2592. Lung Function as an Indicator of Vaccine Enhanced RSV Disease in Cotton Rats

Crystal Jones, PhD; Xiaolan Shen, MS; Xiaoli Ping; Catherine Gallagher;
Jeremy Beech; Cameron M. Douglas, PhD; Michael Citron, MS; Amy Espeseth, PhD;
Merck.& Co., Inc., West Point, Pennsylvania

Session: 269. Pathogenesis and Host-Response Interactions
Saturday, October 5, 2019: 12:15 PM

Background: Respiratory syncytial virus (RSV) infection is the leading cause of lower respiratory tract infections in infants and young children. Seronegative children previously vaccinated with formalin-inactivated live RSV formulated with aluminum (FIRSV) developed vaccine enhanced RSV disease (VERD), which is characterized by fever, wheezing, bronchopneumonia, and airway hyperresponsiveness (AHR). We investigated whether impaired lung function can serve as a marker for VERD in an animal model of RSV infection.

Methods: Uninfected and RSV-infected cotton rats intranasally challenged with 10^6 pfu of RSV A2 were anesthetized with pentobarbital and tracheostomized. A cannula was placed in the trachea and animals were connected to flexiVent™ (Scireq), which is a computer-controlled piston ventilator that analyzes pressure and volume signals in response to an oscillatory waveform applied at the animal's airways. Vecuronium bromide was administered to ventilated animals to prevent independent breathing. To measure AHR, animals were exposed to increasing doses of inhaled methacholine, and methacholine-induced bronchoconstriction was measured.

Results: Two independent studies showed that RSV-infected cotton rats (n = 4) exhibited increased total respiratory system resistance (Rrs) and airway resistance (Rn) following methacholine challenge on days 4 and 6 post-infection compared with uninfected cotton rats (n = 4).

Conclusion: RSV-induced impairment in lung function can be exploited for the development of a more robust and objective method for assessing vaccine safety in a cotton rat model of respiratory disease compared with traditional histopathological analysis.

Disclosures. **All authors:** No reported disclosures.

2593. Human Monoclonal Antibodies Potently Neutralize Enterovirus D68 in both a Clade-Specific and -Independent Manner

Matthew R. Vogt, MD, PhD[1]; Nurgun Kose, BS[1]; Lauren Williamson, BS[2];
Yury A. Bochkov, PhD[3]; James E. Gern, MD[3]; James E. Crowe, Jr, MD[1]; [1]Vanderbilt University Medical Center, Nashville, Tennessee; [2]Vanderbilt University, Nashville, Tennessee; [3]University of Wisconsin-Madison, Madison, Wisconsin

Session: 269. Pathogenesis and Host-Response Interactions
Saturday, October 5, 2019: 12:15 PM

Background: Enterovirus D68 (EV-D68) causes worldwide outbreaks of human respiratory illness with spacio-temporally related outbreaks of acute flaccid myelitis (AFM), a polio-like illness. Numerous seroepidemiology studies show that nearly all humans older than 2 years have EV-D68-neutralizing antibodies in their serum, even in serum collected prior to large outbreaks. However, little else is known about the human antibody response to this virus. We sought to isolate human monoclonal antibodies (mAbs) from B cells in peripheral blood mononuclear cells (PBMCs) of immune subjects, induced by natural infection, to understand human humoral immunity to EV-D68.

Methods: We obtained PBMCs from donors with known infection during the largest ever recorded outbreak, which occurred in the United States in 2014. We used EV-D68 virus isolates from this outbreak in an indirect ELISA to screen immortalized PBMCs for antigen-specificity, then fused them with myeloma cells to create hybridomas.

Results: To date, we have isolated > 60 naturally occurring anti-EV-D68 human mAbs from the B cells of subjects with documented infection. These mAbs exhibit diverse binding affinities when compared across different clades of recent EV-D68 isolates. Many mAbs neutralize EV-D68 quite potently *in vitro*, with [ng/mL] half maximal effective concentrations. Some mAbs neutralize diverse clades of EV-D68, whereas others are highly clade-specific. Binding of antibodies to at least three, but likely more, major antigenic sites on the virus leads to neutralization.

Conclusion: We observed a qualitative difference among antibodies isolated from patients who had natural infection. These differences could contribute to certain individuals being susceptible to respiratory disease and AFM. Our studies of humoral immunity are especially important for a disease with nearly universal apparent seroprotection, in which the virus somehow persistently causes outbreaks across the world. Furthermore, no licensed vaccines or treatments exist for EV-D68. Given the ability of human intravenous immune globulin to protect mice from AFM, these mAbs are being tested for therapeutic benefit *in vivo*, and may have promise in the prevention and/or treatment of EV-D68-related diseases.

Disclosures. **All authors:** No reported disclosures.

2594. Biofilm-Dispersed *Staphylococcus aureus* Exhibits a Distinct *agr*-Independent Host Interaction

Spencer Chang, BS[1]; Vance G. Fowler, Jr, MD, MHS[2];
Batu K. Sharma-Kuinkel, PhD[2]; Felix Medie, PhD[3]; Larry Park, PhD[4];
Yue Zheng, PhD[5]; Michael Otto, PhD[6]; Alexander Horswill, PhD[7]; [1]Duke University School of Medicine, Durham, North Carolina; [2]Duke University Medical Center, Durham, North Carolina; [3]Duke University, Durham, North Carolina; [4]Duke University Department of Medicine, Durham, North Carolina; [5]National Institutes of Health, Betheseda, Maryland; [6]National Institute of Allergy and Infectious Diseases (NIAID), Betheseda, Maryland; [7]University of Colorado Anschutz Medical Campus, Aurora, Colorado

Session: 269. Pathogenesis and Host-Response Interactions
Saturday, October 5, 2019: 12:15 PM

Background: *Staphylococcus aureus* biofilms are a common cause of persistent, life-threatening infections. Dispersal of *S. aureus* cells from established biofilm-based infections is crucial for dissemination within the host, but is poorly understood. We tested the hypothesis that biofilm dispersed *S. aureus* cells have distinct physiology from planktonic cells and are better equipped to evade host immunity in an *agr*-dependent manner.

Methods: Primary murine bone marrow-derived macrophages (BMDMs) were infected with planktonic and biofilm dispersed cells from *S. aureus* USA300 LAC wild type (WT) and USA300 LAC-*agr* knockout (KO). Biofilm dispersed cells were collected via glucose deprivation. Gentamicin protection assays were used to enumerate phagocytosed bacteria and fluorescence microscopy to quantify macrophage viability. A 26-plex immunoassay was used to screen for cytokines and chemokines. Reversed phase high-performance liquid chromatography was used to measure relative phenol-soluble modulin (PSM) levels from macrophage co-cultures.

Results: Compared with planktonic cells, biofilm-dispersed cells in both *S. aureus* WT and KO backgrounds exhibited: (1) ~10-fold less phagocytosis by BMDMs (p = 0.0003; Figure 1); (2) increased macrophage killing (23% vs. 8%; p = 0.0038; Figure 2); (3) stronger pro- (e.g., IFN-y, IL-2, IL-6, IL-17; Figure 3A) and anti- (e.g., IL-10, IL-4, IL-22; Figure 3B) inflammatory cytokine responses from macrophages ($P < 0.05$ for all); (4) significantly higher δ toxin PSM production ($P = 0.0090$; Figure 4) in WT background only.

Conclusion: *S. aureus* biofilm dispersed cells are physiologically distinct from planktonic cells and have a unique interaction with the host immune system. Dispersed cells are more resistant to phagocytosis, have a greater propensity to kill macrophages, and mount stronger pro- and anti-inflammatory responses in an *agr*-independent manner. Dispersed cells also have the ability to produce more δ toxin PSM via well-known *agr*-dependent pathways.

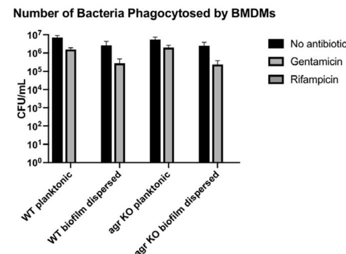

FIGURE 1. Biofilm dispersed cells are more resistant to macrophage phagocytosis independent of *agr* functionality.
Data is representative of mean +/- SD of three independent experiments. Samples with no antibiotic treatment represent the total intracellular and extracellular bacteria. Gentamicin treated samples represent the total intracellular bacteria. Rifampicin treatment is a negative control to confirm that an antibiotic that crosses cell membranes kills the intracellular bacteria.

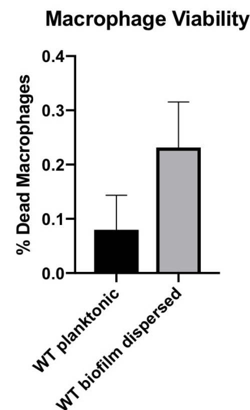

FIGURE 2. Macrophage killing by biofilm dispersed cells is higher than planktonic cells

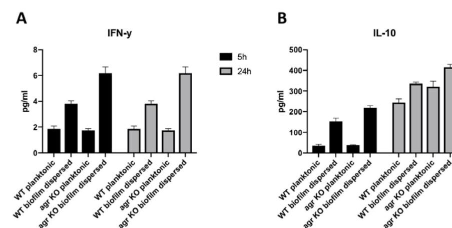

FIGURE 3. Representative data showing more robust pro- and anti-inflammatory cytokine responses to biofilm dispersed cells from both WT and *agr* KO strains at 5 and 24 hours post-infection.