

**Joint Symposia on
Inflammation, Infection, & Immunity**

*June 12, 2019
Atlanta, Georgia*

INVITED SPEAKER ABSTRACTS



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IL-36 cytokines and intestinal inflammation

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The gut epithelium acts to separate host immune cells from unrestricted interactions with the microbiota and other environmental stimuli. In response to epithelial damage or dysfunction, immune cells are activated to produce interleukin (IL)-22, which is involved in repair and protection of barrier surfaces. However, the specific pathways leading to IL-22 and associated antimicrobial peptide (AMP) production in response to intestinal tissue damage remain incompletely understood. Here, we define a critical IL-36/IL-23/IL-22 cytokine network that is instrumental for AMP production and host defense. Using a murine model of intestinal damage and repair, we show that IL-36 γ is a potent inducer of IL-23 both in vitro and in vivo. IL-36 γ -induced IL-23 required Notch2-dependent (CD11b+CD103+) dendritic cells (DCs), but not Batf3-dependent (CD11b-CD103+) DCs or CSF1R-dependent macrophages. The intracellular signaling cascade linking IL-36 receptor (IL-36R) to IL-23 production by DCs involved MyD88 and the NF- κ B subunits c-Rel and p50. Consistent with in vitro observations, IL-36R- and IL-36 γ -deficient mice exhibited dramatically reduced IL-23, IL-22, and AMP levels, and consequently failed to recover from acute intestinal damage. Interestingly, impaired recovery of mice deficient in IL-36R or IL-36 γ could be rescued by treatment with exogenous IL-23. This recovery was accompanied by a restoration of IL-22 and AMP expression in the colon. Collectively, these data define a cytokine network involving IL-36 γ , IL-23, and IL-22 that is activated in response to intestinal barrier damage and involved in providing critical host defense.

Immunologic and Metabolic Alterations in a Nonhuman Primate Model of Pulmonary Hypertension

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While life expectancy of HIV-infected individuals has improved with the advent of anti-retroviral therapy (ART), there remains an increased risk in this population for the development of co-morbidities, coinfections and complications (CCCs). Improvements in the clinical management of HIV infection and associated CCCs requires improved understanding of the complex mechanisms involved in the pathogenesis of these complications. For the past 20 years my research has been focused on gaining an understanding of the pathogenesis HIV-associated cardiopulmonary co-morbidities and opportunistic co-infections, with the goal of developing new therapeutic strategies to prevent or ameliorate these diseases. My work has focused on three major pulmonary diseases associated with HIV infection: 1) Pulmonary Arterial Hypertension (PAH), 2) *Pneumocystis* pneumonia (PCP) and 3) Chronic Obstructive Pulmonary Disease (COPD). Our work has generated unique insights into these disease processes that have revealed paths toward the development of targets for both disease prevention and improved treatments. The progress we have made in understanding critical aspects of the pathogenesis of these diseases has been facilitated by the development of novel, pre-clinical models of each of these major pulmonary diseases, as well our integration with highly collaborative clinical research teams. In the proposed studies, my program will be focused on 1) advancing our understanding of the pathogenesis of HIV-associated PAH with the goal of identifying and testing novel therapeutics aimed at preventing or ameliorating disease progression, 2) understanding the roles of immune dysfunction, long-term ART use and the development of HIV-associated PAH and 3) advancing the development of prophylactic and therapeutic vaccines for the prevention of *Pneumocystis* and related fungal opportunistic infections associated with HIV infection. The development of these studies in highly relevant, pre-clinical models of HIV infection will provide the best possible guidance for the advancement of these therapeutics and vaccine candidates to clinical trial.

Microbiota-rotavirus interactions

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Most viruses first encounter host cells at mucosal surfaces, which are typically colonized by a complex ecosystem of microbes collectively referred to as the microbiota. Recent studies demonstrate the microbiota plays an important role in mediating host-viral interactions and determining the outcomes of these encounters. Mechanistically, these effects can be broadly categorized as reflecting direct bacterial-viral interactions and/or involving microbial impacts upon innate and/or adaptive immunity. Enteric viruses encounter epithelial cells amidst diverse microbiota. We thus hypothesized that our unintentional generation of rotavirus (RV)-resistant Rag1-KO mice reflected microbiota influencing RV infection. Accordingly, such RV-resistance was transferred by co-housing and fecal transplant. Interrogation of microbiotas conferring RV-resistance via antimicrobial agents, heat, and filtration, followed by limiting dilution transplant to germfree mice and subsequent fecal DNA sequencing revealed a central role for segmented filamentous bacteria (SFB), which was sufficient to protect mice against RV infection and associated diarrhea. Such protection was independent of lymphocytes (innate and adaptive), interferon, IL-17, and IL-22. Incubation of SFB-containing feces with RV reduced RV infectivity, suggesting direct disabling of this virus. Additionally, colonization of ileum by SFB induce changes in host gene expression and accelerated epithelial cell turnover, which can reduce RV burden. Thus, irrespective of its effects on immune cells, SFB confers protection against some enteric viral infections and associated diarrheal disease.

Human antibody responses to respiratory pathogens

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The focus of the Mousa Lab is to elucidate the molecular mechanisms by which antibodies combat respiratory pathogens. Our goals are to identify and test new antibody therapeutics for treatment and prevention of respiratory diseases, and to utilize our mechanistic data to design new vaccine candidates. Three pathogens of interest are respiratory syncytial virus (RSV), human metapneumovirus (hMPV), and *Streptococcus pneumoniae*.

RSV and hMPV are two leading causes of lower respiratory tract infection in infants and children, yet there is currently no vaccine available for prevention of RSV or hMPV-induced disease. These viruses are closely related and can cause severe respiratory tract infection in the immunocompromised, such as premature infants and the elderly. We are studying the molecular basis for antibody-mediated immunity to RSV and hMPV to identify the optimal antigenic epitopes for effective vaccine design. We discovered new antigenic epitopes on the RSV fusion (F) protein, one of which was non-neutralizing and elicits antibodies that compete for binding with palivizumab on the post-fusion conformation of the F protein, and another new pre-fusion-specific antigenic site that elicits potentially neutralizing antibodies. For hMPV, we have discovered new antibodies that cross-react between RSV and hMPV F. Additionally, our recent work has established a new panel of hMPV F-specific human mAbs that are potentially neutralizing and one mAb protects against disease *in vivo*. We are further expanding our studies to examine hMPV F antibody responses in both adults and children.

In an additional project, we are isolating human mAbs to *S. pneumoniae*, which is the leading cause of pneumonia mortality globally, despite two currently available vaccines. Our mAbs target virulence factor proteins that are conserved across pneumococcal serotypes. We have isolated mAbs to the PspA and PhtD surface proteins, and have shown that these mAbs bind diverse strains of bacteria, and exhibit opsonophagocytosis activity *in vitro*. Our future work will be to examine these mAbs in an animal model of pneumococcal infection.

Metal Piracy: A successful survival strategy employed by *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae causes the common sexually-transmitted infection, gonorrhea. More than 78 million cases of gonorrhea occur each year worldwide, according to the WHO, and the CDC estimates that there are over 800,000 cases of gonococcal disease every year in the US alone. In addition to increasing incidence of disease, gonococcal isolates are increasingly resistant to antimicrobial therapies, resulting in the recent declaration of *N. gonorrhoeae* as an urgent threat pathogen for which new therapies, and ideally preventative measures, are urgently needed. There is no vaccine to prevent gonorrhea, infections are not protective, and furthermore, infections are associated with significant morbidity, especially among women. Towards identification of suitable therapeutic or vaccine targets, we have focused on well-conserved nutrient transporters that are necessary for growth and survival in the host. The gonococcus produces eight different outer membrane transporters in the TonB-dependent family, and four of these transporters are well-characterized with respect to iron uptake. Of these transporters, TbpA, LbpA and HpuB recognize the human proteins, transferrin, lactoferrin and hemoglobin, respectively, and enable iron uptake directly from these ligands. One transporter, FetA, enables the use of catecholate siderophores. The other four transporters have not been as well characterized, but we recently demonstrated that one, TdfH, enables the gonococcus to bind to the innate immunity protein, calprotectin (S100A8/S100A9), and to subsequently internalize zinc from this zinc-sequestering protein. A second TonB-dependent transporter, TdfJ, also supported zinc internalization. TdfH-expressing *N. gonorrhoeae* cells bind to calprotectin, and more specifically to human and not mouse calprotectin; this binding event supports the growth of the wild-type gonococcal strain in a Zn-dependent manner. Gonococci producing TdfH survive better in neutrophil NETs than do isogenic mutants that do not produce this protein. Specific binding can be demonstrated using SPR when human calprotectin is affixed to the chip and TdfH is used as the analyte. Another human protein, S100A7, is enriched in epithelial cells and usually functions to sequester zinc from invading pathogens. TdfJ-expressing *N. gonorrhoeae* grow in a zinc-dependent manner with this innate immunity protein. S100A7 interacts specifically with TdfJ-producing gonococcal cells in a whole cell binding assay and recombinant *E. coli* producing TdfJ specifically interacts with S100A7, demonstrating that TdfJ is both necessary and sufficient for binding to S100A7. With respect to regulation, both TdfH and TdfJ are zinc repressed and this phenomenon is Zur-dependent. In conclusion, metal acquisition via TonB-dependent transporters enables the gonococcus to overcome nutritional immunity, which is critical to the survival and virulence of this important human pathogen. Because metal transport systems are critical for the survival of the gonococcus, the protein components of these systems are attractive candidates for vaccine development and for therapeutic intervention.

Risk assessment of contemporary swine influenza viruses in murine models of infection

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Influenza A virus (IAV) is endemic in North American swine. A relative of the 1918 pandemic influenza has circulated in swine since that pandemic and remained relatively stable, with some drift, but no reassortment. However, in North America in the 1990's, swine IAV (swIAV) gained an internal gene segment constellation referred to as the triple reassortment internal gene (TRIG) cassette, which quickly became dominant within circulating swIAVs and enabled increased surface protein gene segment reassortment. Regular reverse-zoonotic events followed by antigenic drift in swine resulted in a dramatically increased diversity of HA and NA combinations and today there are nearly a dozen distinct clades of H1N1, H1N2, and H3N2 combinations co-circulating in North American swine. After the 2009 pandemic, reverse zoonosis and subsequent reassortment resulted in the pdmH1N1 origin matrix gene (pdmM) replacing the classical swine origin matrix gene (swM) previously found in the TRIG cassette. The pdmM gene segment rapidly replaces the swM gene segment in North America, suggesting a strong fitness advantage for strains containing the pdmM over the swM gene segment. We hypothesized that the origin of the matrix gene could affect virus replication, pathogenesis, and the subsequent immune responses induced by swIAV infection. To confirm this, we infected BALB/c mice with a panel of H1 and H3 swIAV isolates containing either the pdmM or the swM gene segment. We assessed virus replication, disease, lung pathology, as well as cytokine and chemokine production. Infection of mice with H1 swIAVs containing the pdmM gene resulted in significantly greater morbidity and mortality compared to viruses with the swM segment, while the viruses with the pdmM also consistently replicated at higher levels. The same viruses induced an overall greater proinflammatory response and a greater chemotactic response. While other gene segments such as the NS1 or PB1-F2 traditionally antagonize the immune response, our data suggest that the origin of the matrix gene may contribute to an enhanced immune response against swIAV in mammals.

Protein Interaction Mapping Identifies Human Proteins that Negatively Regulate Ebola Virus Transcription

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How host factors modulate replication of the deadly pathogen, Ebola virus (EBOV), remains incompletely understood. EBOV protein VP30 regulates viral gene expression through interaction with a PPxPxY motif on the viral nucleoprotein (NP). Affinity tag-purification mass spectrometry identified host protein RBBP6 as a VP30 interactor that competes with NP for binding to the largely alpha-helical carboxy-terminal domain of VP30 via its own PPxPxY motif. This impedes viral RNA synthesis. The same mass spectrometry protein-protein interaction data set identified additional PPxPxY-containing host proteins, including hnRNPL, hnRNPUL1 and PEG10 as VP30 interactors. Of these, hnRNPL and PEG10, like RBBP6, inhibit EBOV RNA synthesis. Studies with peptides from the host proteins identifies the motif PxPPPPxY as conferring optimal binding and inhibition of RNA synthesis. Knockdown studies demonstrate that hnRNPL, hnRNPUL1, PEG10 each modulate EBOV infectivity and double knockdown studies support additive effects of RBBP6 and hnRNP L. The host factors are demonstrated to compete with NP for binding to VP30, alter VP30 phosphorylation and modulate viral mRNA transcription. This represents a unique viral replication strategy in which a novel proline-tyrosine motif targets a critical protein-protein interaction to regulate viral gene expression.

Partnering Early Stage Technology

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The exploitation of basic science discoveries in order to produce commercially viable technological and therapeutic innovations is critical for medical progress. Academic centers have increasingly been a major source of early stage inventions and innovations. This presentation will explore how scientists and technology transfer officers can work together to establish practical ways by which innovations can become commercialized.

Protein-nanoparticle universal influenza vaccines

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A widely protective universal influenza vaccine is one top priority for vaccine development. We studied whether conserved influenza structures alone would induce broadly reactive immune responses conferring protection against challenges by different viruses. We first constructed recombinant proteins containing stabilized influenza structures, purified and characterized these proteins. We then generated double-layered protein nanoparticles from these proteins by concentrating proteins into a condensed core by desolvation and coating the conserved surface antigens onto the core surface to form double-layered protein nanoparticles. mAb binding and biochemistry assessment demonstrated that the resulting protein nanoparticles retained influenza antigenicity and native-like structures. Immunization studies in mice demonstrated that these double-layered protein nanoparticles are highly immunogenic, inducing strong antibody and T cell responses to conserved influenza antigens conferring cross protection against different live virus challenges. The results demonstrated that double-layered protein nanoparticles of conserved influenza structures have the potentials to be developed into a universal influenza vaccine.

Impact of age and pre-existing immunity on influenza-specific B cells and induction of enhanced antibody breadth following vaccination in humans.

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Influenza is a highly contagious viral respiratory disease with more than 200 thousand cases reported in the U.S. last season (2017-2018). Annual vaccination is recommended by the World Health Organization with the goal to reduce influenza severity and limit transmission. However, currently available split inactivated influenza vaccines can only elicit protection up to ~60% in well-matched-strain years. Furthermore, vaccine effectiveness frequently varies between different influenza subtypes within a single influenza season for unclear reasons.

Recently, immunological imprinting from early-life influenza infection can prominently shape the immune response to subsequent infections. Here, the impact of pre-existing B-cell memory in the response to quadrivalent influenza vaccine was assessed. Blood samples were collected from healthy subjects (18 to 85 years old) prior to vaccination and 21-28 days after vaccination with quadrivalent influenza vaccine. Samples were assessed for changes in serological antibodies to the hemagglutinin protein (HA) by ELISA and by hemagglutination-inhibition (HAI) assays against the four strains in the vaccine. The number of antigen specific memory B-cells (B_{mem}) were quantified by flow cytometry and the polyclonal B_{mem} response was assessed against the HA of the four vaccine strains. Influenza vaccination greatly induces the HA-specific B_{mem} -cell pool and, despite no differences in antigenicity between the four vaccine components, most individuals are biased towards one of the influenza A or Influenza B vaccine strains. Overall, this study unveils a new mechanism behind the differences in vaccine effectiveness against different influenza subtypes.

Challenges and approaches in developing vaccines against human respiratory syncytial virus (RSV)

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Clinical trials with alum-adsorbed formalin-inactivated human respiratory syncytial virus (FI-RSV) vaccine failed in young children because enhanced respiratory disease resulting in hospitalizations and 2 deaths was observed in vaccines after natural RSV infection. There is no licensed RSV vaccine. Palivizumab, neutralizing monoclonal antibody against RSV fusion (F) protein, has been approved for use in highly risk young infants. Challenges exist in developing effective and safe RSV vaccine candidates which can induce high neutralizing antibodies and prevent enhanced respiratory disease after RSV infection. Soluble fusion protein as RSV subunit vaccine exacerbated pulmonary histopathology after vaccination upon RSV challenge but not when F was presented on virus-like particle (VLP) platforms. Unique combination of Toll-like receptor agonist adjuvants modulated immune responses to F protein or inactivated split RSV vaccination and attenuated pulmonary inflammation after RSV challenge in mice. Also, new sets of mutations in RSV fusion protein were discovered to stabilize and expose the neutralizing epitopes present in pre-fusion conformation of F proteins on enveloped VLP. These approaches are expected to provide insight into developing an effective and safe RSV vaccine candidate.