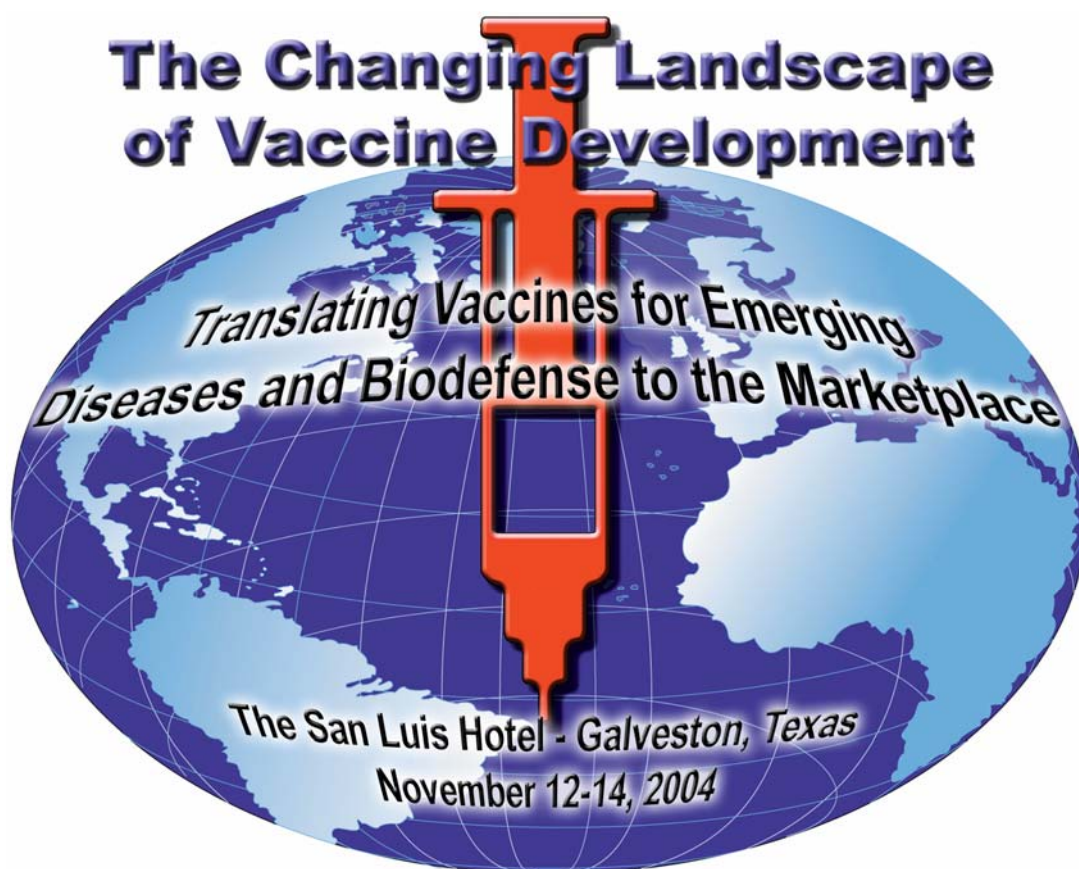


POSTER ABSTRACTS



Posters will be on display for the duration of the symposium.
They are located in the Elissa Room on the 2nd floor of the San Luis Hotel.

GENDER-SPECIFIC PREDICTORS OF GENITAL HERPES VACCINE ACCEPTANCE IN A COLLEGE POPULATION

B.A. Auslander², S.L. Rosenthal², P.A. Succop², L.M. Mills², L.R. Stanberry² and D.I. Bernstein¹

¹University of Cincinnati College of Medicine
Cincinnati Children's Hospital Medical Center
Cincinnati, Ohio

²Sealy Center for Vaccine Development
University of Texas Medical Branch
Galveston, Texas

Vaccines represent one promising method for reducing the STD epidemic. This study evaluated whether the influences on the decision to accept a genital herpes vaccine differed by gender. Five-hundred eighteen college students completed a questionnaire on sexual history, health beliefs, and acceptance of a potential genital herpes vaccine. Each predictor variable plus a gender interaction term were analyzed in separate logistic regression models. Follow-up analyses were performed by gender for outcomes that displayed significant interactions. Results indicated that a prior history of an STD and increased perception of risk for acquiring genital herpes were significant predictors of vaccine acceptance for men, while younger age and concerns about vaccine safety were significant predictors for women. Endorsement of a vaccine strategy targeting sexually experienced people was an influential factor for both genders but was a much stronger one for women. Results suggest that gender-specific strategies may be crucial to genital herpes vaccine acceptance.

IDENTIFICATION OF VACCINE CANDIDATES BY IN SILICO ANALYSIS OF TRYPANOSOMA CRUZI SEQUENCE DATABASE.

V. Bhatia¹, M. Sinha², B. A. Luxon² and N. Garg^{1, 3}

¹Departments of Microbiology & Immunology

²Department of Human Biological Chemistry & Genetics

³Department of Pathology

University Of Texas Medical Branch

Galveston, Texas

GPI-anchored proteins are abundantly expressed in the infective and intracellular stages of *Trypanosoma cruzi*, and recognized as antigenic targets by both the humoral and cellular arms of the immune system. Previously, we demonstrated the efficacy of genes encoding GPI-anchored proteins in eliciting protective immunity to *T. cruzi* infection, suggesting their utility as vaccine candidates. For the identification of additional vaccine targets we screened the *T. cruzi* expressed sequence tag (EST) and genomic sequence survey (GSS) databases. *In silico* analysis of ~2500 sequences identified 348 (37.6%) EST and 260 (17.4%) GSS sequences encoding novel parasite-specific proteins that exhibited no homology in public databases. Of these, 19 sequences exhibited the characteristics of secreted and/or membrane-associated GPI proteins. Eight of the selected sequences were amplified to obtain TcG1-TcG8 genes, that are expressed in different developmental stages of the parasite and conserved in the genome of a variety of *T. cruzi* strains. Flow cytometric analysis of parasites stained with polyclonal sera against TcG1-TcG6 confirmed surface expression of five of the six antigens. All the antigens elicited parasite-specific antibody response in immunized mice, and were capable of producing lytic antibody against the trypomastigote stage of the parasite, a property known to correlate with the immune control of *T. cruzi*. Taken together, our results validate the applicability of bioinformatics in genome mining, resulting in the identification of *T. cruzi* membrane-associated proteins that are potential vaccine candidates.

**CONTRACT RESEARCH ORGANIZATIONS:
SHEPHERDING VACCINE AND DRUG TESTING TO REGULATORY SUBMISSION**

J. E. Bigger, K. A. Marriott, J. E. Estep
Battelle Memorial Institute
Columbus, Ohio

The need for vaccines for biodefense and emerging diseases has changed the landscape for vaccine/therapeutic development and testing. Contract Research Organizations (CRO) must be able to shepherd a vaccine or therapeutic from its development in the hands of the client through the pre-clinical efficacy and safety testing for subsequent submission to the FDA. Now with vaccines against agents for which there can be no human efficacy test, the two animal rule returns the vaccine or therapeutic back to the client or CRO for efficacy testing in two species. A CRO in the emerging disease/biodefense arena must then provide: 1) high biologic containment to cope with the pathogens or toxins for which the clientele will be developing vaccines; 2) select agent licensure to handle the agents; 3) small and large animal capability within biosafety containment; and 4) GLP compliance with 21CFR58.

The Battelle's Medical Research and Evaluation Facility (MREF)'s Biofacility is one of a limited number of U.S. laboratories capable of studying aerosolized etiological agents in animal models under BSL-3 containment with GLP compliance. The MREF facilities are ISO 9001 certified, accredited by AAALAC, and inspected by and compliant with the FDA, DEA, U.S. Army Safety Team, U.S. Army Medical Research and Materiel Command Office of Animal Care and Use Review, and all other regulatory agencies. The MREF encompasses some 85,000 sq ft of office and lab space to include 13 BSL3 animal rooms and 25 BSL2 animal rooms, but not including a 230- x 96-ft. steel barn with two adjoining pasture areas for housing larger research animals using standard agricultural practices. The staff includes scientists with specialty expertise in microbiology, virology, immunology, immunotoxicology, pharmacology, veterinary medicine, pathology, general chemistry, analytical chemistry, molecular biology, inhalation toxicology, and aerobiology, working on a variety of bacteria, viruses and toxins. By specializing in biodefense therapy testing, Battelle offers the expertise which translates to streamlined protocol design, efficient data collection and analysis, and complete regulatory-submission packages, therefore shortening the overall time needed for medical countermeasure development.

RECOMBINANT PLASMINOGEN ACTIVATOR OF *YERSINIA PESTIS*

S. Chauhan¹, A.T. Zemla² and V. L. Motin¹

¹University of Texas Medical Branch
Galveston, Texas

²Lawrence Livermore National Laboratory
Livermore, California

Y. pestis, the etiological agent of plague, is transmitted mainly by fleas and exhibits remarkably efficient spreading from the peripheral site of the flea bite to the lymph node. This is followed by multiplication and further invasion of the circulation. The major role in this process has been attributed to the plague microbe's outer membrane protein called plasminogen activator (Pla). Pla of *Y. pestis* is a surface-located protease that resembles mammalian plasminogen activators in function and converts plasminogen to plasmin by limited proteolysis. The Pla protein belongs to the omptin family of proteases and has a β -barrel topology with 10 transmembrane β -strands and five surface-exposed loops. In this study, we introduced an internal histidine affinity tag (His-Tag) into the pla gene by site-directed mutagenesis. Four constructs were generated based on predicted 3D-models of Pla derived from the known structures of the proteins of the omptin family. The His-Tag fragment was inserted at one of the following locations: the N-terminus of the Pla shortly behind its signal sequence or surface-exposed loops such as L1, L3, and L5. The mutated genes were expressed in *E. coli*, and all four HPla products were found to be located on the cell surface (same as control native Pla). The whole cells producing each of the recombinant HPla's possessed fibrinolytic activity, as judged by the clearing in the fibrin plates. The HPla constructs were compared with the native Pla in the kinetic measurement of plasminogen activation by using chromogenic plasmin substrate, and in the ability to degrade either purified human Glu-plasminogen or the main inhibitor of plasmin in plasma α_2 -antiplasmin (α_2 AP). The accessibility of the His-Tag was tested with corresponding monoclonal antibody using nitrocellulose-immobilized whole cells expressing HPla products. The internally tagged Pla proteins could be purified by single-step, nickel-affinity chromatography in native or denaturing conditions. In addition, HPla proteins were expressed without the signal sequence followed by the product purification from inclusion bodies. The resulting highly pure, but insoluble and inactive HPla products were refolded to an active and soluble form in the presence of rough LPS of *E. coli* and different detergents. The availability of highly purified and active recombinant Pla will allow us to evaluate the feasibility of including this antigen as a component in a modern vaccine against plague.

EMERGENCE OF ATTENUATED WEST NILE VIRUS VARIANTS IN TEXAS, 2003

T. Davis, D. W Beasley, H. Guzman, M. Siirin, R. B Tesh, and A. D. Barrett

Department of Pathology
University of Texas Medical Branch
Galveston, Texas

In order to better understand how West Nile virus (WNV) has evolved since its emergence in North America, our laboratory has undertaken studies to quantify the genetic and phenotypic variation among WNV isolates collected in various regions of North America during different years. Recent genomic sequencing studies describing genetic variation in WNV since its introduction has revealed the continued genetic divergence of isolates collected in 2003 and 2004 in comparison to isolates collected during the early stages of the WNV epidemic. Several of these genetic variants display a small plaque and temperature sensitive phenotype, and reduced replication in cell culture, when compared to isolates collected in Texas in 2002 and New York in 1999. Mouse model studies also indicate that several of these isolates are attenuated in neuroinvasiveness, but not neurovirulence. In order to map the mutations responsible for the observed phenotypic variation, the complete genomes and deduced amino acid sequences of four of the phenotypically distinct WNV isolates have been determined. Many of these isolates share several unique amino acid substitutions and differ by as many as 41 nucleotides and 6 amino acid substitutions from prototype WN-NY99. Reverse genetic techniques are currently being employed to precisely identify the genetic determinants of the observed phenotypic variation in WNV. Results from these studies will help to better understand the relationship between viral evolution and phenotypic variation in an emerging viral population. These findings will also elucidate possible molecular determinants associated with certain WNV phenotypes, which may have important implications for the development of improved live, attenuated WNV vaccine candidates.

REVERSE GENETICS FOR HANTAAN VIRUS

L. Deflube and R. Flick

Department of Pathology
University of Texas Medical Branch
Galveston, Texas

Hantavirus infections are a major public health concern worldwide. Their widespread geographical distribution and their ability to produce serious, often fatal, human disease underline the need of antiviral and prophylactic measures. A reverse genetics system would offer a unique opportunity to identify potential targets for the development of antiviral strategies and for rational vaccine design. The first successful establishment of a reverse genetics technology for Hantaan (HTN) virus, the prototype of the genus *Hantavirus* (*Bunyaviridae* family) was described recently (Flick et al., 2003). This system consists of a reporter gene in sense or antisense orientation, flanked by the 5' and 3' terminal non-coding regions (NCR) of the HTNV L segment. This chimeric cDNA was inserted between an RNA polymerase I (pol I) promoter and terminator-containing plasmid. Following transfection, the cellular pol I generates an artificial vRNA or cRNA segment. The viral proteins necessary for transcription/replication of this RNA minigenome were provided by either HTNV-superinfection or by co-transfected HTN-L/N expression plasmids. This system demonstrated that the viral L and N proteins mediate transcription and replication of pol I-driven HTN minigenomes and confirmed that the L and N proteins expressed are functional. After further optimization of this minigenome rescue system (time course experiments, different ratios between transfected plasmids, sequence changes in the non-coding regions, different backbones for expression plasmids), we have analyzed HTN minigenomes with additional nucleotides on viral genome segment ends to address the question if the bunyaviral polymerase can recognize internally located promoter sequences. This project led us toward the development of a bi-cistronic system as a tool for using hantaviruses as a vector system for vaccine development.

CD8+ T CELLS CLEAR HERPES SIMPLEX VIRUS TYPE 2 (HSV-2) FROM THE FEMALE GENITAL TRACT BY AN IFN- γ DEPENDENT MECHANISM

M. E. Dobbs¹, J. Strasser², C. Chalk², and G.N. Milligan¹

¹University of Texas Medical Branch
Galveston, Texas

²Children's Hospital Research Foundation
Cincinnati, Ohio

HSV-2 infects the genital mucosa and spreads to the sensory ganglia establishing a latent infection. The virus can reactivate resulting in lesions or asymptomatic shedding. The T-cell mediated mechanisms responsible for clearance of HSV-2 from the female genital tract are not fully understood. We focused on the mechanisms by which CD8+ T cells clear virus in the genital tract. An ovalbumin (OVA) -expressing HSV-2 virus was constructed by insertion of the OVA gene into the thymidine-kinase (tk) locus of HSV-2 strain 333. This virus was cleared from the genital mucosa following the adoptive transfer of OVA-specific OT-1 CD8+ T cells. Clearance of HSV-2 tk-OVA by OT-1 T cells was abrogated by *in vivo* neutralization of IFN- γ . In addition, OT-1 T cells genetically deficient in the production of IFN- γ (OT-1IFN γ -/-) were unable to clear HSV-2 tk-OVA from the genital tract in comparison to wild-type OT-1 CD8+ T cells. Similar to OT-1 controls, the OT-1IFN- γ -/- cells produced TNF- α and not IL-4 and IL-5. These data suggest the failure of OT-1 IFN- γ -/- cells to clear virus was due to the absence of IFN- γ rather than a switch to a non-healing Tc2 phenotype. Preliminary studies suggest that the IFN- γ dependent effect was not due to diminished infiltration of innate immune cells to the infected genital tract. Supported by the James W. McLaughlin Fellowship Fund, the NIH Immunology and Mucosal Defense Training Grant, and NIH research grants AI42815 and AI054444.

MAJOR IMMUNOREACTIVE MUCIN-LIKE PROTEINS OF *EHRlichia* SPP. ARE DIFFERENTIALLY EXPRESSED SURFACE GLYCOPROTEINS

C. K. Doyle¹, K. A. Nethery¹, V.L. Popov¹, and J.W. McBride^{1,2,3}

¹Department of Pathology

²Sealy Center for Vaccine Development, and Center for Biodefense

³Emerging Infectious Diseases

University of Texas Medical Branch

Galveston, Texas

Canine monocytic ehrlichiosis is a globally distributed tick-borne disease caused by the obligate intracellular bacterium *Ehrlichia canis* and is a useful model for understanding immune and pathogenic mechanisms of *Ehrlichia chaffeensis*, the causative agent of human monocytotropic ehrlichiosis. Our laboratory is working to identify important immunoprotective antigens as subunit vaccine candidates for ehrlichial diseases. The gene encoding the major immunoreactive 36 kDa protein of *E. canis* was identified by a genomic library screen. Recombinant protein reacted strongly with immune dog sera, migrated larger than predicted by SDS-PAGE, and tested positive for carbohydrate, demonstrating that the protein was glycosylated. Immunoelectron microscopy determined that the gp36 is expressed on the surface and secreted into the morula space by the infectious dense-cored form of the bacteria. The gene encoding gp36 contains 6 tandem repeats encoding 9 amino acids, the serines and threonines of which are predicted to be sites of glycosylation. A single repeat unit expressed as a fusion protein was sufficient for recognition by immune dog serum. Periodate treatment of the fusion protein to modify carbohydrate structures reduced the antibody binding, demonstrating partial dependence on glycosylation for recognition. Alanine substitutions of the serines and threonines of the 9-mer resulted in an electrophoretic mobility shift, indicative of a loss of post-translational modification at these sites. Acidic residues in the repeat region also affected mobility, a consensus that led to the prediction that these proteins are modified on “Yin-Yang” sites with phosphorylation in addition to glycosylation. The mucin-like protein of *E. ruminantium* was recently described to act as an adhesin for tick cells. We predict that the post-translational modifications contribute to protein immunogenicity as well as adhesin function, making mucin-like proteins attractive candidates for ehrlichial subunit vaccines.

CHARACTERIZATION OF A BOVINE HOMOLOGUE OF GRANULYSIN AND NK-LYSIN WITH BROAD SPECTRUM ANTIMICROBIAL ACTIVITY

J. J. Endsley¹, J. L. Furrer², M. A. Endsley¹, M. A. McIntosh², A. C. Maue²,
W. R. Waters³, D. R. Lee², and D. M. Estes^{1,2}

¹University of Texas Medical Branch
Galveston, Texas

²University of Missouri-Columbia,
Columbia, Missouri

³National Animal Disease Center, USDA, ARS
Ames, Iowa

Granulysin and NK-lysin are cationic proteins found in granules of human and swine cytotoxic lymphocytes capable of disrupting microbial membrane integrity. A murine counterpart to granulysin has not been identified to date, indicating the importance of additional models to fully characterize the role of granulysin-like molecules in the immune response to infectious disease. Two partial nucleotide sequences corresponding to the complete functional domain of granulysin and NK-lysin were amplified from bovine peripheral blood mononuclear cell (PBMC) mRNA. Nucleotide identity is significant to granulysin and NK-lysin and predicted amino acid sequence indicates structural and lytic motifs in the core region of the granulysin-like molecules are likely conserved among species. Following stimulation with phorbol ester and calcium ionophore, expression of the bovine gene was detected in CD3+ T cells, CD4+ T cells, CD8+T cells, and WC1+ $\gamma\delta$ T cells, but was absent in CD21+ cells and CD14+ cells. Synthetic human, bovine, and swine peptide corresponding to the carboxyl terminus of helix 2 through helix 3 region of granulysin displayed potent antimicrobial activity against *Escherichia coli*, *Salmonella enteritidis*, and *Staphylococcus aureus*, and *Mycobacterium bovis* BCG. Human and bovine peptides corresponding to helix 2 displayed antimycobacterial activity against *M. bovis* BCG only. Expression of the bovine gene was detected in laser microscopy dissected lymph node lesions from *M. bovis* infected animals. The identification of a biologically active bovine homologue to granulysin demonstrates the application of the bovine model in characterizing the role of granulysin in the immune response to a variety of infectious agents.

GALVESTON IMMUNIZATION SURVEY

B. P. Granwehr, D. H. Freeman, C. B. Turley, L. R. Stanberry and M. G. Myers

Department of Preventive Medicine and Community Health
Sealy Center for Vaccine Development.
University of Texas Medical Branch
Galveston, Texas

Nationwide, 79.4% of children between the ages of 19 and 35 months are immunized (4:3:1:3:3) compared with 74.8% in Texas and 69.2% in the city of Houston. In Galveston, Texas, immunization rates are as low as 56.7%. Poverty, limited access to healthcare, inconvenience, inaccurate assessment of immunization status by parents, and lack of knowledge of the severity of vaccine-preventable diseases present likely barriers to vaccination. Galveston Island Infant Immunization Week was a public health campaign to improve immunization among children of Galveston Island. The Galveston Immunization Survey consisted of an abbreviated version of the telephone-based National Immunization Survey questionnaire designed to demonstrate improvement in vaccination rates of 19-35 month old children with the public health campaign. Of over 3,000 phone numbers called and over 10,000 phone calls completed, only 15 eligible subjects were queried. Racial/ethnic distribution was 40% Caucasian, 33% Hispanic, 20% African-American, and 6.7% mixed race. Gender distribution was 53.3% female and 46.7% male. UTMB was the only provider in 66.7% of subjects with 20% seen only at a local public health clinic, 6.7% by UTMB and other providers, and 6.7% only by other providers. Out of the 15 individuals interviewed, only 2 out of 15 (13.3%) had complete shot records (both from the local public health clinic). Of interest, all of the individuals without shot records were reportedly up-to-date (86.7%), whereas neither of the two who had records was up-to-date. A telephone-based survey, particularly without sub-sampling of provider records, is not an efficient method to gather accurate, comprehensive real-time information about immunization rates for children in the city of Galveston.

ROLE OF INTERFERON ALPHA IN RESPIRATORY SYNCYTIAL VIRUS INFECTION *IN VIVO*

A. Guerrero-Plata¹, S. Baron², J. Poast², P. Adegboyega³ and R. P. Garofalo^{1,2}

¹Departments of Pediatrics

²Department of Microbiology & Immunology

³Department of Pathology

University of Texas Medical Branch

Galveston, Texas

Respiratory syncytial virus (RSV) has been reported to be a poor inducer of type I interferon (IFN-I) and/or also partially resistant to its antiviral activity. However, the role of IFN-I in RSV infection *in vivo* remains elusive. To address this question, BALB/c mice were inoculated intranasally (i.n.) with increasing doses of the IFN-I inducer, a synthetic double-stranded RNA: polyinosinic-polycytidilic acid-poly-l-lysine and carboxy-methylcellulose (poly-ICLC) or murine recombinant IFN-alpha (rIFN-a) or vehicle control. Twenty-four hours later mice were infected i.n. with 107 PFU of RSV. Lung viral titre, clinical illness, body weight loss and lung inflammation were measured after RSV infection. In a separate set of experiments, animals were infected with RSV and 2 days later, they were inoculated with poly-ICLC. Lung IFN-I production was measured at different time points. Our results show that RSV infection alone induced low levels of IFN-I in the lung, becoming undetectable as early as 3 days post-infection. On the other hand, poly-ICLC inoculation resulted in high and sustained levels of IFN-I in the lung. Nonetheless, at day 5 after infection animals treated with poly-ICLC showed more than 90% reduction in viral titer and those treated with rIFN-a showed a 66% to 91% reduction, compared to untreated controls. Despite the reduction in viral replication, we observed an effect of IFN-I on RSV-induced clinical disease, body weight loss or lung inflammation compared to untreated mice. Interestingly, RSV infection before poly-ICLC treatment strikingly inhibited IFN-I production. Our data indicate the existence of a complex interplay between RSV infection and IFN-I production/activity *in vivo*.

RESPIRATORY SYNCYTIAL VIRUS AND HUMAN METAPNEUMOVIRUS INHIBIT CpG-MEDIATED IFN-alpha PRODUCTION BY HUMAN PLASMACYTOID DENDRITIC CELLS

A. Guerrero-Plata¹, A. Casola^{1,2}, R. Pyles^{1,2}, and R. P. Garofalo^{1,2}

¹Department of Pediatrics

²Sealy Center for Vaccine Development

University of Texas Medical Branch

Galveston, Texas

Plasmacytoid dendritic cells (pDC), a key component of the innate immune system, are a major source of interferon-alpha (IFN- α). However the function of pDC in antiviral and immune response against respiratory viral pathogens has not been fully elucidated. Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are major cause of serious lower respiratory tract infections in infants and in the elderly. Thus, the interaction of RSV and hMPV with pDC and their effect on IFN- α production were investigated in this study. Human pDC were isolated from peripheral blood and infected either with RSV or hMPV. IFN- α was detected by ELISA, and additional cytokines by a Luminex-based Bio-Plex system. Expression of Toll-like receptors (TLRs) was assessed by real time RT-PCR. Our results show that human pDC produced high amounts of IFN-I following exposure to RSV (15,828 pg/ml \pm 4,678) or hMPV (11,521 \pm 6,169 pg/ml) for 24 h. On the other hand, pre-exposure of pDCs to RSV or hMPV significantly inhibited the production of IFN- α (60 \pm 7%) in response to in response to the TLR9 ligand CpG-oligodeoxynucleotides. This inhibitory effect appears to be selective for IFN-I since other CpG-induced cytokines, including IL-6, IL-8, IL-10, GM-CSF, IFN-g, IL-1b, MCP-1 and MIP-1b, were not affected by exposing pDC to the viruses. Reduced expression of TLR7 and TLR9 mRNA was observed after viral or CpG stimulation. These findings strongly suggest that pDC can directly recognize and respond to RSV and hMPV, but also suggest that these viruses can selectively interfere with the innate immune responses mediated by pDC.

RIFT VALLEY FEVER VIRUS NONSTRUCTURAL PROTEIN NSs PROMOTES VIRAL RNA REPLICATION AND TRANSCRIPTION IN A MINIGENOME SYSTEM

T. Ikegami, C. J. Peters, and S. Makino

Department of Microbiology & Immunology
Department of Pathology
University of Texas Medical Branch
Galveston, Texas

Rift Valley fever virus (RVFV), which belongs to the genus *Phlebovirus*, family *Bunyaviridae*, has a tripartite negative-strand genome (S, M and L segments) and is an important mosquito-borne pathogen for domestic animals and humans. We established an RVFV T7 RNA polymerase-driven minigenome system, in which T7 polymerase from an expression plasmid drove expression of RNA transcripts for viral proteins and minigenome RNA transcripts carrying a reporter gene between both termini of the M RNA segment in 293T cells. Like other viruses of the *Bunyaviridae* family, replication and transcription of the RVFV minigenome required expression of viral N and L proteins. Unexpectedly, the coexpression of RVFV nonstructural protein, NSs, with N and L proteins resulted in a significant enhancement of RNA replication and transcription. NSs protein expression also increased the RNA replication of minigenomes that originated from S and L RNA segments. Enhancement of minigenome RNA synthesis by NSs protein occurred in BHK-21 cells, which lack type I interferon genes, indicating that the effect of NSs protein on minigenome RNA replication was unrelated to a putative NSs protein-induced inhibition of type I IFN production. Our finding that RVFV NSs protein augmented minigenome RNA synthesis was a sharp contrast to Bunyamwera virus (genus *Bunyavirus*) NSs protein, which inhibits viral minigenome RNA synthesis, suggesting that RVFV NSs protein and Bunyamwera virus NSs protein have distinctly different biological roles in viral RNA synthesis.

COMPUTATIONAL TOOLS FOR ALLERGENICITY PREDICTION

O. Ivanciuc¹, T. Midoro-Horiuti², C. H. Schein¹,
E. Brooks², R. Goldblum², and W. Braun¹

¹Sealy Center for Structural Biology
Department of Human Biological Chemistry & Genetics
²Child Health Research Center
Department of Pediatrics
University of Texas Medical Branch
Galveston, Texas

As genetically modified proteins are introduced into foods, distinguishing allergens from other proteins becomes a critical issue. Similarities in the sequence and structure of allergens may explain clinically observed cross-reactivities between different biological triggers. Using our server SDAP (Structural Database of Allergenic Proteins; <http://fermi.utmb.edu/SDAP/>, [1-3]), we developed a computational approach to determine the potential cross-reactivity of large sequence databases. This data mining approach is based on the PD sequence similarity score calculated with our amino acids descriptors E1-E5 [4]. This computational approach to predict cross-reactivity was applied to Jun a 1 and Jun a 3 allergens from mountain cedar pollen. In order to predict the major histocompatibility complex (MHC) binding affinity of peptides, we developed quantitative structure-activity relationships (QSAR) based on E1-E5. Accurate predictions of the MHC binding affinity can provide fundamental information for vaccine development and for the treatment of allergy, cancer and autoimmune diseases. Our sequence-based QSAR model can be used for the computer-assisted design of T-cell epitopes and to reduce the number of peptides synthesized and assayed. [1] Ivanciuc, O.; Schein, C. H.; Braun, W. *Nucleic Acids Res.* 2003, 31, 359-362. [2] Ivanciuc, O.; Schein, C. H.; Braun, W. *Bioinformatics* 2002, 18, 1358-1364. [3] Ivanciuc O.; Mathura V.; Midoro-Horiuti T.; Braun W.; Goldblum R. M.; Schein C. H. *J. Agric. Food Chem.* 2003, 51, 4830-4837. [4] Venkatarajan, M. S.; Braun, W. *J. Mol. Model.* 2001, 7, 445-453. Acknowledgements. This work was supported by grants from the John Sealy Memorial Endowment Fund (#2535-01), the FDA (FD-U-002249-1), and Texas Higher Education Coordinating Board ATP (004952-0060-2001).

EVALUATION OF NOVEL *BRUCELLA ABORTUS* AND *BRUCELLA MELITENSIS* DELETION MUTANTS AS POTENTIAL VACCINE CANDIDATES IN THE MOUSE AND GOAT MODELS OF BRUCELLOSIS

M. Kahl¹, P.H. Elzer², S. D. Hagius², D. S. Davis¹, A. Den-Hartigh³, R. Tsois³, and T. A. Ficht¹

¹Texas A&M University
College Station, Texas

²LSU AgCenter

Baton Rouge, Louisiana

³Texas A&M Health Science Center
College Station, Texas

Introduction: Unmarked deletion mutants were created in *Brucella abortus* and *Brucella melitensis* via overlapping extension PCR with subsequent *sacB* counter selection as potential superior vaccine candidates for use in large animals. Mutants were first evaluated via competitive infection in the mouse comparing marked mutants (Km^R) with *Brucella* wild type or unmarked mutants (Km^S). Mice were infected non-competitively with the unmarked mutant, to model the rate at which the unmarked mutant clears from the host in the absence of wild type parental strain. The vaccine potential of these strains was first evaluated in mice, followed by safety trials in the pregnant goat model to eliminate candidates that may colonize the fetus and cause abortion. Select vaccine candidates were then evaluated for efficacy in goats (in progress). **Results:** Several mutants have been tested in the mouse model using both competitive and non-competitive infection models that revealed that *Brucella* mutants clear faster in the presence of wild type perhaps as a result of increased competition for survival. Efficacy trials in mice demonstrate that rapid clearing mutants afford less protection (one log relative to naïve mice) at 12 weeks post-vaccination compared to 5 logs as long as 16 weeks post vaccination with slow-clearing, but non-persistent mutants. **Conclusion:** Highly attenuated mutants demonstrate safety in pregnant animals, but due to rapid clearance may not exhibit a high degree of protective efficacy. One slow-clearing mutant did not cause abortion in pregnant goats, and demonstrated a higher degree of protection against infection when used as a vaccine in mice. Future plans include evaluating this mutant further for important correlates of protective immunity, including IFN-gamma, IL12, IL2, and IL10, and use as an improved vaccine in agricultural animals.

**CHARACTERIZATION OF AN INFECTIOUS CLONE OF THE PROTOTYPE
YELLOW FEVER VIRUS ASIBI STRAIN AND THE ROLE OF THE
3'NCR IN INFECTION AND DISSEMINATION IN *Aedes Aegypti***

K. L. McElroy, K. A. Tsetsarkin, D. L. Vanlandingham, and S. Higgs

Department of Pathology
University of Texas Medical Branch
Galveston, Texas

We have constructed the first full-length infectious clone of the prototype strain of yellow fever virus (YFV), Asibi, a mosquito-borne virus endemic in Africa and South America. This clone (YFVASic) produced infectious virus following *in vitro* transcription of linear cDNA and electroporation of RNA into BHK-21 cells, and titers reached 8.5 log₁₀TCID₅₀/ml. The progeny virus exhibits biological characteristics similar to those of the parental Asibi virus including replication kinetics in cell culture, reactivity to flavivirus and YFV-specific antibodies, and high oral infection and dissemination rates in *Aedes aegypti* mosquitoes, the principle vector of YFV. A chimeric infectious clone was constructed that substitutes the Asibi 3' non-coding region (NCR) with that of the YFV 17D vaccine strain (AS/17D3'NCRic), and virus produced from this clone was evaluated for replication, infection and dissemination rates in mosquitoes. While AS/17D3'NCRic virus replication and oral infectivity for *Aedes aegypti* was attenuated compared to the YFVASic virus (23% vs. 72%), the chimeric virus was able to disseminate in a high percentage of infected mosquitoes (73% vs. 83%). This is to our knowledge the first report of an infectious clone of a wild-type YFV strain and the application of YFV infectious clones to studies of vector-virus interactions.

PREDICTING INTERACTING RESIDUES IN THE VENEZUELAN EQUINE ENCEPHALITIS VIRUS (VEEV) ENVELOPE PROTEINS

S. S. Negi, A. A. Kolokoltsov, C. H. Schein, R. A. Davey and W. Braun

Sealy Center for Structural Biology
Department of Human Biological Chemistry and Genetics
Department of Microbiology & Immunology
University of Texas Medical Branch
Galveston, Texas

Venezuelan equine encephalitis virus (VEEV), an alpha virus in the *Togaviridae* family, causes diseases in both human and animals. We are using a combined computational and experimental approach to identify areas of the envelope proteins that are most crucial for viral infection in mammalian cells and particularly for interaction with an as yet unidentified cell surface receptor for the virus. We used our MPACK suite to prepare a model of the E1 protein using as template the crystal structure of that from Semliki Forest Virus. Our PCPmer program was used to analyze aligned sequences of the envelope proteins from various alphaviruses and determine conserved residues and motifs. The variability of residues as a function of their position within the structure, combined with the results of a screen for residues most likely to interact, was used to determine likely sites of contact between the E1 and E2. This new way to predict interacting surfaces in protein structures developed in our group accurately predicts the amino acid residues at protein interface. To test the predictions for the VEEV-envelope proteins, amino acid substitutions were made in pseudotyped murine leukemia virus (MLV) particles that expressed the VEEV envelope proteins on their surface. An immunoprecipitation assay was developed to determine which mutations specifically interfered with interactions between the two envelope proteins. This approach is a paradigm for studying other important protein-protein interactions.

B-CELL EPITOPES OF AN *EHRlichia CANIS* MAJOR IMMUNOREACTIVE GLYCOPROTEIN (GP200) ARE DEFINED BY CARBOHYDRATE AND PEPTIDE DETERMINANTS

K. A. Nethery¹, C. K. Doyle¹, X. Zhang¹, J. W. McBride^{1,2,3}

¹Department of Pathology

²Sealy Center for Vaccine Development

³Center for Biodefense and Emerging Infectious Diseases

University of Texas Medical Branch

Galveston, Texas

Human monocytotropic ehrlichiosis (HME) is a life-threatening emerging tick-borne disease caused by the obligate intracellular bacterium, *Ehrlichia chaffeensis*. *Ehrlichia canis* is genetically and antigenically closely related to *E. chaffeensis* and causes canine monocytic ehrlichiosis (CME), an important veterinary disease clinically similar to HME that is an appropriate surrogate model to study immunity and vaccine efficacy. There is currently no effective vaccine for human or canine ehrlichioses. Glycoproteins have recently been identified in *Ehrlichia* spp. and include three of only eight major immunoreactive proteins that are under investigation as subunit vaccine candidates. The goals of this study were to map the B-cell epitopes of the largest major immunoreactive *E. canis* glycoprotein (gp200) and to determine the contribution of glycans as epitope determinants. Recombinant polypeptides representing overlapping sections of the *E. canis* gp200 were expressed, and their immunoreactivity with anti-*E. canis* dog serum was determined by Western blot. One major B-cell epitope was identified within the N-terminal region, and two major B-cell epitopes were identified within the C-terminal portion of the *E. canis* gp200. Although convalescent anti-*E. canis* dog serum did not recognize any of thirteen overlapping synthetic 14-mer peptides spanning these immunoreactive regions, respective glycosylated recombinant 14-mer peptides were strongly immunoreactive. Periodate treatment (oxidation of carbohydrate groups) eliminated the immunoreactivity of the N-terminal gp200 epitope with early (28 d.p.i.) immune dog serum. However, immunoreactivity of the two C-terminal epitopes was not affected by periodate treatment, indicating that the glycan determinants of these epitopes may not include the carbohydrate ring structures. These results are the first to demonstrate a contribution of glycans to the immune recognition of a major immunoreactive protein of a pathogenic bacterium.

DEVELOPMENT OF A NOVEL VACCINE DELIVERY SYSTEM FOR BRUCELLOSIS

K. Nielsen, D. Davis, T. A. Ficht, and A. C. Rice-Ficht

Department of Veterinary Pathobiology
Texas A&M University
College Station, Texas

Brucellae spp. are gram-negative intracellular pathogens that are the causative agents of brucellosis in Bovidae and that may be transmitted to the human population. Among the infected animal populations in the US are wildlife populations in the Greater Yellowstone Area. Disease transmission among the elk and bison populations principally occurs through contact with a contaminated aborted fetus. Currently, two cattle vaccines are commercially available for brucellosis, *B. abortus* S19 and *B. abortus* RB51. However, neither vaccine provides better than 25% protection against abortion in elk or 30% protection in bison. Microencapsulation of the current S19 vaccine boosts the immune response by extending release of the organism and increasing host exposure time to the vaccine. Capsules averaging 400µm in diameter were made using alginate coated with either a poly-L-lysine or a mixed poly-L-lysine/vitelline protein B (VpB) coat and loaded with at least 3.5×10^9 live S19 CFU/ml capsules. (VpB) is a non-immunogenic eggshell protein isolated from *Fasciola hepatica* that is highly resistant to proteolytic breakdown in the host. A sample population of Texas native Red Deer (*Cervus elephus*), the model system for elk, was vaccinated with 2 ml of capsules, constituting 7×10^9 CFU per animal. Animals immunized with encapsulated S19 showed significantly higher immune responses over a 7-week time course when compared with those receiving unencapsulated S19. Other attenuated strains of *Brucella* spp. are under study in this delivery system that may serve as a model for vaccine delivery to other species including the human population.

A VIRUS-LIKE PARTICLE SYSTEM FOR BUNYAVIRUSES: A TOOL FOR ANALYSING GENOME PACKAGING

A. K. Överby^{1,2}, R.F. Pettersson², R. Flick¹

¹Department of Pathology
University of Texas Medical Branch
Galveston, Texas

²Ludwig Institute for Cancer Research
Karolinska Institute
Stockholm, Sweden

The packaging of viral RNA segments into progeny viruses is a crucial step within the infection cycle of bunyaviruses. To study cis-acting elements involved in bunyaviral genome packaging, we used our recently developed minigenome rescue system for Uukuniemi (UUK) virus, as a model. We determined the influence of the different non-coding regions (NCR) in packaging, using two different systems; a helper virus-driven and a plasmid-based system generating virus-like particles (VLP). In the helper virus-driven system we determined an increased packaging efficacy for the UUK L minigenomes compare to the S- and M-NCRs-derived constructs, resulting in increased reporter gene activities after several passages. A mutational analysis was performed to further map and characterize the bunyaviral packaging signal. To be able to identify and analyze genome packaging signals from pathogenic bunyaviruses, e.g. Crimean-Congo hemorrhagic fever and Rift Valley fever virus, requiring high-containment environment we developed a complete plasmid-driven minigenome rescue/packaging system. This allows the study of these viruses under BSL2 conditions. For this we transfected the glycoprotein expression plasmids together with minigenome components into suitable cell lines. Subsequently, cells were analyzed for reporter gene expression as an indirect method to characterize VLP production. Reporter gene expression could be detected in VLP-infected cells, which proves that the UUK glycoproteins can form infectious VLP and that the minigenome can be packed into these VLPs. We could detect glycoprotein in the supernatant by immunoblot and the VLP showed striking similarities to UUK virus when we visualized them with electron microscopy.

ROLE OF MONOMER-DIMER DYNAMIC EQUILIBRIUM IN REGULATION OF IL-8-INDUCED NEUTROPHILIA

L. Rajagopalan¹, A. Guerrero-Plata², R. Garofalo^{2,3} and K. Rajarathnam¹

¹Department of Human Biological Chemistry & Genetics and Sealy Center for Structural Biology,

²Department of Pediatrics

³Department of Microbiology & Immunology

University of Texas Medical Branch

Galveston, Texas

The chemokine interleukin-8 (IL-8) functions as a pro-inflammatory agent by recruiting neutrophils from the circulation to areas of tissue injury and/or infection. IL-8 is usually released by cells at the injured site, diffuses through tissue to the vasculature and binds proteoglycans on the endothelial cells lining blood capillaries, then recruits circulating neutrophils by binding to neutrophil chemokine receptors CXCR1 and CXCR2. Although IL-8 is monomeric at low concentrations, it can dimerize at the high local concentrations generated during tissue injury and infection. Initially believed to function as a dimer, IL-8 was shown in 1994 to be fully functional in the monomeric form. To date, the importance of dimerization in IL-8 function has not been investigated. Our studies, using wild-type and a trapped monomeric variant of IL-8 in mice, address the role of monomer-dimer dynamics of IL-8 in spatial and temporal regulation of neutrophil recruitment in vivo. Our results indicate that the IL-8 monomer-dimer equilibrium is essential for optimal neutrophil recruitment. We will discuss possible roles of the monomeric and dimeric forms of IL-8 in different processes such as proteoglycan binding and receptor binding, leading to neutrophil recruitment.

U.S. GIRLS' FIRST TIME USE WITH A MICROBICIDE-LIKE PRODUCT

S. L. Rosenthal, M. B. Short, P. K. Sunder and L. R. Stanberry

University of Texas Medical Branch
Galveston, Texas

Introduction: Adolescents are a particularly high-risk group; 1 in 4 sexually experienced U.S. adolescents will acquire an STD. One option for adolescent girls may be topical microbicides, products in development which adolescent girls would place in their vagina to prevent STD acquisition. There may be specific issues associated with adolescent use. The purpose of the current analysis was to describe adolescent girls' first time use with a microbicide-like product.

Methods: Girls were enrolled in a 6 month study and asked to use a vaginal moisturizer (either a pre-filled applicator with gel - *Replens*, or a suppository - *Lubin*). At 3 months, girls were asked if they had used the product and, if so, if and when they discussed use/study with their partner. Users were asked if partners waited during product insertion and, if so, if partners became impatient. Users were also queried about post-coital cleaning behavior in relation to product use and about frequency of product use with the first-time use partner. **Results:** Currently, 111 girls have completed their 3 month interview. These 111 girls were 23% non-Hispanic White, 34% African American, 41% Hispanic, and 2% other, with a mean age of 17.9 yrs. (14 - 21 yrs.). Of the 111 girls, 91 had sexual intercourse with a boyfriend/sexual partner in the first 3 mos. of the study and of these, 68 had used a product at least once in that time. Users were significantly older than non-users (18.7 vs. 17.7 yrs; Wald $\chi^2=4.9$, $p=0.03$), but did not differ in terms of race/ethnicity. A significantly greater proportion of users than non-users talked with their partners about the product/study (97% vs. 70%; $\chi^2=14.6$, $p<0.001$). With regard to first time use, 59 users (87%) discussed product use with their partner. 14 (24%) discussed the product before use, 7 (12%) after use, and 38 (64%) before and after use. Most users ($n=47$; 69%) reported that they did something to clean up after this sexual episode, but only 18 (38%) reported cleansing differently due to product use. "New" behaviors were similar to others' usual behaviors (e.g. showering, wiping). One additional girl reported not cleaning after sexual intercourse, but douching several days later due to leakage at that time. Fifty-nine users (88%) continued to have sex with their first-time use partner, and of those, 51 (86%) used the product sometimes with that partner and 8 (14%) used it always. **Conclusion:** Adolescent girls appear willing to try and to discuss a topical vaginal product with their partners. Conversations were mostly positive, with girls who talked to partners about use/study being more likely to use the product. Younger girls were less likely to use the product; thus developmentally oriented interventions to support their use may be needed. If microbicides require additional cleansing after use, it may be possible to integrate them into existing post-coital behaviors. However, consistent messages about the negative impact of douching should also be given. Finally, microbicide use may mimic condom use for adolescents, in that use may be intermittent.

USE OF WEST NILE VIRUS REPLICONS IN CELL CULTURE FOR ANTIVIRAL DRUG SCREENING

S. L. Rossi, Q. Zhao, V. O'Donnell and P. W. Mason

University Texas Medical Branch
Galveston, Texas

West Nile virus (WNV) is the etiologic agent of the largest epidemic of viral encephalitis in US history. There is no licensed vaccine for human use to prevent this disease or antiviral treatment to combat infection. Since WNV is a BSL 3 agent, viral assays to identify and develop antiviral compounds are inherently dangerous and cumbersome. Genetically engineered subgenomic, self-replicating RNA molecules (replicons) have been established for hepatitis C virus as useful surrogates for antiviral screening. We have engineered WNV replicons using similar technologies, transfected RNA transcripts into mammalian cells, and demonstrated the establishment of replicon replication. Replicon genomes in transfected cells are incapable of spreading to naïve cells but can be passed onto daughter cells during cell division. These replicons lack the structural protein encoding regions of WNV, but contain a selectable marker gene (Neo; providing resistance to G418) and a reporter gene (GFP or firefly luciferase). We have been able to select replicon-bearing clonally derived cell lines that can be maintained for dozens of passages by the presence of G418 contained in the growth media. To validate the replicon's ability to be used a surrogate for live virus infections, we treated stably transfected cells with established antiviral compounds: ribavirin, mycophenolic acid and 6-azauridine. These known antiviral compounds inhibited reporter gene and antigen levels within 48 hours of treatment without significantly compromising cell viability. These assays have also been scaled down to a 96-well format, demonstrating the feasibility of large-scale, high-throughput screening for antiviral candidates.

PARENTAL ACCEPTANCE OF ADOLESCENT VACCINES WITHIN A SCHOOL BASED HEALTH CENTER

R. E. Rupp, M. B. Short and S. L. Rosenthal

Department of Pediatrics and Sealy Center for Vaccine Development
University of Texas Medical Branch
Galveston, Texas

Introduction: Vaccines for sexually transmitted infections (STIs) are in development and adolescents will be likely recipients. It is unknown whether parents would find SBHCs an acceptable venue for their child to receive an STI vaccine. This study evaluates parental attitudes about a hypothetical STI vaccine for genital herpes (HV) vs. a non-STI meningococcal vaccine (MV) in a SBHC.

Methods: A telephone questionnaire was administered to parents of 14-18 year-olds enrolled in the SBHC. The parents responded to questions regarding their attitudes towards the SBHC (e.g., quality of care, agreement with care), beliefs about the diseases and vaccines (e.g., susceptibility, severity, stigma and shame) and their willingness to have their child vaccinated at the SBHC.

Results: The majority of parents would have their adolescent receive the vaccines in the SBHC (MV-75%, HV-62%). Attitudes and beliefs were analyzed for significance. The final logistic regression model revealed that parental attitudes toward the SBHC were predictive for MV. For the HV vaccine, attitudes toward the SBHC and belief in a universal vaccine strategy were predictive for the HV.

Conclusion: Although the majority would have their teen receive the hypothetical HV (62%), the number is significantly lower than the MV (75%). Important factors in whether parents would have their child vaccinated with the HV in the SBHC included their attitudes toward the care in the SBHC and the belief in universal vaccination of all children. Efforts to enhance STI vaccine uptake in SBHCs should focus on promoting positive perceptions of the SBHC among parents and on establishing universal recommendations

**ROUTE OF INOCULATION INFLUENCES T CELL PRIMING:
AN INTRADERMAL ENVIRONMENT PROMOTES A STRONG PROTECTIVE
TYPE-1 RESPONSE AGAINST MONOCYTOTROPIC *EHRlichia***

H. L. Stevenson, N. Ismail and D. H Walker

Department of Pathology
University of Texas Medical Branch
Galveston, Texas

Human monocytotropic ehrlichiosis (HME) is an emerging infection in humans caused by *Ehrlichia chaffeensis*, an obligately intracellular bacterium, and is the most prevalent life-threatening tick-borne disease in North America. Our objective is to gain a better understanding of the immune responses associated with resistance and susceptibility to ehrlichial infection employing two animal models of mild and severe HME using different routes of inoculation with a highly virulent *Ehrlichia* isolated from *Ixodes ovatus* ticks (IOE). C57BL/6 mice were inoculated intradermally (i.d.) or intraperitoneally (i.p.) with identical high doses of IOE. I.d. inoculation establishes a milder, persistent infection in mice, with all mice surviving past 21 days. In contrast, i.p. inoculation caused an acute, severe and fatal infection with mice succumbing to the disease by day 8 post-infection (p.i.). Analysis of the Th1/Th2 phenotype of the immune response shows IOE i.d. inoculation stimulates a strong, protective cell mediated type-1 response characterized by generation of a substantial number of IOE-specific IFN-gamma producing CD4+ Th1 cells on day 7 p.i., a low level of serum TNF-alpha, and less IL-10 production. In contrast, i.p. infection resulted in a weak CD4+ Th1 response and a late burst of TNF-alpha and IL-10. While i.p. infection with IOE resulted in extensive focal hepatic necrosis and apoptotic cell death on day 7 p.i.; the i.d.-infected mice only developed mild hepatic monocytic and lymphocytic infiltration on days 4 and 7 p.i. that cleared by day 14 p.i. Real-time PCR analysis of different organs showed that lethal i.p. infection is associated with a higher bacterial load at earlier time points compared to the non-lethal i.d. infected mice. On day 7 p.i. the bacterial burden in both groups was comparable and a low level of ehrlichiae was detected in the i.d.-infected mice 21 days p.i. Our data suggests the route of inoculation determines host response and IFN-gamma secreting CD4+ Th1 cells are responsible for protection; this is a significant development in ehrlichial immunology and effective vaccine development.

CO-EXPRESSION OF HN AND F GLYCOPROTEINS OF HUMAN PARAINFLUENZA VIRUS TYPE 3 (hPIV3) IN AN ALPHAVIRUS-BASED REPLICON SYSTEM IS A POTENT VACCINE AGAINST hPIV3 INFECTION WITH UNIQUE FEATURES

N. Vasilakis¹, A. X.Y. Mo¹, S. Bhargava, S. L. Rossi¹,
T. Zamb, S. A. Udem and G. R. Kovacs¹

Viral Vaccine Discovery, Wyeth-Lederle Vaccines
Pearl River, NY

¹ Present addresses: Department of Pathology
University of Texas Medical Branch,
Galveston, Texas (N.V, and S.L.R)
Antigenics,
Lexington, Massachusetts (A.X.Y.M)
Office of Biodefense Research Affairs
National Institute of Allergy and Infectious Diseases
Bethesda, Maryland (G.R.K)

We constructed several Venezuelan Equine Encephalitis Virus (VEEV) replicon particles (VRP) expressing the internal proteins of human parainfluenza virus type 3 (hPIV3), including the nucleoprotein (NP), matrix (M), phosphoprotein (P) and C (VRP-NP, -P, -M and -C), as well as two surface glycoproteins, hemagglutinin-neuroaminidase (HN) (VRP-HN) and fusion (F) (VRP-F) proteins, or co-expressing both HN and F (VRP-HN/F). Their efficacy against hPIV3 infection was evaluated in the Syrian hamster animal model. These studies showed that intranasal or intramuscular administration of replicons encoding the internal proteins, NP, P, M and C provided no protection against hPIV3 disease, whereas replicons co-expressing the surface glycoproteins HN and F protected against hPIV3 replication in both the upper and lower respiratory tracts, and induced significant serum neutralizing titers. Administration of replicons expressing the HN protein alone, were only able to fully protect the LRT, whereas replicons expressing only the F protein failed to protect either the upper or lower respiratory tract. Furthermore, we demonstrated that VRP-HN/F vaccination affords complete protection of the LRT and partial protection of the URT with doses as low as 1×10^4 infectious units (IU). Immunogenicity studies in mice indicated that VRP-HN/F immunization induced a higher percentage of IgA producing cells in the cervical lymph nodes, as compared to VRP-HN cell induction. The enhanced efficacy of VRP-HN/F, versus VRP-HN, may be due to its unique capacity for inducing syncytial formation thus generating larger infected foci that could synthesize additional HN and F proteins, affording prolonged antigen expression, leading to increased immune responses.

ANTIVIRAL STRATEGIES AGAINST VIRAL HEMORRHAGIC FEVER VIRUSES: EXPLORING GENE SILENCING MECHANISMS TO IDENTIFY POTENTIAL ANTIVIRAL TARGETS

P. Walpita¹, A. Groseth², D. Stein³, H. Feldmann², R. Flick¹

¹Department of Pathology
University of Texas Medical Branch
Galveston Texas

²Canadian Science Center for Human and Animal Health, Special Pathogens Program
Winnipeg, Manitoba, Canada

³AVI Biopharma
Cornvallis, Oregon

We have appraised gene silencing as one of several approaches to identify potential targets to develop rational antiviral strategies for a selected group of viruses. In this report we describe a comparison of three small hairpin RNA (shRNA) delivery systems for their ability induce gene silencing evaluated in a plasmid-mediated minigenome rescue system: The shRNAs which are processed into siRNA like molecules *in-vivo* were targeted to three different regions of the Ebola virus Reston (EBOVR). Our results indicate that RNA polymerase I (pol I)-driven shRNA mediated gene silencing was comparable to that induced by conventionally used vectors which are engineered to express shRNAs under pol III control. The shRNAs generated *in-vitro* from a truncated T7 promoter were equally effective in preventing gene expression. As expected, the level of silencing mediated by shRNAs was target dependent.

FUNCTIONAL ANALYSIS OF THE WEST NILE VIRUS NS4B PROTEIN USING SITE-DIRECTED MUTAGENESIS

J. A. Wicker¹, D.W.C. Beasley², M.C. Whiteman² and A.D.T. Barrett¹

¹Department of Pathology

²MD/PhD Combined Degree and Microbiology & Immunology Graduate Programs
University of Texas Medical Branch
Galveston, Texas

West Nile Virus (WNV) is a member of the genus *Flavivirus* in the family *Flaviviridae*. Since 1999, WNV has spread throughout North America and while most cases in humans are asymptomatic or mild, WNV is capable of causing serious disease in the elderly and immunocompromised. The WNV genome is a positive-sense RNA molecule approximately 11kb in length encoding a single polyprotein that is cleaved by a combination of viral and host proteases to produce three structural and seven nonstructural proteins. While the structural proteins have been relatively well characterized, certain small hydrophobic nonstructural proteins such as NS2A, NS4A, and NS4B have not been assigned concrete functions but are thought to associate in a vaguely defined replication complex. NS4B is a small hydrophobic protein approximately 27kd in size that is hypothesized to participate both in viral replication and evasion of host innate immune defenses. To investigate the function of NS4B, recombinant WN viruses with point mutations engineered into distinct regions of the NS4B protein are being used to gain a better understanding of critical residues involved in mediating interactions between NS4B and other viral or host proteins. One mutation has been found to attenuate virulence of WNV in our mouse model.

Supported by the State of Texas Advanced Research Program

DEVELOPING NEW ALLERGEN VACCINE USING PHYLOGENETIC APPROACH

L. Xie, M.D., T. Midoro-Horiuti, M.D., Ph.D., O. Ivanciuc, Ph.D.,
C. Schein, Ph.D., W. Braun, Ph.D. and R. M Goldblum, M.D.

Departments of Pediatrics and Human Biological Chemistry and Genetics
University of Texas Medical Branch
Galveston, Texas

Rationale: The molecular biodiversity of plants may provide a basis for exploring new vaccines, based on hypoallergenic homologues of allergens. Methods: A library of sequences for Jun a 1 homologues was created by sequencing genomic DNA from 140 *Juniperus/Cupressus* species. 80 Pinaceae sequences were mined from GeneBank. Mutational analysis of IgE epitopes was carried out by Alanine substitution in synthetic peptides and tested by immunoassay. The physico-chemical characteristics of amino acid sequences of IgE epitopes were calculated as property distance (PD) values, which can be used for compare the similarity between homologues. Results: The sequences of linear IgE epitopes 2 and 3 of the Jun a 1 homologues from *Juniperus/Cupressus* species are highly conserved in Cupressaceae family. However, sequence variants were found within epitopes 1 and 4 regions. In Pinaceae, variants with high PD values were found at each epitope site. Mutational analysis by Alanine substitution within epitope 4 showed that single amino acid changes resulted in loss or reduction of human IgE binding. Furthermore, there are some Pinaceae sequences in our library have similar amino acid changes in one of those sites. Conclusions: The high PD values for some substitutions in IgE epitopes, combined with mutation analysis, suggest that some Jun a 1 homologues are good candidates for hypoallergenic cedar pollen vaccines. These findings suggest the feasibility of identifying structural variants within the pollen of naturally occurring variants.