylic acid (SA) and is mediated by the NPR1 protein. NPR1 functions as a co-transcriptional activator and its expression alone does not lead to the activation of SAR but rather primes the plant for the defense response. We have transformed 'Carrizo' citrange and 'Duncan' grapefruit plants with the Arabidopsis NPR1 gene in an attempt to induce broad-spectrum disease resistance in citrus. The regenerated plants have normal phenotypes. Analysis of these plants indicates that some lines show higher expression levels of the PR1 gene (considered a marker for SAR) compared to non-transgenic plants. A few lines were also evaluated for their response to citrus canker. Although complete resistance was not observed, the transgenic plants, on average, had reduced lesion development compared to non-transgenic plants. These lines are being further evaluated for resistance to Huanglongbing (HLB). In addition, a grapefruit line is being evaluated under field conditions for performance and response to natural infection of pathogens.

(18) Biochemical studies of HIV-1 subtype B and C proteases: I50L, I50V, and I84V variants

Marty A. Fernandez[1], Roxana M. Coman[1], Maureen M. Goodenow[2], Ben M. Dunn[1]
University of Florida 1Department of Biochemistry and Molecular Biology, 2Department of Pathology, Gainesville, FL

The human immunodeficiency virus (HIV) is characterized by significant genetic diversity, and is divided among types, groups, subtypes, and recombinant forms. The protease inhibitors (PIs) used for HIV treatment were designed for HIV-1 subtype B protease (PR), and information regarding resistance to current PI treatment is largely limited to subtype B PR, which predominates in North America and Western Europe, but accounts for only approximately 12% of the global HIV pandemic. Subtype C, A and D viruses predominate worldwide. We performed kinetic analyses of subtype B and C PRs and the I50L, I50V, and I84V variants in order to compare resistance levels of these mutants in each PR subtype, and to understand what roles the naturally occurring polymorphisms in HIV-1 C PR play in drug resistance upon acquisition of the mutations. We determined their Ki values against 8 clinically used PIs. We found that HIV-1 subtype C PR exhibited comparable Ki values to subtype B PR for the inhibitors tested, and that the naturally occurring polymorphisms found in subtype C PR, in combination with the mutations, can influence drug resistance and could provide for a higher level of resistance to PIs when compared to subtype B PR. No significant changes were seen in catalytic efficiencies between B and C PR for both wild type and the I50L mutant. All Ki values determined were in the low-nanomolar to sub-nanomolar range indicating tight binding of the inhibitors. We also obtained crystals of the unbound subtype C I84V and I50L mutants, which were analyzed at a resolution of 1.8 Å.

(19) Investigating evolutionary and ecological factors underlying HIV-1 epidemic history in east Africa by Landscape Phylodynamics

Rebecca Gray[1], Marco Salemi[1], Andy Tatem[2], Oliver Laeyendecker[3,4] Maureen M. Goodenow[1], Thomas C. Quinn[3,4]
[1]Department of Pathology, Immunology, and Laboratory Medicine, U.F., Gainesville, FL
[2]Department of Geography and Emerging Pathogens Institute, U.F., Gainesville, FL
[3]Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD
[4]School of Medicine, Johns Hopkins University, Baltimore, MD

The factors responsible for modern disease epidemics are multi-factorial and involve ecological, political, social, as well as evolutionary influences. Here we introduce a new framework, called "landscape phylodynamics", that combines genetic sequence data analysis of pathogens based on coalescent theory (phylodynamics) with geographic and historical data about the territory where the pathogen is found. "Landscape" refers to the myriad ways in which humans affect and are affected by environment, and includes geography, land use, infrastructure and migration on a temporal scale. We apply the framework to provide a comprehensive analysis of the social, ecological, and evolutionary causes behind the early emergence of HIV-1 subtypes A and D epidemic in east Africa. By using Bayesian skyline plots inferred from HIV-1 genealogies of the gag and env gene, we show that subtypes A and D were introduced into Uganda in the 1950s - 1960s, preceding the earliest known cases of HIV-1 infection in the region by at least a decade. Both subtypes grew exponentially during the 1970s, coincident with high migratory out-flux from and political upheaval in Uganda. Phylogeographic analysis of viral gene flow also revealed that Uganda was the epicenter of the viral epidemic within east Africa. Furthermore, geographic analysis indicated that east African infrastructure was better developed than in neighboring countries

in central Africa, offering a possible explanation for the low prevalence of HIV-1 for decades in the Democratic Republic of Congo, in contrast to the almost immediate exponential growth of the virus upon introduction into east Africa. In conclusion, our analysis illustrates how a "landscape phylodynamic" approach, which couples phylodynamic inference with geographic data, can offer a powerful framework for understanding and interpreting past epidemics as well as predicting newly emerging outbreaks of infectious diseases.

(20) Episodic Compartmentalization of HIV-1 in Breastmilk One Year Post-partum

R. R. Gray[1], M. Salemi[1], G. M. Aldrovandi[2], K. Semrau[3], D. M. Thea[3], L. Kuhn[4], M. M. Goodenow[1,5]

[1]Department of Pathology, University of Florida, Gainesville, FL
[2]Childrens Hospital Los Angeles, University of Southern California, CA
[3]Boston University, Boston, MA
[4]Columbia University, New York, NY
[5]Department of Pediatrics, University of Florida, Gainesville, FL

Background: Breastfeeding remains an essential practice for most HIV-infected women in low resource settings because risks associated with weaning outweigh benefits of transmission reduction. Therefore, it is essential to better understand how postnatal transmission occurs so that effective strategies to prevent transmission can be devised while breastfeeding. However, no studies have been published to date describing the molecular evolution of the virus in breast milk. *Methods*: We analyzed longitudinally sampled HIV-1 envelope sequences from plasma and left and right breastmilk of six women participating in the prospective Zambian Exclusive Breastfeeding Trial. Each tissue was sampled at >3 timepoints during the first year post-partum. Phylogenies were inferred using a Bayesian framework. Compartmentalization of the virus within tissues was analyzed employing a modified version of the Slatkin-Maddison test and a test for population substructure. Maximum likelihood estimates of the non-synonymous/synonymous rate ratio for major internal branches in the phylogeny were obtained to investigate selective pressure. *Results:* The Bayesian maximum clade phylogeny was inferred for each patient. The Slatkin-Maddison test indicated evidence for compartmentalization of the virus between plasma and breastmilk ($p<0.0001$) for all patients. Significant population structure was detected between the breastmilk and plasma virus and between the left and right breastmilk virus ($p<0.01$) for at least one timepoint. In general, compartmentalization increased with time after delivery. This pattern, found in all patients, suggests episodic compartmentalization of the virus in the breastmilk. Branches under positive selection were detected for all patients, and in general, were more frequent later in infection. *Conclusions:* The emergence and loss of compartmentalization of the breastmilk virus indicates a much more complex population dynamics underlying HIV-1 evolution within this tissue than previously identified. The reemergence of distinct viral variants and the presence of positive selection at later timepoints suggest that the breastmilk environment is variable and may select for different genotypic and phenotypic features. Understanding the complex population dynamic underlying HIV evolution within these tissues may be essential to uncover the molecular mechanisms responsible for the differential risk of transmission associated with duration and exclusivity of breastfeeding.

(21) Full-Genome Evolutionary Patterns Analysis of West Nile Virus

*R. R. Gray[1], L. A. Santos[2], N. M. C. Veras[3], *M. Salemi[1].*

[1]Department of Pathology, Immunology, and Laboratory Medicine, U.F., Gainesville, FL
[2]Advanced Public Health Laboratory, Goncalo Moniz Research Center, Oswaldo Cruz Foundation, Salvador, Bahia, Brazil
[3]Instituto de Biologie, Universidade de Brasilia, Brasilia, DF, Brazil

Background: West Nile Virus (WNV) is a mosquito-borne RNA virus member of the Flaviviridae family. It was first isolated in Uganda in 1937 and has been associated with severe and even fatal diseases in humans with an overall mortality rate of about 10%. Several human and equine outbreaks have been recorded in portions of Africa, southern Europe, Asia and, since 1999, North America. Despite a number of phylogenetic studies have already been published on WNV, a detailed analysis of phylogenetic signal/noise and evolutionary patterns in different genes is still lacking. *Methods:* We analyzed the 104 WNV full genome sequences available in GenBank. Best-fitting nucleotide substitution models, the molecular clock hypothesis, positive versus purifying selection and phylogenetic signal to noise ratio were investigated with both maximum likelihood and Bayesian-based methods. Full-genome alignments as well as three structural and eight non-structural genes were evaluated separately. *Results:* The NS3 and NS5 genes contained the highest phylogenetic signal to noise ratio. In contrast, the structural genes showed little phylogenetic signal, particularly in the North American dataset. The relaxed clock model fitted the data best, although median evolutionary rate estimates were similar to those previously reported using strict-clock methods. Several sites in the non-structural genes were identified as evolving under positive selection. Most importantly, in all analyses, the concatenated NS3NS5 dataset provided the same resolution as the full genome alignments. *Conclusions:* Current phylogenetic studies are frequently based on genes containing poor phylogenetic signal/noise ratio. Our results suggest that a greater emphasis should be placed in studying NS3 and NS5 regions to elucidate WNV evolutionary dynamics.

(22) Dynamics of Effective Population Size of Hiv-1 Correlates with Disease Progression in Natural Infection

**R. R. Gray[1], M. Salemi[1], J. Sleasman[2], M. M. Goodenow[1]*

[1]Department of Pathology, Immunology, and Laboratory Medicine, U.F., Gainesville, FL
[2]Department of Pediatrics, University of South Florida, Tampa, FL

We studied viral evolutionary patterns in longitudinal samples from nine therapy naïve pediatric subjects to determine robust predictors of progression to AIDS during the natural course of human immunodeficiency virus type 1 (HIV-1) infection. HIV-1 intra-host evolution was monitored by sequencing multiple clones of the viral envelope over time. A Bayesian Markov Chain Monte Carlo approach was used to infer non-parametric estimates of HIV-1 effective population size (Ne) during infection which was compared with clinical information including viral load and CD4 counts. Individuals were classified into three groups based on immune parameters: consistently high CD4 percentage (>25%) throughout infection; initially high CD4% followed by a irreversible decline (<15%); and persistently low CD4% (<25%) throughout the course of the study. Subjects who either maintained a high CD4% throughout infection or began the study with low immune counts exhibited a constant and low Ne throughout infection. In contrast, individuals with initially high CD4% followed by a crash exhibited a spike in viral Ne >2000 ~ 6-12 months prior to immune failure. Viral load taken at the last timepoint showed a lognormal relationship with the peak Ne value. A model based on immune system pressure, viral population size, net adaptation rates, and target cell availability demonstrated that Ne is a robust indicator to predict immunological failure in HIV-infected individuals. These novel findings could inform optimal initiation of costly and toxic drug therapies.

(23) Bacillus anthracis Toxins Paralyze Neutrophil Actin-based Motility

Sarah E. Guilmain[1], Russell L. During[1], Wei Li[1], Conrad Quinn, Wei-Jen Tang[2] and Frederick S. Southwick, MD[1]

[1]Division of Infectious Diseases, College of Medicine, U.F., Gainesville, FL 32610, Center for Disease Control, Atlanta, GA

[2]Ben May Department for Cancer Research, University of Chicago, Chicago, IL 60637

Inhalation anthrax results in high-grade bacteremia and shock that is accompanied by a blunted rise in the peripheral neutrophil count and a paucity of neutrophils in infected pleural fluid. Edema toxin (ET) is one of the major Bacillus anthracis virulence factors and consists of the adenyl cyclase, edema factor (EF) and protective antigen (PA). Relatively low concentrations of ET (100-500ng/ml) significantly impair human neutrophil chemokinesis, chemotaxis and the ability to polarize. These changes are not accompanied by a significant change in CD11/CD18 receptor expression with ET or LT alone, however the combination of the two toxins causes a significant decrease in receptor expression. Toxin treatment is also associated with a 30% reduction in FMLP-stimulated F-actin content. ET treatment also reduces the speed of Listeria monocytogenes intracellular actin-based motility by 30%, and decreases actin filament tail length by 50% in HeLa cells. These defects in actin assembly are accompanied by >20 fold increase in intracellular cyclic-AMP and a >4 fold increase in the phosphorylation of protein kinase A (PKA). Our results demonstrate that ET and the combination of ET + LT is able to suppress human neutrophil migration and polarity, and these defects are a consequence of a decrease in the adhesion receptor and a reduction in actin assembly, that in turn may be mediated by high intracellular levels of cyclic-AMP and the phosphorylation of PKA.

(24) Stigma and Additional Factors Influencing the Effectiveness of TB Contact Investigations among Mexicans Living in Florida

P. Hamsho-Diaz, MD, MA; K. Simpson, MSHSE; M. Lauzardo, MD, MSc

Southeastern National Tuberculosis Center, U.F., College of Medicine, Gainesville, FL

(25) The role of electrical potentials in H+ V-ATPase-coupled membrane energization of disease vector mosquitoes

William R. Harvey, Kenneth M. Sterling and Bernard A. Okech

Whitney Laboratory, University of Florida, St. Augustine, FL

H+ V-ATPases impose voltages across many types of plasma membranes in the alimentary canal of Anopheles gambiae and Aedes aegypti larvae. The voltages are used to drive uptake of essential amino acids by Na+-coupled amino acid symporters (co-transporters) and to rid cells of toxic acids by exchange of intracellular H+ for extracellular Na+ via Na+/H+ antiporters (exchangers). These voltage-driven transporters of mosquito larval epithelia are unlike those of most vertebrate epithelia which use Na+ concentration differences to absorb nutrients from the alimentary canal and to secrete toxic acids into it. More than two dozen of the voltage-driven amino acid transporters and other voltage-driven carriers have been cloned and several if them have been used to prepare antibodies. We have prepared brush border membrane vesicles from whole larvae and shown by antibody labeling that they contain three of the transporters that are essential for amino acid uptake, pH regulation and Na+ conservation – all necessary for mosquito survival. We are arranging to cooperate with an industrial firm to use the BBMV preparation in a screen for inhibitors that can be developed as new environmentally safe and inexpensive mosquitocides.

(26) Response of Phlebotomus papatasi (Diptera: Psychodidae) to Commercial Mosquito Traps in Southern Egypt

D.F. Hoel[1], D.L. Kline[2], S.S. El-Hossary[3], H.A. Hanafi[3], N. Watany[3], E.Y. Fawaz[3], B.D. Furman[3], P.J. Obernauer[4], J.A. Hogsette[2], U.R. Bernier[2], D.E. Szumlas[5]

[1]Program Mngr., Medical Entomology Collaborations, NMCPHC Det., CMAVE, Gainesville, FL
[2]Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL
[3]U.S. Naval Medical Research Unit No. 3, Cairo, Egypt
[4]Departments of Entomology and Nematology, University of Florida, Gainesville, FL
[5]Centers for Disease Control and Prevention, Atlanta, GA

Phlebotomus papatasi (Scopoli) is the primary Old World vector of the etiological agents responsible for cutaneous leishmaniasis from North Africa east into India and a vector of several phleboviruses. The World Health Organization has recently recognized leishmaniasis as an important neglected tropical disease, affecting the health of over 12 million people throughout 88 countries worldwide. We tested four commercial adult mosquito traps marketed for homeowner use in residential settings against a CDC light trap, routinely used for sand fly surveillance for efficacy in collecting sand flies. Our traps included the Mosquito Magnet (MM) Pro, the Sentinel 360 mosquito trap, the BG-sentinel mosquito trap, and the Mega-Catch trap. The BG-sentinel and CDC light traps were baited with 2 kg dry ice nightly, Sentinel 360 and Mega-Catch traps were not, while the MM Pro produced its own CO2. Traps were not baited with optional lures (lactic acid, octenol baits) that are recommended for some models. Traps were rotated through five sites in a 5x5 Latin square experiment in a small farming village in the Nile River Valley 10 km north of Aswan, Egypt. Four repetitions were conducted during the height of the sand fly season (June, August (2x) and September, 2007) at a site in which P. papatasi is abundant and Leishmania-free. 6,440 sand flies were collected over four trials, 6,037 of which were P. papatasi (93.7%). The BG trap collected significantly more ($P < 0.05$) P. papatasi than the Mega-Catch and Sentinel 360 traps and more than the MM Pro and CDC light trap. Order of success and trap means (±SE) were: BG trap, 142.1 (45.8) > MM Pro, 56.8 (40.1) > CDC trap, 52.3 (27.5) > Mega-Catch trap, 38.2 (28.5) > Sentinel 360 mosquito trap 12.6 (8.2). Results indicate new, commercial traps are a suitable substitute for CDC light traps in sand fly surveillance programs. Unlit, CO2-baited commercial traps performed much better than lit commercial traps lacking CO2 production.

(27) First Responders Online: Problem Solving and Critical Thinking Training For Emergency Personnel Responding To Pathogen Outbreaks

Tracy Irani[1], Maria Gallo[2], Lisa Hightower[1], Ricky Telg[1], Brian Myers[1]
[1]Agricultural Education and Communication Department, 305 Rolfs Hall, U.F., PO Box 110540, Gainesville, FL 32611
[2]Agronomy Department, 303 Cancer/Genetics Research Complex, Gainesville, FL 32610

Over 40 years of research into the psychology of risk and crisis management shows that dealing with human factors related to how emergency responders solve problems and work together in teams is of crucial importance to insure optimal outcomes and mitigation of risk. A criticism of conventional emergency responder training has been that while such training may do a good job of helping participants understand how to deal with a known crisis, it is inadequate at preparing responders for the next crisis. In these situations, team process skills, effective crisis management and communication, and the ability to draw on critical thinking skills to facilitate effective decision making become crucial components of the emergency response process. First Responders Online is a prototype training component focused on the development of effective problem solving and team process skills among emergency responders, scientific researchers and other personnel who would be involved in biodefense or emerging infectious disease emergencies. The training is made up of four modules: critical thinking, persuasion and the media, problem solving, and group problem solving. Case studies and multimedia components have been integrated into the modules to make the material more dynamic and interactive. A wiki has been created to allow participants to discuss with each other skills they are learning, in effect building an online community. College students studying in academic fields focused on disease control and pathogen outbreaks may also benefit from going through the training. The modules offer students hands-on curriculum in critical thinking and problem solving, as it applies to emergency pathogen outbreak situations. This project was developed by the Scientific Thinking & Educational Partnership (STEP) program, and funded by the Emerging Pathogens Institute and the Institute of Food and Agricultural Sciences (IFAS).

(28) Tubercle Resorption and Epithelial Remodeling In Fathead Minnows Exposed To Ethinylestradiol

A.S. Kane[1], J.D. Salierno[2], J.C. Wolfe[3]
[1]Environmental Health Program, College of Public Health & Health Professions; and Emerging Pathogens Institute; U.F., Gainesville, FL
[2]Department of Biological and Allied Health Sciences, Fairleigh Dickinson Univ., Madison, NJ
[3]Environmental Pathology Labs, Sterling, VA

The reduction of nuptial tubercles in male fathead minnows resulting from exposure to environmental estrogens is well documented. However, knowledge regarding tubercle alterations at the cellular level is poorly understood. In this study, histological tubercle data was analyzed from a study in which male fathead minnows, Pimephales promelas, were exposed to 0, 20 or 40 ng/L 17α-ethinylestradiol (EE2) for 21 days. The focus was to investigate EE2-exposed alterations in male fathead minnow nuptial tubercle morphology. Fish exposed to EE2 had fewer tubercles compared with control fish (ANOVA, $p \leq 0.001$). This, in turn, was related to the increased incidence of tubercle resorption (Kruskal-Wallis AOV, $p \leq 0.001$). Upon further histological evaluation of resorbed tubercles, several significant differences were observed between non-exposed and EE2-exposed fish. EE2-exposed fish exhibited increased tubercle hypoplasia and atrophy (Kruskal-Wallace AOV, $p = 0.007$), along with decreased stratum spinosum cellular hypertrophy and keratinization (Kruskal-Wallace AOV, $p \leq 0.009$).

In addition, dermal cellular pigmentation was marginally increased in EE2-exposed fish (Kruskal-Wallace AOV, $p = 0.06$). These alterations occurred concurrently with significant decreases in 11-ketotestosterone, testosterone, and estradiol (11-KT, T, and E2 respectively, ANOVA, $p \leq 0.03$). This analysis confirmed the regression in tubercles observed under light microscopy and describes significant cellular changes in tubercle structure resulting from hormonal alterations in fish exposed to estrogenic compounds. In addition, this is the first report to describe tubercle regression through the use of histological analysis in fathead minnows. Extensive remodeling of these secondary sex characteristics, known to be under hormonal control, has ramifications for male reproductive success and provides novel insights into the cellular physiology that underpins the formation and regression of keratinized tubercles in fish.

(29) Using Cell-Free Protein Expression for Ricin Detection

Ruba Khnouf[1], Qian Mei[2], Shouguang Jin[3], Hugh Fan[1,2]
[1]Biomedical Engineering, University of Florida
[2]Mechanical and Aerospace Engineering
[3]Molecular Genetics and Microbiology

Keywords: Cell- free, protein expression, ricin, 96- well plate, luciferase

Cell-free protein expression has been investigated to replace conventional cellular protein expression methods for obtaining various proteins. The method is based on the fact that cells are not needed for synthesizing proteins as long as the machinery necessary for protein synthesis such as ribosomes and amino acids are available. One of the methods developed to sustain protein synthesis reactions for a longer period of time and as a result increase protein expression yield is supplying these reactions with the energy and amino acids necessary for protein synthesis through a semipermeable membrane. The process is called continuous exchange cell-free (CECF) protein synthesis. We have successfully designed and fabricated a 96 well-plate where CECF protein synthesis can be applied and have shown the improvement of protein yield over a regular microplate. In addition we have successfully synthesized six proteins that can be optically detected as they are being synthesized in the designed plate. Ricin functions as a protein synthesis inhibitor by catalyzing the ricin/sarcin loop in 28s ribosomal subunit. We have successfully shown that ricin can be detected through its mechanism of action; the difference between a positive control where no ricin is added and one where ricin is added would show a reduction in the amount of protein expressed. We have shown the reduction in luciferase expression by testing the resulting luminescence when adding ricin and comparing it to the amount of luminescence when no ricin is present in the reaction. Using the microplate we have developed, the luminescence can be measured straight from the expression device using a plate reader.

(30) Activation of Cd4+Cd25+ Regulatory T Cell Suppressor Function By Analogs Of The Selecting Peptide

Joseph Larkin III 1,2, Andrew Rankin1, Cristina Cozzo Picca1 and Andrew J. Caton1
1The Wistar Institute, Philadelphia, PA 19104 and 2 Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611

CD4+CD25+Foxp3+ regulatory (Treg) cells can undergo both thymic selection and peripheral expansion in response to self-peptides that are agonists for their T cell receptors (TCRs). However, the specificity by which these TCRs must recognize peptide:MHC complexes in order to activate Treg cell function is not known. We show that CD4+CD25+Foxp3+ Treg cells can mediate suppression in response to peptides that are only weakly crossreactive with the self-peptide that induced their formation in vivo. Moreover, suppression could be efficiently activated by peptide analogs that were inefficient at inducing CD69 up-regulation, and that also induced little or no proliferation of naïve CD4+CD25-Foxp3- T cells expressing the same TCR. These findings provide evidence that self-peptide-specific CD4+CD25+Foxp3+ Treg cells can exert regulatory function in response to self- and/or pathogen-derived peptides with which they are only weakly crossreactive.

(31) The Effect of Foreign Birth and Race on All-Cause Mortality in Patients Diagnosed With Tuberculosis

M Lauzardo1 K Palmer[2]
[1]The University of Florida, Southeastern National Tuberculosis Center, Gainesville, FL
[2]Karolinska Insitutet, Stockholm, Sweden

(32) Isolation and Characterization of Vibrio vulnificus Strains from Tilapia Fish in Bangladesh

Z.H. Mahmud[2], A.C. Wright[3], M.S. Islam[2], J.G. Morris[1], and A. Ali[1]
[1]Emerging Pathogens Institute, University of Florida, Gainesville, FL 32610
[2]International Centre for Diarrhoeal Disease Research, Bangladesh
[3]Department of Food Science and Human Nutrition, U.F., Gainesville, FL 36611

V. vulnificus, a gram-negative bacterium distributed worldwide in estuarine and marine environments, can cause serious human illnesses, including wound infections, septicemia and gastroenteritis. People with underlying chronic diseases acquire the bacterium upon consumption of raw and undercooked seafood, especially oysters and fish. While oysters are considered to be a major reservoir of V. vulnificus in the U.S., necrotizing wound infections associated with injuries caused by aquacultured fish such as tilapia are increasingly being recognized as a reservoir of this bacterium, as evidenced by the recent report of 62 cases of V. vulnificus infection in Israel

attributed to the handling and eating of tilapia. A single case of V. vulnificus infection (upon consumption of tilapia) has also been reported from Canada. To meet the growing demand for protein, policymakers in a number of countries, including Bangladesh are encouraging their citizens to increase aquaculture production of tilapia. To assess the potential health risk from V. vulnificus linked to the handling and consumption of tilapia fish in aquaculture environments in areas such as Bangladesh, we screened coastal aquaculture ponds, and tilapia, for the presence of V. vulnificus for a three month period beginning in May, 2008. During our study period, we collected a total of 42 tilapia fish and water samples from coastal aquaculture ponds. As controls, we also collected 24 tilapia fish and water samples from fresh water aquaculture ponds. While none of the tilapia fish and water samples obtained from fresh water aquaculture ponds yielded V. vulnificus, 24 (57%) of 42 samples collected from coastal aquaculture ponds yielded a total of 33 V. vulnificus isolates. A battery of biochemical tests revealed that all 33 V. vulnificus isolates belong to biotype 1. PCR and ribotyping analyses confirm that all of the strains possess virulence gene, vvhsp, and belong to five ribotypes. This is the first report of isolation of V. vulnificus from tilapia in Bangladesh. At this point, none of the isolates showed evidence of antimicrobial resistance; however, given the known heavy (and unregulated) usage of antibiotics in aquaculture settings, subsequent development of resistance would not be unexpected. Currently there are no data on rates of illness associated with V. vulnificus in Bangladesh. Our findings suggest the need for caution among persons working in coastal aquaculture, and the need for surveillance for V. vulnificus infections in these and other high-risk populations.

(33) Isolation and Characterization of Phage Display scFv Antibodies to Flagella of Escherichia coli O157:H7

J.L. Martin, T. Finnegan, and P.A. Gulig*
Department of Molecular Genetics and Microbiology, University of Florida.

Compared with traditional antibody techniques, phage display enables the rapid isolation of recombinant antibodies. We used the Tomlinson J single chain F variable (scFv) phage display library to isolate reagents that recognize Enterohemorrhagic E. coli EDL933, which causes hemorrhagic colitis and hemolytic uremic syndrome. After three rounds of panning on crude flagella, 18 of 22 phage clones were positive by ELISA. In a Western blot these clones reacted with a band corresponding to the flagellin protein. Some clones reacted with only the upper region of the band, suggesting that they may recognize carbohydrate modification of the flagella. Placing the phagemids in a suppressor-free E. coli failed to yield scFv protein for most clones. DNA sequencing of these phagemids identified three unique clones, and the clones that failed to produce scFv protein had two internal stop codons. We repaired these stop codons and recovered scFv protein, but only one scFv reacted with flagella. We are manipulating the sequence of the other clone to restore its antigen-binding properties. The phage clones were examined for specificity by ELISA with whole cells of O157:H7 strain EDL933 and K12 strain MG1655. One clone was specific for O157:H7, while the other also cross-reacted with K12. Neither clone reacted with whole cells of an isogenic flagellin mutant. The scFv proteins from these clones did not react with whole cells expressing flagella possibly due to low avidity. We also used a high throughput scFv screen to directly isolate functional scFv-secreting clones without first screening phages. Approximately 200 clones were induced to produce scFv in 96 well cultures and tested by a high throughput ELISA with flagella. Sixteen positive clones were verified in a Western blot for reactivity with the flagellin, demonstrating the usefulness of this high throughput method. We are genetically engineering these scFv clones to optimize their usefulness.

(34) Bacterial Resistance Issues in Wound Care and Wound Dressings

A. Mikhaylova[1], B. Liesenfeld[1], D. Moore[1], J. Vella[1], R. Carr[1], J. Olderman[1], C. Batich[1,2], G. Schultz[1,2]
[1]Quick-Med Technologies, Inc.
[2]University of Florida

The emergence of bacteria resistant to various agents has been a developing public health issue for some time. Overuse of antibiotics, in healthcare as well as in the food supply, is considered a likely cause for the emergence of more and more strains of antibiotic resistant bacteria. Bacterial strains have also demonstrated the ability to generate resistance to silver – a common active ingredient in antimicrobial products such as wound dressings. Scientists have identified at least three genes associated with silver resistance (Sil- E, R and S). Additionally, there is evidence in the literature that strains of organisms with resistance to a specific agent also display a higher tolerance to other antimicrobial agents, which is attributed the development of efflux pump mechanisms (cellular mechanisms that can expel certain antimicrobials before they are able to act on their cellular targets). We provide an overview of how bacteria acquire and transmit resistance. Consideration is also provided on how this may affect the healthcare provider, and the types of products utilized to prevent infections now and in the future. Quick-Med has developed an antimicrobial technology based on surface bound cationic polymeric materials (NIMBUS). This technology was tested by exposing 10 consecutive generations of E. coli to the material in such as manner as to recover survivors. The results of the experiments showed that the bacteria did not generate resistance to NIMBUS after prolonged and repeated exposure. We attribute the lack of resistance development to the mechanism of kill, which is through disruption of the cellular membrane, rather than by targeting specific intracellular targets. We conclude that high molecular weight antiseptics anchored to a surface pose the lowest possible risk of inducing bacterial resistance. *References:* Wound Repair Regen. 2005 Jul-Aug;13(4):412-21. "Comparison of in vitro disc diffusion and time kill-kinetic assays for the evaluation of antimicrobial wound dressing efficacy." Gallant-Behm et al. Appl. Environ. Microbiol. 2008 Aug;74(15):4825-34. Epub 2008 May 30, "In vitro study of the effect of cationic biocides on bacterial population dynamics and susceptibility." Moore LE, Ledder RG, Gilbert P, McBain AJ

(35) Use of Integrated Malaria Management Reduces Malaria in Kenya

Bernard A. Okech[*1,2,5], *Isaac K. Mwobobia*[2], *Anthony Kamau*[5], *Samuel Muiruri*[6], *Noah Mutiso*[6], *Joyce Nyambura*[2], *Cassian Mwatele*[2], *Teruaki Amano*[2], *Charles S. Mwandawiro*[2]

[1*]Environmental Health Program, Department of Epidemiology and Biostatistics and Emerging Pathogens Institute, U.F., Gainesville, FL 32610,USA

[2]Eastern and Southern Africa Centre for International Parasite Control

[3]Kenya Medical Research Institute(KEMRI), P. O. Box 54840, Nairobi, Kenya

[4]Centre for Biotechnology, Research and Development, KEMRI, P. O. Box 54840, Nairobi, Kenya

[5]African Medical Research Foundation

[6]Division of Vector-Borne Diseases (DVBD), Ministry of Health, Nairobi, Kenya

The integrated malaria management (IMM) practices was assessed among community members of Mwea division, central Kenya using a knowledge attitude and practices (KAP) survey. The KAP study evaluated case management practices at the home and hospitals, personal protection measures and vector control methods. Concurrently, malaria infection prevalence in the outpatient department at the referral hospital in the area was examined. The indoor resting population of the major malaria mosquito and their sporozoite infection status were evaluated. The daily risk of malaria transmission in the entire division was calculated using the entomological inoculation rates (EIR). Three hundred and eighty nine households in Mwea division participated in the KAP study while 90 houses were surveyed for EIR. Ninety eight percent of the households knew about malaria while approximately 70% of households knew its symptoms and its management methods. 97% of households went to a health center for malaria diagnosis and treatment. Also 81% used anti-malarial medicines bought from local pharmacies. Almost 90% of households reported owning and using an insecticide treated bed net and 81% reported buying the nets within the last 5 years. The mosquito reduction measures reported included in order of preference, environmental management (35%), mosquito repellent and smoke (31%) insecticide canister sprays (11%), and window and door screens (6%). The management of malaria cases, personal protection and mosquito reduction measures comprise an integrated malaria management (IMM) package. Over the last 4 years prior to this study, the malaria cases in the community hospital reduced from about 40% in 2000 to less than 10% by 2004 and by the year 2007 malaria cases decreased to zero while a onetime cross-sectional malaria parasite survey in 2005 detected no Plasmodium infection in 300 primary school children in the area. Mosquito vector populations were low and variable within the study villages. The EIR remained low and was estimated at 0.011 infectious bites per person per day. The use of malaria control tools in an integrated in central Kenya might have influenced the decreased malaria cases in the district hospital and in the school children. A vigorous campaign emphasizing IMM should be adopted and expanded in Mwea division and in other areas with different eco-epidemiological patterns of malaria transmission. With sustained implementation and support from community members integrated malaria management can reduce malaria significantly in affected communities in Africa.

(36) The Genomics Division within Uf's Icbr Offers the Means for Specialized Genetic Data Acquisition at a Reasonable Cost

D.G. Ostrow[1], *E. Almira*[1], *G. Clark*[1], *D. A. Moraga*[1], *S. Norton*[1], *R. Shaw*[1], *S. Shanker*[1]

[1]Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

The ICBR's Genomics Division allows researchers to generate, store, and analyze genomic data without creating costly technical infrastructure within their own labs. The Genomics Division is composed of five groups: 1) DNA Sequencing using Sanger and next-generation technologies, 2) Fragment Analysis, 3) Gene Expression, 4) Real-Time PCR, and 5) SNP Genotyping. Together, we provide access to researchers across campus to technology including genome sequencing on the 454 and SOLiD instruments, gene expression measurement on the Agilent and Affymetrix platforms, and high throughput SNP genotyping on the Illumina Bead Station. We are also able to provide expertise to assist with experimental design, data collection, and analysis. Having these resources on campus saves valuable time and money and allows researchers to focus on generating scholarly publications and external funding opportunities.

(37) Infection of Alveolar Macrophages by Canine Influenza Virus Potentiates Tnf-Alpha Repsonse to Lipopolysaccharide and Induces Cell Death

J.R. Powe and W.L. Castleman

Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL

Secondary bacterial pneumonia is an important cause of morbidity and mortality in dogs naturally infected with canine influenza virus (CIV). We tested the hypothesis that prior infection of alveolar macrophages with CIV induces cell death and augments the macrophage TNF-alpha response to bacterial components such as lipopolysaccharide (LPS), thereby contributing to the severity of disease seen in CIV infection with secondary bacterial pneumonia. Canine alveolar macrophages were isolated from mongrel dogs and were incubated in culture with CIV (Influenza A/Canine/FL/04) for 1 hour. LPS was added to culture media at 0, 3 or 6 hours after virus incubation. Samples of supernatant were collected at 0, 3, 6, 12, and 24 hours after virus incubation and TNF-alpha levels were measured by ELISA. TNF-alpha levels in virus-inoculated, LPS exposed cells were above those seen in cells inoculated with virus alone, or mock-inoculated cells exposed to LPS. Additionally, cells inoculated with virus lost viability as measured by trypan blue assay by 24 hours after inoculation. The results are

consistent with the hypothesis that infection of alveolar macrophages with CIV contributes to disease severity in secondary bacterial pneumonia by inducing cell death and augmenting macrophage TNF-alpha response to bacterial components such as LPS.

(38) Mhc-I And Mhc-Ii Restricted T Cells Important For A Broadly-Effective Aids Vaccine

R. Pu, E. Sato, J.K. Coleman, and J.K. Yamamoto
Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Objective: Protective vaccines for human (HIV-1) and feline (FIV) immunodeficiency viruses must provide protection against viruses of multiple heterologous subtypes. Prototype and commercial dual-subtype FIV vaccine, consisting of inactivated subtype-A and -D viruses, confer protection against both homologous- and heterologous-subtype strains. Passive-transfer studies using antibodies from vaccinated cats revealed homologous-subtype protection can be mediated by vaccine-induced antibodies. However, cross-subtype protection was not mediated by vaccine-induced antibodies. Hence, the cellular-immune mechanisms of this vaccine protection were evaluated to provide insights to the immunity needed for a broadly-effective HIV-1 vaccine. *Methods:* Three adoptive-transfer (A-T) studies were performed with semi-inbred donor-recipient pairs that were MHC-II-matched by mixed leukocyte reaction (MLR). Magnetic-cell-purified B-cell, T-cell, CD4+ T-cell, and CD8+ T-cell populations from vaccinated donors were transfused intravenously to MLR-matched recipients one day before challenge with homologous FIVPet (Studies 1-2) or heterologous-subtype-B pathogenic FIVFC1 (Study 3). FIV infection was determined by immunoblot analysis for FIV-specific antibodies and by virus isolation of PBMC and lymphoid tissues using reverse-transcriptase and proviral-PCR analyses. Protective mechanisms were characterized by MHC-I/II sequencing, T-cell functional phenotyping, and functional epitope mapping. *Results:* In Studies 1-2, 7 of 8 recipients of T cells, 2 of 3 recipients of CD4+ T cells, and 2 of 3 recipients of CD8+ T cells from vaccinated/MLR-matched cats were protected against FIVPet, but eight recipients of PBS, B cells from vaccinated/MLR-matched donors, or T cells from nonvaccinated donors were unprotected. Protected recipients and donors were generally matched at MHC-I sequence, while unprotected recipients and donors were unmatched at MHC-I or MHC-II sequence. In Study 3, 3 of 5 recipients of T cells from vaccinated/MLR-matched donors were protected against FIVFC1, but five recipients of PBS or MLR-unmatched T cells were unprotected. The vaccine-induced CD4+ T-cell and CD8+ T-cell populations had FIV-specific interferon-□, granzyme-A+, granzyme-B, and perforin responses, suggesting the role of CD4+ granzyme-A+/perforin+ cytotoxic T lymphocytes (CTL) and CD8+ granzyme-B+/interferon-□+ CTL, respectively. Furthermore, functional epitope mapping of virus proteins using CFSE proliferation analysis suggest that both CD3+CD4+ T cells and CD3+CD8+ T cells from vaccinated donors recognize the same epitopes. The use of semi-inbred cats also provided more consistent responses to the specific epitopes when the cells were from animals with the same feline MHC haplotype. *Conclusions:* Vaccine-induced protective immunity was mediated by MHC-restricted, FIV-specific CD4+ granzyme-A+/perforin+ CTL and CD8+ granzyme-B+/interferon-□+ CTL. Functional epitope mapping suggest that both CD3+CD4+ T cells and CD3+CD8+ T cells from vaccinated donors frequently respond to the same virus epitopes for maximum functional response. These are the first studies to utilize the FIV/semi-inbred-cat model for characterizing the cellular immune correlates of vaccine protection against a naturally occurring, AIDS-inducing, multi-subtype lentivirus. The findings from this model should provide important insights for developing a broadly effective HIV-1 vaccine. This work was funded by NIH R01-AI30904.

(39) Comparative Analysis of Different Typing Methods on Yersinia pestis Strains Isolated from Endemic Foci of Plague in the Republic of Georgia

Chythanya Rajanna[1,2] Tamara Revazishvili[2], Mohammed H. Rashid[2], Lela Bakanidze[3], Nikoloz Tsertsvadze[3], Paata Imnadze[3], J. Glenn Morris, Jr.[2], and Alexander Sulakvelidze[1,2]
[1]Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, USA
[2]Emerging Pathogens Institute, University of Florida, Gainesville, USA
[3]National Center for Diseases and Public Health, Tbilisi, Republic of Georgia

Forty Y. pestis strains isolated from endemic foci of plague in the Republic of Georgia, and 6 Y. pestis strains isolated in neighboring former Soviet Union countries were analyzed for their genetic relatedness by pulsed field gel electrophoresis (PFGE), Multi Locus Variable Number Tandem Repeats Analysis (MLVA) and Multilocus sequence typing (MLST). In addition, the DNA of 11 Y. pestis strains isolated in the United States, and published nucleotide sequences from Y. pestis strains KIM, CO92 and 91001, were compared to those of the 46 strains in our collection, using MLST based on sequence data from the 16S rRNA, hsp60, glnA, gyrB, recA, manB, thrA and tmk loci. Also, loci from four virulence genes (caf1, lcrV, psaA and pla) were sequenced and analyzed. Two clusters of strains were identified by MLST. One contained predominantly (95%) Georgian strains, and the other contained predominantly (95%) non-Georgian strains. PFGE also identified two major clusters and, in agreement with the MLST data, it also clustered the Georgian Y. pestis strains separately from the non-Georgian strains. MLVA also separated Georgian strain from non Georgian strains better than MLST and PFGE and it identified several subgroups among strains that were indistinguishable by both MLST and PFGE. From the analysis it appears that MLST is ideal for identifying genetic relatedness between species, MLVA for intra-species and PFGE for both inter and intra species relatedness.

(40) Draft Genome Sequences of Two Georgian Yersinia Pestis Isolates

Chythanya Rajanna[1,2], Tamara Revazishvili[1], Lela Bakanidze[3], Nikoloz Tsertsvadze[3], Paata Imnadze[3], J. Glenn Morris, Jr.[1], Oscar. C. Stine[4], Henry. S. Gibbons[5], and Alexander Sulakvelidze[1,2]

[1]Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

[2]Emerging Pathogens Institute, University of Florida, Gainesville, FL

[3]National Center for Diseases and Public Health, Tbilisi, Republic of Georgia

[4]University of Maryland, MD

[5]US Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD

Yersinia pestis, the cause of plague, exists today in discrete, geographically defined foci each of which presents different microevolutionary pressures. We have obtained draft genome sequences of two Y. pestis isolates (G3768 and G1670) isolated from mountain voles (Microtus arvalis) from the Republic of Georgia. In silico analysis of VNTR, CRISPR regions indicate that these strains are highly similar to previously determined sequences from another Georgian strain G8786, previously grouped with other Y. pestis subsp. caucasus strains. The pMT1 plasmid resembles that of another Georgian strain previously sequenced and the sequenced Y. pestis subsp. caucasus strain Pestoides F (also known as Y. pestis subsp. microtus), being approximately 30 kb larger than the pMT1 plasmids of most Y. pestis strains. Like most Antiqua and Medeivalis biovar strains, G3768 and G1670 lack the bacteriophage-encoding locus YPO2271-2278. Both the sequenced strains further resembles Antiqua and Medeivalis strains in that its genome encodes the entire flagellar locus at YPO0739-0754, and a repeat within this region resembles most closely that found in another microtus strain 91001. YPO0757-0780 are missing in this strain. In general, our draft genome sequences confirm the microarray data of Hinchliffe et al. for a related Georgian strain G8786 (Genome Res. 2003, 13:2018-2029). Single nucleotide polymorphism analysis revealed this strain to be highly similar to Pestoides F, several SNPs were unique to G3768 among sequenced strains. Other notable features include the aspA gene contains a non-synonymous SNP that also occurs in Pestoides F that leads to replacement of leucine at position 363 with serine. G3768 also contains an lcrV gene that closely resembles Y. pestis subsp. pestis strains. The effect of these substitutions on virulence or host specificity of G3768 and G1680 is unknown.

(41) West Nile Virus Infection Alters Midgut Gene Expression in Culex pipiens quinquefasciatus Say (Diptera: Culicidae)

Stephanie L. Richards, Sheri L. Anderson, Jennifer S. Erickson and Chelsea T. Smartt

University of Florida/IFAS, FMEL, 200 9th St. S.E., Vero Beach FL

Alterations in gene expression in the midgut of female Culex quinquefasciatus exposed to West Nile virus were investigated. Fluorescent Differential Display was used to analyze gene expression differences in Cx. quinquefasciatus midgut tissue before and after exposure to a known titer of WNV. Results from the display revealed 26 cDNA bands exhibiting reproducible differences after feeding on infected blood, of these 21 showed an increase in expression and 5 showed a decrease in expression as a result of WNV presence. Eleven cDNA bands exhibiting increased expression following infection with WNV were cloned. Several of these were novel transcripts and did not match any sequences in the Genbank. VectorBase, Pfam and BLAST database searches revealed one clone, CQ G12A2, shares identity (85%) with a leucine-rich repeat-containing protein from Cx. quinquefasciatus and shares 42% identity to Toll-like receptors from Aedes eagypti. Leucine-rich repeats are sequence motifs present in many proteins that participate in protein-protein interactions. Here we present the first cDNA clone isolated from female Cx. quinquefasciatus midgut tissue whose protein product may interact with WNV and play a role in disease transmission.

(42) Age And Race-Specific Rates Of Initial Hpv Vaccine Initiation Among Females Aged 9 – 20 Enrolled In Florida Medicaid

Robert L. Cook, Jianyhi Zhang, Heather G. Steingraber, Teresa L Kauf, Babette A Brumback, Tina A Arcomone, Chris Mallison

Department of Epidemiology and Biostatistics, Department of Pharmaceutical Outcomes and Policy, and the Florida Center for Medicaid and the Uninsured, University of Florida, and the Florida Agency for Health Care Administration

Background: The Gardasil HPV vaccine was approved by the FDA in June, 2006, and recommended by the Advisory Committee for Immunization Practices in March, 2007. Specific recommendations are to vaccinate girls aged 11-12, with catch-up vaccination for women up to age 26. The objectives of this study were to determine age- and race-specific rates of uptake of the HPV vaccine during the initial year after vaccine approval. *Methods*: Using administrative Medicaid data for the period 7/1/06 –8/31/07, we identified all enrolled females aged 9 – 20 (n=416,829). Overall and group-specific rates of first HPV vaccine administration were calculated by dividing the number of females who received their first HPV vaccine by the number of enrolled women within each time interval. Rates were determined for consecutive 2-month intervals and adjusted to rates per 1000 woman-years. The work was supported in part by a research grant from Merck. *Results*: 9612 women received their first HPV vaccination through Florida Medicaid during the follow-up period. Vaccination rates increased over time and were still increasing at censorship. During the final 2-month interval (July-August, 2007), vaccination rates were highest in girls aged 11-12 (175 vaccinations per 1000 woman-years), followed by ages 13-15 (151 vaccinations), ages 16-18 (73 vaccinations), 9-10 (37 vaccinations), and were lowest in women aged 19-20 (3.1 vaccinations per 1000 woman years). When examined by race/ethnicity, vaccination

rates at each time point were lowest among black women compared to white and Hispanic women. *Conclusion*: Vaccination rates increased initially after the ACIP recommendation, mostly among females aged 11 – 15. Few women aged 19 – 20 had received the vaccine, despite recommendations and access to vaccine coverage. Strategies to improve HPV vaccination rates to young women seen in internal medical practice may be needed. These initial data also suggest the possibility of racial disparities in HPV vaccine uptake. Future analyses will examine trends over the subsequent year and examine individual, provider, and system level variables associated with HPV vaccine uptake.

(43) Microbiological Surveillance of Animal Source Methicillin-Resistant Staphylococcus aureus in species of veterinary interest in Central Florida

Shannon Roff, Katherine Maldonado, DVM, Carmen Glotfelty, Maureen T Long, DVM, PhD
University of Florida College of Veterinary Medicine, Department of Infectious Disease and Pathology Gainesville, FL

Although methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of nosocomial and community acquired infections in human populations, little information exists regarding the significance of MRSA in animal populations. Further concerns include transfer of MRSA between animals and humans in close contact. Initial studies performed at the University of Florida determined the prevalence of MRSA within the companion animal population of Florida, through nasal swabs collected from hospitalized horses, dogs, and cats. Six samples were colonized with MRSA and further studies on virulence genes indicated the presence of human community acquired MRSA with two USA 300 isolates that were positive for the Panton-Valentine leucocidin PVL virulence gene and were positive for the Staphylococcus chromosome cassette IVa. Four other MRSA were had variable virulence profiles. Additional culturing on 50 nasal swabs collected from stray dogs and cats, representing animals with minimal veterinary care and minimal human contact. No MRSA was found in any of these samples. In a hospital barn of a large milking herd, no cattle with gram positive mastitis were positive for any MRSA. The prevalence of S. aureus was similar to that observed in the previously analyzed hospitalized dogs, cats, and horses; however MRSA was not detected in the milk any gram positive mastitis samples or animals with limited human contact. Research Support Sources: Emerging Diseases Research and Test Program, University of Florida, Merck Merial Student Scholars, Student Support Sources: IDEXX Laboratories

(44) Cats, Rats, and the Prevalence of Microparasites in an Urban Landscape

Manojit Roy[1], Robert D. Holt[1] and Gregory E. Glass[2]
[1]Department of Zoology, U.F., Gainesville, FL 32611
[2]Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205

Wild city rats carry a large variety of zoonotic pathogens, and raise "spillover" risk to human populations living in urban areas. Domestic and feral cats prey on these rats. Theoretical studies show that such predation can potentially influence pathogen prevalence in host populations. We are conducting both empirical and theoretical studies of this phenomenon. Field data on Baltimore Norway rats (Rattus norvegicus) reveal that both hantavirus prevalence and predation occur predominantly among juvenile rats weighing 100-200g; cats usually avoid large adult rats (>200g), and nestlings stay hidden in underground burrows. We incorporate these features in a simple stage-structured rat population model with three age classes: young, juvenile and adult, which loosely correspond to <100g, 100-200g and >200g by body weight, respectively. The pathogen in our model circulates only among juvenile rats, and is modeled using SEIR (Susceptible-Exposed-Infectious-Recovered) dynamics and frequency-dependent transmission. Cats are modeled as a generalist predator attacking only juvenile rats with a saturating functional response. An interesting outcome of this model is that the mean pathogen prevalence exhibits a hump-shaped relationship with predation pressure for a wider range of predator foraging choices than reported earlier with generic SIR models (Holt and Roy, AmNat. 2007; Roy and Holt, TPB 2008), including when cats selectively attack only infectious rats. Early data from field studies conducted in our Baltimore residential study area (Glass et al. unpubl.) appear to display such hump-shaped pattern between cat numbers and SEOV (Seoul Virus) prevalence in rats. Future extensions to the theoretical work will incorporate crucial details of the empirical system, such as spatial and social structure in the rat population.

(45) Nisin, Rosemary and Ethylenenediaminetetraacetic Acid Affect the Growth of Listeria monocytogenes on Ready- To-Eat Turkey Ham Stored at 4°C for Sixty-Three Days

A. Ruiz[1], S. K. Williams[1], A. Hinton, Jr.[2], G. E. Rodrick[3], N. Djeri[1]
[1]Department of Animal Sciences, P.O. Box 110910, University of Florida, Gainesville, FL 32611-0910, [2]USDA ARS Richard B. Russell Agricultural Research Center, Athens, GA,
[3]Food Science and Human Nutrition Department, University of Florida, Gainesville, FL

The objectives of this study were to determine the anti-Listeria and general antimicrobial properties of nisin, rosemary, and Ethylenenediaminetetraacetic acid (EDTA) alone and in combination on Listeria monocytogenes inoculated on ready-to-eat vacuum packaged diced turkey ham, and to ascertain the effects of the treatments on pH and objective color. The turkey hams were cut into 0.5 cm pieces, inoculated with a L. monocytogenes cocktail containing five strains of the bacterium, and treated with either 1) no treatment and no inoculum (negative control), 2) inoculum only (positive control), 3) 0.5% nisin, 4) 20mM EDTA, 5) 1% rosemary, 6) 0.5% nisin + 20mM EDTA, 7) 0.5% nisin + 1% rosemary, 8) 0.5% nisin + 20mM EDTA + 1% rosemary, or 9)

20mM EDTA + 1% rosemary. All samples were vacuum packaged, stored for 63 days at 4 °C ± 1° and analyzed at one week intervals for total aerobes, L. monocytogenes, lactic acid organisms, pH and objective color. Nisin, nisin with rosemary, nisin with EDTA and nisin with rosemary and EDTA treatments reduced ($P < 0.05$) L. monocytogenes counts by 4.42, 4.20, 3.73, and 4.11 log cfu/g, respectively, when compared to the positive control, on Day 0. L. monocytogenes counts remained less than 2.75 log cfu/g for ham treated with nisin through 63 days storage. The EDTA and rosemary treatments alone and in combination were ineffective in inhibiting growth of L. monocytogenes. Although none of the treatments completely eliminated L. monocytogenes, the results indicated that ready-to-eat turkey ham will have significantly lower L. monocytogenes counts when treated with nisin alone or in combination with rosemary and/or EDTA.

(46) In-vitro pharmacodynamics of TR-700, the active moiety of prodrug TR-701 a novel oxazolidinone, in a kill-curve model

M. Sahre[1], S. Schmidt[1], A. Barbour[1], K.H. Rand, MD[2], H. Derendorf, PhD[1]
[1]Department of Pharmaceutics, University of Florida, Gainesville, Florida, USA
[2]Department of Pathology, University of Florida, Gainesville, Florida, USA

Background: The aim of this study was to determine the effectiveness of TR-700, the active form of its prodrug TR-701, a novel oxazolidinone, against three strains of methicillin-resistant Staphylococcus aureus (MRSA), that are also resistant to linezolid. *Methods:* Three strains of S. aureus (CM/05, ATCC 33591, NRS 271) were studied in this experiment. The minimum inhibitory concentrations (MIC) for TR-700 were determined according to the microbroth dilution method as standardized by CLSI to be 0.5, 0.25 and 4 µg/mL respectively. Cultures of each strain were grown in Mueller-Hinton broth (MHB) at static concentrations of 0, 0.25, 0.5, 1, 2, 4, 8, 16 times their MIC. Serial dilutions of samples taken every 2 hours were made, plated on sheep blood agar (SBA) and allowed to incubate for 20 hours at 37° C. A modified Emax model was fit to the kill curve data and allowed to estimate EC50 values for each strain. For S. aureus NRS 271 an additional Hill factor of 1.4 was included. *Results:* TR-700 showed bacteriostatic activity against all three strains with maximum reductions of 0.18 log, 0.28 log, and 1.73 log for S. aureus CM/05, S. aureus ATCC 33591 and S. aureus NRS 271 respectively. The estimated EC50 values for TR-700 were found to be 0.14, 0.07 and 1.32 µg/mL respectively. *Conclusion:* The estimated EC50 values correlate well with previously determined MIC values (r2=0.99). The described PK/PD model will allow integration of these pharmacodynamic properties of TR-700 with its pharmacokinetic profile to facilitate dose selection for future clinical trials.

(47) Exotic Perkinsus sp. protozoa in an imported Vietnamese ornamental clam, Tridacna crocea, maintained in a home aquarium

Barbara J. Sheppard. D.V.M., Ph.D., Dipl. A.C.V.P.[1] and Christopher S. Dungan, M.S.[2]
[1]Department of Infectious Diseases and Pathology PO 110880, College of Veterinary Medicine, U.F., Gainesville, FL 32611
[2]Maryland Department of Natural Resources, Cooperative Oxford Laboratory, 904 S. Morris Street, Oxford, MD 21654

An adult, hermaphroditic Tridacna crocea ornamental clam imported from Vietnam into the U.S.A. became terminally moribund with sloughed byssal tissue and incomplete extension of the poorly responsive mantle, and was necropsied. Necropsy findings included emaciation, visceral mass edema, and rare multifocal, 1-mm diameter, off-white to light tan gill nodules. Histopathology revealed marked inflammation and necrosis within the visceral mass and gills, with interstitial edema and atrophy of glandular, gonadal, and muscular tissues. Inflamed tissues contained large numbers of 10-15 µm extracellular, spherical organisms with signet-ring morphology consistent with Perkinsus spp. trophozoites. The organisms often formed clusters of 2-4 cells, and were surrounded by a host reaction consisting of a 1-4 µm rim of amorphous eosinophilic material and 2-4 host haemocytes. Incubation of infected host tissues in alternative Ray's fluid thioglycollate medium (ARFTM) confirmed the presence of Perkinsus sp. hypnospores that stained blue-black with Lugol's iodine. Perkinsus sp. organisms, including P. olseni and P. marinus which are internationally reportable, are highly pathogenic destructive protozoa capable of disrupting ecosystems populated by naïve molluscs within the U.S.A., and negatively impacting both domestic and international shellfish industries. This is the first report of an exotic Perkinsus sp. pathogen in an imported ornamental clam maintained long term in a home aquarium. However, ongoing research indicates that T. crocea from Vietnam are commonly infected with such organisms. Veterinarians, aquarium facility mangers, and veterinary clients with hobby aquariums should use appropriate caution and responsible disposal practices for clam carcasses, and water in which imported ornamental clams have been housed. Such practices will reduce the possibility of dispersing viable, exotic Perkinsus sp. organisms into domestic waters.

(48) Rabies in Florida Bats

Danielle Stanek, Lillian Orciari , Valerie Mock, Pamela Yager
Department of Health, State of Florida, Center for Disease Control, Atlanta, GA

Background: Bats currently represent 10% of animals testing positive for rabies in Florida, and the majority of endemic human rabies cases in the US are caused by bat variants. However in 2006, less than half (35%) of rabid bats reported nationally were identified by species, and we have limited knowledge of bat rabies epizootiology. Improved knowledge of current bat rabies epizootiology may help identify risk factors leading to exposures in people and domestic pets, and improved disease control. *Methods:* Data from 248 rabid bats collected by Florida Department of Health from 1953-1973 were compared to data from 195 rabid bats of 1,742 total bats submitted from 1994-2006. A subsample of recent submissions from representative species was typed by the

Poxvirus and Rabies Branch of Centers for Disease Control and Prevention (CDC) using RT-PCR amplicons from the rabies virus N gene and compared with archival Florida bat samples from the 1980's. Alignment of the sequences was performed using Bioedit 7.0. A phylogenetic tree was produced using the Mega 4.0 software and the neighbor-joining algorithm and bootstrap analysis of 100 replicates. *Results:* In the twenty year period between 1953-1973, L. intermedius (Li) represented 75.8% of bats identified as rabid in Florida, compared with 11.9% of speciated cases from 1994-2006. Brazilian free-tailed bats (Tadarida brasiliensis) represented 5.2 % of historical cases, and 51.3% of current speciated cases. The number of positive bats peaked during August for both time periods. The lowest proportion of positive bats for 1994-2006 was in June. Four distinct clades containing L. borealis (Lb) rabies virus variant were identified which included most samples from L. seminolus (Ls), Li and Lb bats. Virus variants from Lb and Ls bats appear more related to L. cinereus (Lc) bats then variants from other Lc bats. Two clades of Tb rabies virus variants primarily included Tb samples and clustered tightly together. Only archival Tb samples were found in Tb clade 2.

(49) Vibrio Vulnificus Clade Is Associated With Systemic, As Opposed To Local, Infection in a Mouse Model of Disease

Thiaville, P.C. [1], K.L. Bourdage[1], M. Evans[2], A.C. Wright[2], V.J. Harwood[3], and P.A. Gulig[1]*
[1]Department of Molecular Genetics and Microbiology
[2]Department of Food Science and Human Nutrition, University of Florida
[3]Department of Biology, University of South Florida

Vibrio vulnificus is the leading cause of reported seafood-related death in the U.S. with death from sepsis. V. vulnificus also causes wound infection that often, but not always, leads to sepsis. For as many people who consume contaminated shellfish, only a small fraction experience systemic disease. A major question is why some people experience severe skin infection without sepsis and who the incidence of systemic disease is so low after consumption of contaminated seafood. A variety of genetic methods have been used to genotypically characterize V. vulnificus strains. A clear pattern has emerged – there are two clades of V. vulnificus, one primarily represented among strains of clinical derivation and another that is most often isolated from environmental strains. This relationship suggested that the clinical clade is more virulent for humans and is the environmental clade. We therefore examined a large collection of V. vulnificus strains that had been genotyped for virulence in our subcutaneously inoculated iron dextran-treated mouse model. In this model, nearly all strains, regardless of origin or genotype have the ability to cause a high level of localized skin infection. However, not all strains have the ability to cause lethal systemic infection measured as liver CFU. We determined that the clinical-type clade of V. vulnificus is significantly more proficient at causing systemic liver infection than is the environmental-type clade. This was often observed by significant differences in CFU/g tissue; however, the greatest statistical differences were when the frequencies of detectable systemic infection were compared. These results demonstrate that the two major clades of V. vulnificus differ not only in their sources of isolation but in their ability to cause systemic lethal disease. Identifying the genetic differences between the clinical- and environmental-type clades will lead to the understanding of the virulence mechanisms that are responsible for lethal, systemic infection of humans.

(50) Live Attenuated Influenza Vaccine Decreased Flu Related Absenteeism One Year after Administration in a School Based Immunization Program in Alachua County, Florida

Cuc Thi-Hong Tran MPHc[1], Krystin Engelhardt MPHc[1], Parker A. Small Jr. MD[2], Emily Wilson MPH[3], Thomas Belcuore M.S[3], and J. Glenn Morris Jr. MD , MPH &TM[1,4]
[1]University of Florida's College of Public Health and Health Professions
[2]University of Florida's College of Medicine
[3]Alachua County Health Department
[4]Emerging Pathogens Institute, Gainesville, FL

Background: Schools are virus exchange system ultimately spreading the virus to the whole community. Children are "super spreaders". They shed more virus and do so for longer periods of time than adults. Computer models have suggested that immunizing school children could protect the whole community. This study assessed if there was a decrease in flu related school absenteeism one year after Live Attenuated Influenza Vaccine (LAIV) was administered at a time when the inactivated vaccine was shown to be only 44% effective. 3 *Methods:* 25% (n=5,198) of Alachua County K-8 school children were immunized with LAIV during the fall of 2006. A year later, flu related absenteeism was evaluated for a thirteen week period (January 7th - April 13th,2008). This time frame was determined by the health department as the height of a flu outbreak. Children in grades kindergarten and 10-12 acted as controls for this time period, having not received the vaccine. *Results:* Absenteeism of students in grades 1-9 (76.4%), 25% of whom had been immunized, was lower than the control (78%), who had not been immunized (P=<.0001). *Conclusions:* Live Attenuated Influenza Vaccine immunization decreased school absenteeism during a flu outbreak one year after vaccine administration, even though the virus had drifted (the vaccine strains do not match the circulating strains). Given the apparent broader protection provided by LAIV, immunizing school children *might* reduce the severity of the next flu pandemic.

(51) Bacteriophage Based Prevention of Foodborne Disease is Effective in a Mouse Model and Prevents Weight Loss Seen in Infected Animals Treated with Placebo or Antibiotic

Lee Visone[1], M. Ukhanova[1], T. Abuladze[2], A. Sulakvelidze[1,2], V. Mai[1]*
[1]Univ. of Florida, Gainesville FL, [2]Intralytix, Baltimore, MD

The past decade has seen an increasing emergence of pathogenic bacteria resistant to many currently available antimicrobial agents. An approach for beneficial use of microbes which has received very little research attention is the use of bacteriophages to target potentially pathogenic bacterial species in the GI tract. Prior to the discovery and widespread use of antibiotics, it had been suggested that bacterial infections could be treated by administration of bacteriophages. We examined the effect of pre- and post-exposure administration of a L. monocytogenes-specific phage preparation, designated LMP-102, in mice experimentally-infected with the L.m. strain 370 (a nalidixic acid-resistant mutant of ATCC strain 49594, which is derived from the Scott A strain). C57B6 mice were pretreated daily for 3 days with either PBS or LMP-102 (via oral gavage), challenged with L. monocytogenes strain Lm 370 (ca. 105 CFU/mouse) and sacrificed after three days during which either treatment with PBS or LMP-102 was continued or an antibiotic was administered. Listeria counts in tissue homogenates were determined using selective media while overall gut microbiota composition was analyzed by 16S rDNA based profiling. The concentrations of L. monocytogenes in the (i) small intestine, (ii) cecum, (iii) colon (fecal material collected from the large intestines), (iv) liver, and (v) spleen were significantly reduced ($p = <0.05$) in both the phage and the antibiotic treated groups. In addition, the weight loss observed in the PBS treated group as well as in the antibiotic treated group was prevented by phage administration. Bacteriophage treatment had no detrimental effects on clinical health markers or on gut microbiota composition. These results suggest a potential for novel bacteriophage based prevention of listeriosis and possibly other foodborne diseases.

(52) Reduced Pathogenesis by High Levels of Drug-resistant HIV Replication in vivo

Wallet MA, Saporta S, Sleasman JW and Goodenow MM
Department of Pathology, Immunology and Laboratory Medicine

Background. HIV infection results in immune activation which may contribute to disease progression. The causes of T cell activation [TCA] versus monocyte/macrophage activation [MMA] were investigated.
Methods. A longitudinal study including plasma and peripheral blood cells at baseline and after 18-36 weeks combination antiretroviral therapy [ART] from 40 HIV+ perinatally-infected adolescents compared with 21 healthy controls [HC] was performed. Therapy resulted in immune reconstitution in all HIV+ subjects, but almost half (19 of 40) developed discordant therapy outcome, characterized by viral rebound to pretreatment levels [viral failure/immune success (VF/IS)]. Immune activation was determined by ELISA for soluble plasma factors sCD27, sCD14 and TNF and by FACS analysis of activated T cell subsets CD4+CD45RO+ and CD8+CD45RA+CD27-CD11a++. Microbial translocation [MT] was measured by LAL assay for LPS and EndoCAb. Statistical analysis was performed in SAS. *Results.* TCA but not MMA was related to CD4+ T cell decline and TCA was elevated in HIV non-controllers (VL ≥ 104). MT was associated with MMA but not CD4+ T cell activation or frequency. The occurrence of MMA in HIV+ subjects was associated with ethnicity, where African American subjects were far less likely to develop the outcome. Longitudinal analysis of HIV+ subjects before and after ART revealed that TCA resolves after 18-36 weeks of ART, while both MT and MMA persist. In a VF/IS subset, TCA was reduced and CD4+ T cell frequency was improved despite persistent viremia, while MT and MMA were unchanged. *Conclusions.* T cell activation and monocyte/macrophage activation are parallel, unrelated outcomes of HIV infection. TCA results from uncontrolled viremia and leads to CD4+ T cell decline. MMA results from MT and genetic predisposition to the outcome. Reduced viral pathogenesis following ART in VF/IS subjects is sufficient to reduce TCA and allow immune reconstitution despite persistent viremia, MT and MMA.

(53) Utilizing Mechanism-Based Pharmacokinetic/Pharmacodynamic Models to Understand and Prevent Antimicrobial Resistance

B. Wu and H. Derendorf
Department of Pharmaceutics, University of Florida, Gainesville, FL

Background: Despite decades of antimicrobial usage, the underline relationship between antimicrobial drugs and the development of drug resistant has not fully been delineated. Current observations show that increase usage of antibiotics leads to increase of drug resistance, indicating less than ideal dosage were recommended for suppressing the resistant microbial population. Several mechanisms of resistance have been published in the literature. We have developed new mechanism-based pharmacokinetic/pharmacdynamic (PK/PD) models mimicking various resistance mechanisms to describe in vitro drug-bacteria kill curve relationship. *Methods:* In vitro kill-curve of Escherichia coli 204 (E. Coli II) following ciprofloxacin treatment at 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 125 times the minimum inhibitory concentration (MIC) totaling 193 data points were obtained from a previously published data. The in vitro system simulated the clinical half-life of ciprofloxacin (4 hours) by constant dilution of media with a flow rate of 7 mL/hr in a 40 mL unit. The monoexponential decline rate was fixed for modeling PD. The resistant mechanisms implemented in the mathematical models include (1) susceptible-dormant conversion, (2) susceptibleresistant conversion , and (3) dual effects of dormant and resistant mechanisms. All parameter estimates for each model were computed using all dose levels fitted simultaneously using Adapt II software. Model selection criteria were based on AIC values, residual plots, and visual evaluation of observe versus predicted plots. *Results/Conclusions:* The E. Coli kill curve after ciprofloxacin treatment was best described by a heterogeneous bacterial population that include a dose dependent inter-conversion between susceptible and dormant population as well as an emergent of resistant population with its own growth fitness and kill rate (Model 3). The PKPD models infer that mechanisms of antimicrobial resistance are complex and require the consideration of multiple bacterial defense mechanisms simultaneously to describe the drug exposure-bacterial killing relationship.

(54) FIV-cat model for selecting viral proteins for HIV-1 and FIV vaccines

Janet Yamamoto, James Coleman, Ruiyu Pu, Jay Levy.
University of Florida, Gainesville, Florida USA
University of California, San Francisco, California

Background: Vaccines for HIV-1 and FIV must provide protection against viruses of multiple heterologous subtypes. Prototype and commercial inactivated dual-subtype FIV vaccines (FIV-IDV of subtypes A and D) confer protection against homologous- and heterologous-subtype strains. In a recent report, HIV-1 recombinant CA-p24 vaccine conferred cross-protection against FIV in cats. Current studies were performed to confirm and expand on the reported work, identify other cross-protective HIV-1 proteins, and to explore the immune mechanism(s) of cross-protection. *Methods:* Pathogen-free cats were immunized 3X with FIV-IDV, HIV-1 or FIV recombinant protein vaccines, adjuvant (Ribi or FD-1 plus LPS/IL-12), or PBS and were challenged 3 wk later with FIV. Vaccine proteins were FIV p24, FIV RT-p62, FIV MA-p15, HIV-1 p24, and HIV-1 RT-p66. Infection was determined by immunoblot analysis for FIV antibodies and by virus isolation from PBMC and lymphoid tissues using viral RT and PCR analyses. Protective mechanisms were characterized by passive-transfer studies and by virus-specific IFNγ ELISpot or CFSE proliferation of the T cells. *Results:* Protection rates were 40/64 (62%, $p<0.001$ compared to controls) for HIV p24, 20/21 (95%, $p<0.001$) for IDV, 4/25 for FIV p24, 0/3 for FIV p15, 0/22 for adjuvant control, and 0/37 for PBS control against 15 CID50 of either subtype-A, recombinant subtype-A/B, or subtype-B FIV. At challenge doses of 25-100 CID50, protection rates were 2/8 for HIV-p24, 2/8 for HIV-p24+p66, 3/7 for HIV-p66, 1/8 for FIV-p24, 0/3 for FIV-p15, 2/7 for FIV-p24+p62, 5/7 (71%) for FIV p62, 2/4 for HIV-p24+FIV-p62, 0/4 for adjuvant, and 0/4 for PBS. Strong IFNγ and CD4/CD8-CFSE responses to HIV or FIV protein peptides were observed in corresponding vaccinated cats. HIV p24-vaccinated cats recognized many HIV p24 peptide pools, which were also recognized by HIV-infected human long-term survivors. Neither HIV-p24 nor FIV-p24 vaccine-induced antibodies conferred passive-transfer protection. In contrast, IDV-induced antibodies conferred passive protection against homologous FIV but not against heterologous-subtype-B FIV. *Conclusions:* Based on the FIV-cat model, the most effective vaccine is FIV-IDV followed in order by FIV-p62, HIV-p66, HIV-p24, FIV-24, and FIV-p15. Thus, this cross-protection model may be useful in selecting HIV proteins and epitopes important to include in a broadly-effective T cell-based HIV-1 vaccine. This work was funded by NIH R01-AI065276.

(55) Porphyromonas gingivalis and human oral epithelium interaction

Chao Liu, Caroline Jermanus, Ralee Spooner, and Özlem Yilmaz
Departments of Periodontology and Oral Biology, U.F., Gainesville, FL 32610

The microbiota of the human oral mucosa consists of a myriad of bacterial species that normally exist in commensal harmony with the host. Porphyromonas gingivalis, an aetiological agent in severe forms of periodontitis (a chronic inflammatory disease), is a prominent component of the oral microbiome and a successful colonizer of the oral epithelium. This Gram-negative anaerobe can also exist within the host epithelium without the existence of overt disease. Gingival epithelial cells, the outer lining of the gingival mucosa, which function as an important part of the innate immune system, are among the first host cells colonized by P. gingivalis. This study highlights the recent work and developments implicating the co-existence and intracellular adaptation of
P. gingivalis in these target host cells. Specifically, recent findings on the putative mechanisms of persistence, intercellular dissemination and opportunism are highlighted.
These new findings may also represent an original and valuable model for mechanistic characterization of other successful host-adapted, self-limiting, persistent intracellular bacteria in human epithelial tissues.

(56) HIV-1 Env V1-V2 Length Polymorphism Predicts Combination Antiretroviral Therapy Outcome in HIV-Infected Children

Li Yin[*1], *Steven McCready*[1], *Amanda Lowe*[1], *Joseph Oshier*[1], *John W. Sleasman*[2] *and Maureen M. Goodenow*[1]
[1]College of Medicine, University of Florida, Gainesville, FL, USA
[2]All Children's Hospital, College of Medicine, University of South Florida, St. Petersburg, FL, USA

Although HIV-1 env polymorphism correlates with disease progression, a relationship to combination antiretroviral therapy (ART) is unknown. HIV-1 env V1V2 length quasispecies were evaluated by spectratyping. Correlation of V1V2 length and extent of length polymorphisms with clinical status was determined via Spearman Rank Order Correlation test (SROCt) for 14 untreated children. Association of pretherapy viral complexity with therapy outcome was assessed using Mann-Whitney S test (MWSt) by comparing V1V2 length diversity in pretherapy viruses between children (n=5) who achieved viral suppression and those (n = 9) who failed viral control after ART. Change in V1V2 length distribution was followed one year posttherapy. V3 genotype between viral successes and failures was compared by MWSt, and correlated to V1/V2 lengths by SROCt. Number of HIV-1 env V1V2 length variants in pretherapy viruses ranged from 1 to 16 among individuals. V1V2 length extended from 273 to 360 bp among all variants across subjects, but clustered in a related length range within each individual. Although pretherapy V1V2 length or length polymorphisms were unrelated to pretherapy CD4% or viral levels, a significant relationship between V1V2 length diversity and therapy outcome was identified ($p = 0.02$). Children with more diversified pretherapy V1V2 length quasispecies developed complete viral suppression, while subjects harboring viruses with uniform or limited number of V1V2 length variants failed to control viral replication posttherapy. Net V3 charge was independent of length in V1, V2 or V1V2, but related to CCR5 coreceptor use in viral success or CXCR4 use in viral failure ($p = <0.001$). Distribution and diversity of V1V2 length was stable in all viral successors and 6/9 viral failures over one year posttherapy. Genomic diversity in pre-therapy env, coupled with CCR5 coreceptor use, correlates with favorable response to ART, and can serve as a novel surrogate marker for therapy outcome.

UF | UNIVERSITY of FLORIDA

The Foundation for The Gator Nation