Basic Science Research Presentations
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COMPARISON OF GENETICALLY DIVERSE RVFV STRAINS USING A MOUSE MODEL

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Rift Valley fever virus (RVFV) is a vector-borne zoonotic pathogen endemic to Sub-Saharan Africa and the Arabian Peninsula that causes severe disease in livestock and humans. RVFV is a significant threat to US livestock and public health. RVFV has low genetic diversity with a ~3% maximum amino acid sequence divergence. Using an LD_{50} approach in a BALB/c mouse model, we compared the virulence of 2 wildtype strains (128B-15 and SA01), 1 recombinant strain rescued via reverse genetics (rSA51), and the attenuated strain MP-12. Groups of 5 mice were challenged subcutaneously with 10-fold serial dilutions of a given RVFV strain ranging from 1000 plaque forming units (pfu) to 0.1pfu and monitored for survival for 10-12 days. We found that strains 128B-15 and SA01 had equivalent LD_{50} values (LD_{50} =7.8pfu), while the LD_{50} value of rSA51 was slightly higher (LD_{50} =150pfu). All mice challenged with 1000pfu of 128B-15 or SA01 succumbed or were euthanized by day 3, while rSA51 challenged mice succumbed or were euthanized by day 5. All mice challenged with 1000pfu of MP-12 survived for the study duration. Differences in LD_{50} between these wildtype RVFV strains may be attributed to a higher virulence of these viruses in BALB/c mice or to greater diversity of virus subpopulations in field isolates 128B-15 and SA01 compared to rSA51; this requires further investigation. Results from this study will be used to determine the efficacy of RVFV vaccine candidates against diverse RVFV strains and to determine the role of RVF viral glycoproteins on protective immunity.

A novel open-field task for the analysis of episodic-like memory components

Sarah L. Stuebing

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Episodic memory requires the binding of multiple memory elements, including what event occurred, where the event occurred, and when the event occurred. The decline of any part of the episodic memory system results in the memory loss often associated with aging and dementia. This deterioration is also a valuable predictor of preclinical dementia onset, as episodic memory decline precedes a clinical diagnosis of dementia of the Alzheimer's type by over half a decade. As such, understanding episodic memory and its substituents is essential to better targeting future pharmaceutical and behavioral therapies for aging and dementia. Rats provide a unique avenue for studying episodic-like memory, the non-human episodic memory counterpart, as the main pathways of this memory system are analogous in rodents and primates. Though rodent episodic-like memory models have been developed, they have not analyzed the underlying components of episodic memory. This project aimed to develop a novel rodent episodic-like task through which the what, when, and where substituents of episodic memory could be assessed. Results indicated that rats are able to learn the object recognition (what), spatial navigation (where), and timing (when) elements, but do so at different rates. The animals were also able to integrate these episodic memory components to accurately obtain food reward, even when presented with conflicting component information, indicating successful binding and application of the episodic memory components. With the validation of this model, future research will strive to elucidate where within its substituents episodic memory loss begins to better focus aging and dementia therapies.

Porcine alveolar macrophages obtained from genetically edited pigs are resistant to in vitro infection of porcine reproductive and respiratory syndrome virus (PRRSV)

Rachel Bardot

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Porcine Reproductive and Respiratory Syndrome Virus (PRSSV) is one of the most economically important diseases in the global swine industry, costing producers an estimated $660 million annually. PRRSV is genetically diverse and incorporates a number of receptors for entry into permissive cells. The virus has a tropism for cells of the monocyte/macrophage lineage. Cluster of Differentiation 163 (CD163) is considered the primary receptor on porcine alveolar macrophages (PAMs). CRISPR/Cas9 was used to knock out (KO) CD163 in pigs. In previous work we demonstrated that the CD163 KO pigs were resistant to infection with one PRRSV isolate. However, the breadth of resistance to other isolates is unknown. In vitro infection of PAMs allows for testing of multiple isolates and does not require additional live animals. Alveolar macrophages obtained from three week old KO pigs (n = 3) and from normal or wildtype (WT) pigs (n = 2) were challenged with serial dilutions of 12 different PRRSV isolates. All PAMs from the normal pigs showed the presence of virus replication; whereas, all KO pigs were negative for presence of infection. This study further supports the hypothesis that the CD163 KO genotype may provide resistance to all PRRS isolates circulating in the field.

Functional differences of NleB glycosyltransferase orthologs among enteric bacterial pathogens

Kangming Chen

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Many bacterial pathogens use a type III secretion system (T3SS) to inject virulence factors (effectors) into host cells to modulate immune responses. NleB, a conserved T3SS effector, is a glycosyltransferase that modifies several host proteins with N-acetyl-D-glucosamine (GlcNAc) to block antimicrobial responses. Here we compared the activities of *Escherichia coli*, *Citrobacter rodentium*, and *Salmonella enterica* NleB orthologs (named SseK in *S. enterica*) on the pro-inflammatory NF-κB pathway. Activation of this pathway is normally enhanced by the association of the glycolysis enzyme GAPDH with the adaptor protein TRAF2. We found that GAPDH was modified by enterohemorrhagic *E. coli* NleB1, *C. rodentium* NleB, and *S. enterica* SseK1, but not by enteropathogenic *E. coli* NleB1, NleB2, SseK2, or SseK3. EHEC/EPEC NleB1, *C. rodentium* NleB, and SseK1/3 prevented IκBα degradation and suppressed NF-κB p65 nuclear translocation. EHEC NleB1, *C. rodentium* NleB, and SseK1 inhibited TRAF2 polyubiquitination to greater extents as compared with the other NleB orthologs. Our data suggest that NleB orthologs that modify GAPDH with GlcNAc have higher inhibitory activities against the NF-κB pathway, as compared with orthologs that do not.

Attenuation of porcine reproductive and respiratory syndrome virus (PRRSV) by inactivating the ribosomal frameshifting products nsp2TF and nsp2N: Implication for the rational design of vaccines

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The nsp2, nsp2TF and nsp2N share the N-terminus PLP2 domain that was previously identified to possess innate immune suppression function. In this study, two recombinant viruses, KO1 and KO2, were further analyzed. KO1 was generated by partial inactivation of nsp2TF expression, while for KO2, the expression of both nsp2TF and nsp2N were knocked out. A multiplexed digital mRNA profiling array assay was employed to detect the expression of 579 immune genes in MARC-145 cells infected with wild-type (WT) virus, KO1 or KO2 mutants. In comparison to that of WT virus, a total of 81 and 72 differentially expressed immune genes was upregulated in cells infected with KO1 and KO2, respectively. The upregulation of representative genes was validated by qRT-PCR using samples from WT and mutants-infected MARC-145 cells, as well as from lung tissues from WT and mutants-infected pigs. Upregulated immune gene expression was correlated with increased NK cell activity and T-helper cell response. Furthermore, vaccination with KO1 and KO2 mutants enhanced homologous protection against a challenge infection, as was evident from reduced lung lesions and viral loads. Our data strongly implicate PRRSV nsp2TF/nsp2N in viral immune evasion and demonstrate that nsp2TF/nsp2N-deficient viruses are capable of generating protective immune responses. Thus, manipulation of nsp2TF/nsp2N expression could be used in the rational design of improved PRRSV vaccines.

Influence of PB1-F2 on the pathogenicity of H1N1 swine influenza virus in mice and pigs

Jinhwa Lee

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PB1-F2 is encoded by alternative translation within segment 2 of influenza A virus genome. Previous studies have shown that PB1-F2 affects influenza virus pathogenicity in a strain and host dependent manner. However, the contribution of PB1-F2 to the pathogenicity of H1N1 swine influenza viruses (SIVs) remains unknown. In this study, we investigated effects of PB1-F2 on the pathogenicity of a highly virulent H1N1 SIV in mice and pigs. Two mutant viruses were generated in the genetic background of A/swine/Kansas/77778/2007 (KS07) using reverse genetics: one is KS07_K/O in which PB1-F2 is knocked out, and the other one is KS07_N66S which contains a single mutation at PB1-F2 position 66 (N/E) that is correlated with virulence in mice. KS07_K/O showed similar pathogenicity in mice as the KS07, whereas KS07_N66S displayed enhanced virulence when compared to the other two viruses. In pigs, similar levels of virus replication in lungs and nasal shedding were observed in animals infected with each of the three viruses. The pigs infected with the KS07 virus had higher levels of GM-CSF, IFN-γ, IL-6 and IL-8 at 3 and 5 days post infection (dpi) and lower levels of IL-2, IL-10 and IL-12 at 1 dpi than those infected with the KS07_K/O virus. Taken together, our results indicate that PB1-F2 has no effects on virus replication and pathogenicity of the virulent H1N1 SIV in mice and pigs, but seem to modulate innate immune responses in pigs, and single substitution at PB1-F2 position 66 (N/E) plays a critical role in virulence in mice.

Immunoproteomic Characterization of *Streptococcus agalactiae* vaccine candidates

Guangjin Liu

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Streptococcus agalactiae, also referred to as Group B Streptococcus (GBS), causes pneumonia, septicemia and meningitis in humans, mastitis in cows and meningoencephalitis in fish. GBS isolates are divided into nine antigenically and structurally unique capsular polysaccharide (CPS) serotypes. The CPS antigens provide protection against homologous challenge, but are ineffective in against other serotypes. Thus we are developing a universally effective GBS vaccine.

Here we separated the total cellular proteins of a GBS strain, GD201008-001, isolated from cultured tilapia in China, using two-dimensional electronphoresis (2-DE) and characterized immunoreactive proteins using rabbit hyperimmune sera, convalescent guinea pig sera and GBS-infected tilapia sera as primary detection antibodies.

We identified 16 proteins, including 13 novel immunoreactive proteins of GBS. Nine immunoreactive proteins were expressed in *E.coli* BL21 (DE3) and were probed with sera. Four recombinant proteins, the serine-rich repeat glycoprotein 1, the branched-chain alpha-keto acid dehydrogenase (BKD) subunit E2, the 5'-nucleotidase family protein and the ornithine carbamoyltransferase, reacted with all three sera. These proteins are conserved in multiple serotypes and independent of host species, thus are considered to represent universal GBS vaccine candidate antigens.

Assessment of Ornithodoros Tick Parasitism: Lipocalin Proteins as Targets for Detecting Anti-tick Antibodies in Domestic and Feral Swine

Hailey Clemons-Mueller

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Ornithodoros argasid (soft body) ticks are capable of transmitting a variety of infectious agents, including African swine fever virus (ASFV), East African human relapsing fever virus; blue tongue virus; epizootic bovine abortion virus; infectious bovine rhinotracheitis virus, and under some circumstances, West Nile virus. Experimental infections using several native North American *Ornithodoros* spp. have demonstrated the ability to become infected with and transmit ASFV. This project uses salivary lipocalin proteins as targets for detecting anti-tick antibodies for US ticks in both domestic and feral swine. It is predicted that tick feeding will reflect the geographical distribution of tick species that serve as vectors. Lipocalin sequences from *Ornithodoros coriaceus* and *Ornithodoros parkeri* were selected based on the presence of the Biogenic Amine Binding motif ($CDVX_{7-17}$ EL [W/Y] X_{3-30} C).The same motif is present in a South African species, *O. Moubata*. Salivary glands from *Ornithodoros turicata* were sequenced at Lawrence Livermore and compared with the sequences from the other species. Nucleotide sequences for lipocalins were codon optimized, commercially synthesized, then cloned into pCR2.1 and pHUE plasmids. This resulted in a histidine tag addition to be used for affinity purification. Expression was then done in E. coli (BL21(DE3) competent cells). Purification was completed using a modified denaturing CAPS- Sarkosyl protocol with nickel columns. SDS-PAGE results show the proteins at the expected size of 27kDa. Western Blot has been carried out with feral sera from areas potentially inhabited by ticks, yielding positive results. Incorporation into ELISA and Luminex multiplex serological assays is in progress.

Alterations of the fecal microbiome in nursery pigs after co-challenge with porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV)

Rebecca Ober

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Evidence has shown that the gastrointestinal microbiome plays an important role in response to infectious disease. In nursery pigs, porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) co-infections are widespread with a significant impact on the pork industry. To assess the impact of microbiome on co-infection with PCV2 and PRRSV, fecal samples were collected from twenty nursery pigs prior to challenge and at 48 days post-infection. Pigs were selected due to weight gain after co-infection and were separated into "Best" and "Worst" performing groups. Fecal microbiome was assessed utilizing the Lawrence Livermore Microbial Detection Array. Microarray results revealed a shift in two bacterial families after co-infection when comparing these groups. In the worst performing group, there was a significant increase in the proportion of pigs with members of the family Spirochaetaceae between days 0 and 48 post-infection ($p = 0.0007$; Fisher's exact test); in contrast, there was no significant change in the proportion best performing pigs detected during this time ($p = 0.37$; Fisher's exact test). Additionally, best performing pigs had a decrease in the proportion of pigs with members of the family Acidaminococcaceae detected in feces between days 0 and 48 dpi ($p = 0.07$; Fisher's exact test). Whereas, the proportion of worst performing pigs with this family detected did not change post-challenge. This study provides insight on how fecal microbiome shifts in pigs that have high and low weight gain after co-infection and suggests a role of fecal bacteria contributing to disease outcome.

Widespread detection and characterization of Porcine parainfluenza virus 1 in pigs in the United States

Rachel Palinski

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Porcine parainfluenza virus-1 (PPIV-1) was first identified in 2013 in slaughterhouse pigs in Hong Kong, China. The pathogenesis or endemic potential of PPIV-1 has not yet been assessed nor has the virus been formally identified outside of China. Here, metagenomic sequencing was used to assemble two complete genomes of PPIV-1 from nasal swabs collected from swine in Oklahoma and Nebraska. The genomes were 91.18-96.21% identical to the previously published Chinese strains. Phylogenetic analysis of a 1720bp segment of the HN gene from 13 U.S. samples suggests moderate genetic variability between strains with nucleotide identities from 89.5-100%. Comparison of the PPIV1 U.S. and Hong Kong samples yielded an 84.8-96.4% identity. Further molecular analysis by real-time RT-PCR (qRT-PCR) identified 17 positive samples out of 279 (6.1%) lung homogenate, oral fluid, or nasal swab samples from nine states from pigs with acute respiratory disease. Eleven nursery pigs from a naturally infected herd were monitored for virus replication and pathogenesis. No clinical signs of illness were apparent however qRT-PCR detected PPIV-1 in nasal swabs from seven pigs and the lungs of one animal. In situ hybridization identified PPIV1 RNA in the nasal respiratory epithelium and trachea to a lesser extent. Serological analyses using immunoprecipitation coupled to PCR detection and ELISA demonstrated seroconversion and further analysis of 60 swine serum samples resulted in 55.0% and 63.3% seropositivity, respectively. Taken together, the results confirm the widespread presence of PPIV-1 in the United States swine herd.

ASFV p54 epitope mapping using polyclonal swine sera and monoclonal antibodies

Vlad Petrovan

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African swine fever virus (ASFV) is a large enveloped virus belonging to the family *Asfarviridae*. The envelope protein p54 is among the most immunogenic ASFV proteins, and although anti-p54 antibodies are produced after natural infection or vaccination, there is little information on the antigenic epitopes that they recognize. The goal of this study was to identify p54 epitopes using polyclonal swine sera and monoclonal antibodies (mAbs). The p54 protein, from amino acid (aa) 54 to 183, was divided into 3 overlapping fragments of 55 aa each. The nucleotide sequences were commercially synthesized, cloned into pHUE expression vector, and transformed into BL21 (DE3) *E.coli* cells. After expression and purification, the recombinant proteins were used as antigens in indirect ELISA and Western blotting (WB) to test mAbs and polyclonal swine sera. The mAbs were produced against the Georgia/07 strain, while the polyclonal sera originated from pigs immunized with an alphavirus-based replicon particle expressing the p54 whole protein, based on the BA71V strain.

The majority of the polyclonal sera reacted, by WB only, to the p54 region between aa 83 and 143. Among the mAbs tested, 5 reacted by ELISA and WB with the regions between aa 54 and 143. Seven mAbs did not react with any fragment or the whole protein, probably due to four aa differences between p54 from BA71V and Georgia/07. The mAbs and the pig sera recognized overlapping regions. Future studies will be directed at fine mapping of the identified regions.

Discovery of Alternative Conjugates in Fluorescence Microsphere Immunoassay (FMIA): A Useful Tool in the Veterinary Diagnostic Laboratory

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Fluorescence microsphere immunoassay (FMIA) is an immunological tool which can be used for the detection of multiple targets simultaneously with high sensitivity and specificity. This technology has been applied to the diagnosis of numerous infectious diseases in humans and animals. Species-specific biotin-conjugated IgG have been commonly used as a capture antibody for the detection of IgG in traditional FMIA and ELISA. Previous studies in our laboratory have shown that FMIA is a diagnostic tool to detect cattle and sheep IgM and IgG antibodies to several Rift Valley fever virus (RVFV) recombinant proteins, including the major surface glycoprotein, Gn, the nonstructural proteins, NSs and NSm; and the nucleoprotein, N. Conjugates A, G, and L, proteins of microbial origin, and a genetically engineered recombinant form of Protein A and G, called Protein A/G, bind to mammalian immunoglobulins. However, the uses of these proteins as a capture antibody in FMIA are uncommon. One limitation of the current FMIA is that it relies on IgG as a capture antibody that may sometimes produce high backgrounds. The objective of these studies are the development of multiplex FMIA for the detection of IgG antibodies in serum samples from a broader range of animal viruses with low background using antibody binding proteins A, G, A/G, and L. For the production of recombinant antigens, structural or nonstructural proteins of several viruses were expressed in *E. coli.* After successfully conjugation of proteins to Luminex MagPlex® polystyrene, carboxy- lated microsphere beads, the target antigens were assembled into a single multiplex and tested against sera from experimentally infected animals. The results showed that protein A, G, A/G and L are responsive to IgG in sheep, bovine and porcine. Therefore, a FMIA has been developed to detect several newly emerged viruses: RVFV, BVDV, PRRSV, and PCV2 specific IgG antibodies using alternative conjugates A, G, A/G, and L with very low or no background. The use of conjugates A, G, A/G, and L in FMIA would be an alternative powerful strategy for the detection of viral infection in veterinary diagnostic laboratory.

Rift Valley Fever Virus Quasispecies during Infection of Ruminants and Influence of Host Alternation on Population Diversity

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Rift Valley Fever (RVF) is a mosquito-borne, zoonotic, viral disease that primarily affects ruminants and humans. The RVFV (family *Bunyaviridae* and genus *Phlebovirus*) genome is composed of the L, M, and S segments which are negative-sense or ambisense, single-stranded RNA. In general, the high mutation rate of RNA viral replication produces a genetically diverse virus population (quasispecies) that increases the virus' ability to adapt to diverse selective pressures; however, the mosquito-vertebrate alternating host lifecycle might impose a fitness trade-off which restricts the viral genome evolution. To characterize the quasispecies diversity of RVFV, tissue samples were collected from sheep and cattle experimentally infected with RVFV strains Kenya 128B-15 and Saudi Arabia (SA) 01-1322 propagated in mosquito cells and analyzed on the Illumina Miseq platform. The full length viral genome including the untranslated regions was amplified by RT-PCR from inoculum virus and tissue samples and subjected to sequencing and single nucleotide variant (SNV) analysis. In comparison to SA01-1322, Kenya 128B-15 produced higher diversity in the quasispecies population during *in vitro* and *in vivo* replication with higher number of SNVs mainly in the M segment. Kenya 128B-15 maintained some of the SNVs from the inoculum during its *in vivo* replication, but SA01-1322 did. Subsequent passage of Kenya 128B-15 from the infected tissues in mosquito cells led to a reduction in the quasispecies diversity, indicating that selective pressure in insect cells may restrict genome evolution. The present study demonstrates the dynamics of RVFV quasispecies and the selective pressure of host alternation on RVFV evolution.