Basic Science Presentations
Phi Zeta Research Day
March 10, 2015, 1:15-3:30pm
Frick Auditorium, Mosier Hall

1:15 – 1:30 **Megan C. Niederwerder** – Association of clinical outcome following PCV2/PRRSV challenge with microbiome and immunological response

1:30 – 1:45 **Deepthi Uppalapati** – Systemic and/or Local Gene Therapy with a Nanoparticles-containing Angiotensin II Type 2 Receptor (AT2R) Significantly Attenuate Lung Cancer Allograft Growth in Mice

1:45 – 2:00 **Jennifer L. Wright** – Reactive Hyperemia: Effects on Skeletal Muscle Capillary Hemodynamics

2:00 – 2:15 **Maria V. Murgia** – Epitope mapping of African swine fever virus p30 using monoclonal antibodies and sera from immunized pigs

2:15 – 2:30 **Jingjiao Ma** – Characterization of Uncultivable Bat Influenza Virus Using Replicative Synthetic Virus

2:30 – 2:45 **Break**

2:45 – 3:00 **Yanhua Li** – A unique mechanism of protein-directed trans-activation of ribosomal frameshifting in arteriviruses

3:00 – 3:15 **Yuekun Lang** – Mouse model for the Rift Valley Fever virus MP12 strain infection

3:15 – 3:30 **Giselle Cino** – PRRSV infection of pigs that lack B and T cells shows that infection of pulmonary macrophages is necessary but not sufficient for lung pathology.

5:00 – 6:00 pm **Reception and Awards Ceremony** Frick Auditorium and Foyer, 2nd Floor, Mosier Hall

- Initiation of New Members to Phi Zeta
- Announcement & Presentation of Awards Recognizing Research & Scholarship Accomplishments
- Closing Comments

Association of clinical outcome following PCV2/PRRSV challenge with microbiome and immunological response

Megan C. Niederwerder

Author(s): Megan C. Niederwerder, Crystal J. Jaing, Raymond R. R. Rowland

PRRSV and PCV2 contribute to a range of immunological outcomes and polymicrobial disease syndromes in swine. Clinical outcome following co-infection with PCV2 and PRRSV can vary drastically, from acute death to a complete lack of overt disease. To investigate differences in microbiome and immunological response associated with clinical outcome, 95 six-week old pigs were co-infected with PCV2 and PRRSV. At 70 days post-infection (dpi), 20 representative pigs were selected as having the best or worst clinical outcome based on average daily gain (ADG) and the presence of clinical disease. ADG for the worst clinical outcome group (0.475±0.15 kg) and the best clinical outcome group (0.837±0.04 kg) were significantly different ($p<0.001$). Worst clinical outcome pigs had prolonged and greater levels of viremia as measured by qPCR. Mean PRRSV and PCV2 viremias were significantly higher on 28 dpi ($p<0.02$) and 14 dpi ($p<0.05$), respectively. Fecal and lung samples collected at 70 dpi were analyzed with DNA microarray technology. PCV2 was detected in worst clinical outcome fecal and lung samples at a higher rate compared to best clinical outcome samples (44% vs 10% and 100% vs 80%, respectively). However, no significant association between fecal or lung microbiome with clinical outcome has been detected to date. Worst clinical outcome pigs had less PRRSV neutralizing antibody and greater levels of PCV2 non-neutralizing antibody. Overall, this study suggests that the response to PRRSV and PCV2, as opposed to the presence of secondary pathogens, appears to be the most important factor in determining clinical outcome following co-infection.

Systemic and/or Local Gene Therapy with a Nanoparticles-containing Angiotensin II Type 2 Receptor (AT2R) Significantly Attenuate Lung Cancer Allograft Growth in Mice

Deepthi Uppalapati

Deepthi Uppalapati[1], Susumu Ishiguro[1], Nabil A. Alhakamy[2], Cory Berkland[2], Masaaki Tamura[1].
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Targeted delivery, high transfection efficiency and low toxicity are important challenges in conventional cancer treatments. Recent research by Kawabata *et al. (Cancer Res. 72, 2012)*, has highlighted many advantages of intratracheally administered HIV-1 TAT peptide formulated nanoparticle vector (dTAT-NP) as a local therapeutic approach with high gene transfection efficiency and low toxicity. In this study, we examined the new dTAT-NP formulation to leverage the efficiency for tumor-targeted gene delivery in the setting of intravenous (IV) or/and intratracheal (IT) administration using mouse bronchioloalveolar carcinoma model. Addition of a small amount of albumin (2mg/ml) to the original dTAT-NP formulation increased the nanoparticle stability in biological fluid such as serum containing cell culture medium without losing gene transfection efficiency. In orthotropic tumor allograft models in immunocompetent mice, a single IV administration of new dTAT-NP encapsulating plasmid DNA such as the pTRAIL, pAT2R or microRNA, pmiR34a, attenuated murine Lewis lung carcinoma (LLC) tumor growth without showing any significant adverse effects on mouse health conditions. This clarifies that this new dTAT-NP formulation is suited for IV treatment. Further, a single or co-treatment of IV or/and IT administration of the dTAT-NP containing pAT2R significantly attenuated human H358 xenograft growth by two-fold in the lung confirming that dTAT-NP pAT2R gene therapy is effective in inhibition of the developed human lung bronchioloalveolar carcinomas in SCID mice. Taken together, the current mouse studies suggest that our newly developed dTAT-NP pDNA formulation is applicable as a systemic formulation for the treatment of lung cancer.

Reactive Hyperemia: Effects on Skeletal Muscle Capillary Hemodynamics

Jennifer L. Wright

Author(s): Jennifer L. Wright, Scott K. Ferguson, Clark T. Holdsworth, Trenton D. Colburn, Thomas J. Barstow, Timothy I. Musch, David C. Poole

Post-occlusive reactive hyperemia (PORH) is the commonly accepted non-invasive diagnostic test used for evaluating endothelial function in humans. In contrast to conduit brachial artery blood flow, little is known regarding capillary hemodynamics during PORH. An understanding of microcirculatory behavior post occlusion is fundamental to interpreting the PORH response. Based on previous results found in humans we tested the hypothesis that mechanically induced ischemia of the spinotrapezius muscle would increase the percentage of capillaries supporting red blood cell (RBC) flux during reperfusion. Capillary hemodynamics were measured via intravital microscopy in young male Sprague-Dawley rats at baseline (BL), during mechanical feed artery occlusion (OCC, 1 min and 5 min) and subsequent reperfusion. Mean arterial pressure and heart rate were not altered (both $P>0.05$). As expected, the percentage of capillaries supporting RBC flux was reduced during 1 min OCC (BL: 90±3, OCC: 2±2%, $P<0.05$) and 5 min OCC (BL: 87±3, OCC: 0±0%, $P<0.05$). At 30 s reperfusion the percentage of capillaries supporting RBC flux decreased relative to BL following 1 min OCC (BL: 90±3%, reperfusion: 85±3%, $P<0.05$) and 5 min OCC (BL: 87±3, reperfusion: 29±3%, $P<0.05$). Thus during reperfusion the percentage of capillaries supporting RBC flux neither exceeded nor even returned to BL levels up to 5 min post OCC thereby demonstrating impaired capillary function and thus blood-myocyte O_2 and substrate flux. Consequently, the PORH state in the spinotrapezius muscle of the rat differs markedly from physiological contraction-induced hyperemia found in humans.

Epitope mapping of African swine fever virus p30 using monoclonal antibodies and sera from immunized pigs

Maria V. Murgia

Author(s): Maria V. Murgia, P. Wu, W. Jia, R. R.R. Rowland

African swine fever virus is an enveloped virus belonging to the family *Asfarviridae*.
Its virion is composed of more than 34 polypeptides and, among them, p30 is one of the most immunogenic. Although anti-p30 antibodies are produced after natural infection or vaccination, there is no information on the immunogenic epitopes they recognize. The purpose of this study was the identification of p30 immunogenic epitopes using monoclonal antibodies (mAbs) as well as sera from immunized pigs. The ASFV p30 protein, from amino acid (aa) 61 to aa 204, was divided into five overlapping fragments of approximately 50 amino acids each and their nucleotide sequence was commercially synthesized. The synthesized fragments were then cloned into pHUE expression vector and transformed into chemically competent *E.coli* cells. The recombinant proteins were expressed *in vitro*, purified and used as antigen in indirect ELISAs. Each protein fragment was tested against a panel of 22 mAbs as well as sera from pigs immunized with a defective alphavirus replicon particle expressing ASFVp30 (RP-30). *Two mAb recognized the region from aa 61 to 91, three from aa 91 to 110, six from aa 111 to 130, and three from aa 143 to 160. A more heterogeneous response was observed when sera from RP-30 immunized pigs were used, most of which recognized the p30 region from aa 61 to 160. Overall, the mAbs and the pig sera recognized overlapping regions. Future studies will be directed at the fine mapping of the identified regions.*

Characterization of Uncultivable Bat Influenza Virus Using Replicative Synthetic Virus

Jingjiao Ma

Author(s): Jingjiao Ma, Bin Zhou, Qinfang Liu, Bhupinder Bawa, Juergen A. Richt, David E. Wentworth, Wenjun Ma

Bats harbor many viruses, which are periodically transmitted to humans resulting in outbreaks of disease (e.g., Ebola, SARS-CoV). Recently, influenza virus-like sequences were identified in bats of H17N10 and H18N11; however, the viruses could not be cultured. This discovery aroused great interest in understanding the evolutionary history and pandemic potential of bat-influenza. Using synthetic genomics, we were unable to rescue the wild type bat virus, but could rescue a modified bat-influenza virus that had the HA and NA coding regions replaced with those of A/PR/8/1934 (H1N1). This modified bat-influenza virus replicated efficiently in vitro and in mice, resulting in severe disease. Additional studies using a bat-influenza virus that had the HA and NA of A/swine/Texas/4199-2/1998 (H3N2) showed that the PR8 HA and NA contributed to the pathogenicity in mice. Unlike other influenza viruses, engineering truncations hypothesized to reduce interferon antagonism into the NS1 protein didn't attenuate bat-influenza. In contrast, substitution of a putative virulence mutation from the bat-influenza PB2 significantly attenuated the virus in mice and introduction of a putative virulence mutation increased its pathogenicity. Mini-genome replication studies and virus reassortment experiments demonstrated that bat-influenza has very limited genetic and protein compatibility with Type A or Type B influenza viruses, yet it readily reassorts with another divergent bat-influenza virus, suggesting that the bat-influenza lineage may represent a new Genus/Species within the Orthomyxoviridae family. Collectively, our data indicate that the bat-influenza viruses recently identified are authentic viruses that pose little, if any, pandemic threat to humans; however, they provide new insights into the evolution and basic biology of influenza viruses.

A unique mechanism of protein-directed trans-activation of ribosomal frameshifting in arteriviruses

Yanhua Li

Author(s): Yanhua Li, Emmely E. Treffers, Sawsan Napthinee, Ali Tas, Longchao Zhu, Brian L. Mark, Peter A. van Veelen, Andrew E. Firth, Ian Brierleye, Eric J. Snijder, and Ying Fang

Among the repertoire of mechanisms that viruses use to control or regulate their gene expression, non-canonical translation plays an important role, in particular for positive-strand RNA viruses whose genomic RNA serves a dual function as mRNA and genome. Recently, we uncovered that porcine reproductive and respiratory syndrome virus (PRRSV), and apparently most other arteriviruses, use an unusual -2 frameshifting (-2 PRF) signal directing efficient expression of a transframe protein (nsp2TF) from an alternative reading frame overlapping the viral replicase gene. The signal is also capable of directing -1 PRF, resulting in a truncated version of nsp2 (nsp2N) due to a stop codon adjacent to the shift site in the -1/+2 frame. The -2 and -1 PRF were found to occur with surprisingly high efficiencies (20% and 7%). Unusually, this arterivirus PRF signal lacks an obvious stimulatory RNA secondary structure. The minimal RNA sequence required for PRF was mapped within a 34-nucleotide region that includes the slippery sequence (G_GUU_UUU) and a downstream conserved CCCANCUCC motif. Remarkably, both frameshifts depend on the expression of a viral protein, specifically nsp1β, a PRRSV replicase subunit. Interaction of nsp1β with the PRF signal was demonstrated. Embedded in nsp1β's papain-like autoproteinase domain, we identified a highly conserved, putative RNA-binding motif that is critical for PRF transactivation. These studies demonstrate for the first time that a protein can function as a transactivator of ribosomal frameshifting. This could be a more widely employed mechanism, which might include viral strategies to regulate viral gene expression and/or modulate host cell translation upon infection.

Mouse model for the Rift Valley Fever virus MP12 strain infection

Yuekun Lang

Author(s): Yuekun Lang, Yonghai Li, Jinhwa Lee, Jamie Henningson, Jingjiao Ma, Yuhao Li, Nan Cao, Haixia Liu, William Wilson, Juergen Richt, Mark Ruder, Scott McVey, Wenjun Ma

Rift Valley fever virus (RVFV) is the causative agent of Rift Valley fever (RVF) and classified as a Category A pathogen and select agent. To date there is no commercial, fully licensed vaccine available in the U.S. for human or animal use and effective antiviral drugs have not been identified. The RVFV MP12 strain is a vaccine strain commonly used in laboratories that is categorized as a BSL-2 pathogen. In order to evaluate antivirals or vaccines in a BSL-2 facility, it is crucial to develop small animal models that are susceptible to MP12 strain infection. Herein, we investigated susceptibility of six mouse strains (129S6/SvEv, STAT-1 KO, 129S1/SvlmJ, C57BL/6J, NZW/LacJ, BALB/c) to the MP12 virus via an intranasal route of inoculation with an infectious dose of 1.58×10^6 PFU/mouse. There was severe weight loss and obvious neurological clinical signs with 50% mortality in the STAT-1 KO mice, whereas inoculation of the other 5 strains of mice did not result in obvious weight loss. Interestingly, there was neurological disease observed at the end of the study (14 days post infection, dpi) in two BALB/c mice. Furthermore, virus replication was detected in brains and livers of the STAT-1 KO mice on 3 dpi and 6 dpi. Histopathological lesions were also found in livers and/or brains of the MP12 infected STAT-1 KO mice that were euthanized on 3 dpi and those euthanized due to severe clinical disease. Taken together, the STAT-1 KO mouse strain is susceptible to MP12 virus infection and develops disease.

PRRSV infection of pigs that lack B and T cells shows that infection of pulmonary macrophages is necessary but not sufficient for lung pathology

Giselle Cino

Author(s): Ada Giselle Cino-Ozuna, Catherine L. Ewen, and Raymond R.R. Rowland

PRRSV is a macrophage-tropic virus responsible for more than $500 million/yr in losses, a primary result of the consequences of respiratory disease. The mechanism for the induction of interstitial pneumonia during acute PRRSV infection is unknown. One possibility is destruction of pulmonary alveolar macrophages (PAMs) by the virus. Alternatively, the activation of T-cells may culminate in the induction of cytokine storm. The purpose of this study was to investigate pulmonary lesions in SCID-pigs that naturally lack B- and T-cells. Seven SCID-pigs and two normal littermates were infected with PRRSV at 21-days of age. Pigs were sacrificed 10 dpi and PRRSV nucleic acid was measured by PCR in serum, PAMS, and BALF. The levels of PRRS-associated cytokines (IL-1α, IL-6, IL-10, TNF-α), IL-18, IL-8, GM-CSF, and T- and B-cell cytokines (IL-2, IL-4, IFN-γ) were measured in BALF and serum using a MAGPIX® instrument. At necropsy, the two normal pigs had moderate mononuclear interstitial pneumonia with increased CD3+ T-cells, typical of PRRS. Two SCID-pigs had a moderate suppurative interstitial pneumonia with Gram+ bacteria and two had very mild non-lymphocytic interstitial pneumonia. No lesions were observed in the lung of the remaining three SCID-pigs. PRRSV PCR showed that all pigs were productively infected, including the presence of virus in PAMs and BALF, with no statistical differences. TNF-α, IL-8, and IL-18 were increased in serum and BALF, but only in normal and SCID pneumonic pigs. These results demonstrate that lung lesions in normal pigs result from T-cell recruitment, whereas in SCID-pigs lung lesions are triggered by overwhelming secondary bacterial infection.