Methods. miRNA from serum and RNA from PBMCs were acquired from n = 40 participants in a prospective cohort of Filipino septic patients: n = 15 developed septic shock and n = 17 developed renal failure. RT-qPCR was done to measure the expression of 21 sepsis-associated miRNAs. Differentially expressed miRNAs (DEMs) for each outcome was identified, followed by gene target prediction for each DEM. Gene expression microarrays covering 18,616 genes were also performed to identify differentially expressed genes (DEGs; $P < 0.05$, log FC> |0.3|) for each outcome. Significant miRNA-gene pairs were selected by evaluating the overlap of the predicted gene targets of the DEMs with the DEGs for each corresponding outcome. Given the gene-silencing mechanism of miRNAs, overlap analysis was performed on only the downregulated DEGs when the specific DEM was upregulated (and vice versa).

Results. Septic participants who developed shock, compared with those who did not, had higher expression of 1 DEM, miR-223-5p, and downregulation of 20 DEGs. *NUS1* was the only predicted gene target of miR-223-5p that was also downregulated in septic shock. Participants who developed renal failure, vs. those who did not, had lower expression 6 DEMs and upregulation of 6 DEGs. *KPNA4* is a gene target of the DEMs, miR-126-5p, and miR-181a-5p, that was also upregulated in renal failure.

Conclusion. Significant miRNA–gene pairs related to worse clinical outcomes in sepsis were identified: miR-223-5p with *NUS1* for shock and either miR-126-5p or miR-181-5p with *KPNA4* for renal failure. While the biological significance of these miRNA-gene pairs still needs to be evaluated, these findings can potentially help future efforts in developing prognostic markers or therapeutic targets for shock and renal failure in sepsis.

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

405. Serum Antibody Responses Against Carbapenem-Resistant *Klebsiella pneumoniae* in Infected Patients

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Background. Capsular polysaccharide (CPS) of Carbapenem-resistant *K. pneumoniae* ST258 (CR-*Kp*) is a potential vaccine target. CPS of these isolates generally falls within 2 homology groups named clade 1 and clade 2. We and others have made antibodies (Abs) that act against clade2 CR-*Kp* but failed to make therapeutic Abs against clade1 CR-*Kp*. Previous studies had shown that studying patient's antibody responses could help in identifying suitable candidates for developing immunotherapies. Thus, we sought to identify potential vaccine candidates by investigating the humoral response CPS in CR-Kp-infected patients.

Methods. 24 CR-*Kp* isolates and corresponding serums were collected from inpatients at Stony Brook Hospital. CPS was isolated and purified by size-exclusion column chromatography from CR-*Kp* strains 34 (clade 2), 36 (clade 1), and 38 (clade-*Other*). Anti-CPS Abs in patient's serum were detected by enzyme-linked immunosorbent assay (ELISA) and bulk Abs from positive serum were purified using an affinity column. These Abs were tested for activity against CR-*Kp* by serum bactericidal and agglutination assays.

Results. 50% of clade2 CR-*Kp*-infected patients had humoral responses against CPS34. 77% of clade 1-infected patients sera cross-reacted wtih CPS34, but none of them developed Abs against CPS36. Interestingly, 90% of clade1 and 60% of clade 2-infected patients, respectively, showed Abs binding to CPS38. Thus, we selectively purified Anti-CPS Abs from two clade-*Other*-infected patients and observed that they were cross-reactive with all three CPS. Further, these Anti-CPS Abs agglutinated all tested CR-*Kp* isolates (34, 36, and 38) when compared with control human IgG ($P < 0.005$). Additionally, these Anti-CPS Abs promoted killing of clade2 bacteria and inhibited the growth of clade1 bacteria in Ab-mediated serum bactericidal assay. These data elucidate that humoral responses developed in clade-*Other* CR-*Kp*-infected patients have therapeutic potential.

Conclusion. With the unavailability of effective antimicrobials for CR-*Kp*, approaches like developing novel anti-CPS vaccine could serve as an alternate therapy. Our data suggest that *developing immunotherapies targeting CPS38 could potentially provide protection across both clade1 and clade2 bacteria in clinical settings.*

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

406. Cloning Antibodies Against Kawasaki Disease from Acute Plasmablast Responses

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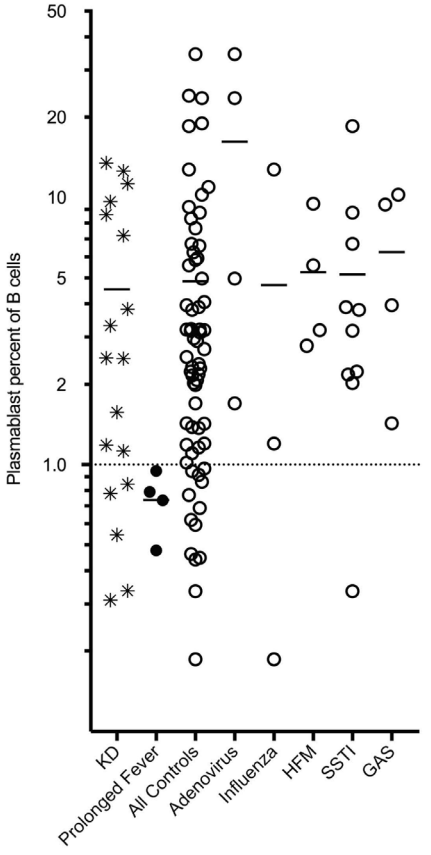
Background. Kawasaki Disease (KD) is a childhood vasculitis, marked by prolonged fevers and coronary artery inflammation/aneurysms in near one-quarter of those untreated. The cause remains unknown; however, epidemiologic and demographic data support a single preceding infectious agent may lead to KD. Plasmablasts (PBs) are a stage of transitional B-cells that lead to plasma cells, the long-lived antibody-producing cells of the bone marrow. After initial infection, peripherally circulating PB populations are enriched for cells with antibodies against the preceding infection. We have recently published data showing children with KD have similar PB responses to children with infections. We sought to define the antibody characteristics, including clonality, of these PBs during KD.

Methods. We used antibody repertoire next-generation sequencing to characterize memory and PB populations. Additionally, pairing of heavy and light chains was performed with Chromium Single Cell Gene Expression (10x Genomics, Pleasanton, CA) using the Human B cell Single Cell V(D)J Enrichment Kit.

Results. From subject 24, antibody sequences using VH4-34 and a 19 amino acid length complementarity determining region 3 showed a massive expansion between day 4 and 6 of fever. Chromium single-cell sequencing produced over 946 heavy and light chain paired sequences. Sequence comparison showed 40% of sequences demonstrated markers of clonal expansion, which represented 100 clonal groups. Seven other KD subjects are being processed and comparative analysis will be presented.

Conclusion. This clonal expansion within plasmablast populations supports that Kawasaki disease is caused by an infection. Antigen targeting of these monoclonal antibodies is currently being explored.

FIGURE 1: PBs in KD are similar to infectious diseases.



Plasmablast levels, as a percentage of overall B cell number, were compared between children with KD (star), prolonged fever (closed circle), and febrile control groups as marked (open circles). Abbreviations: Hand-foot-and-mouth (HFM), Skin and Soft tissue infections (SSTI), and Group A streptococcal pharyngitis (GAS). (figure 2 in Martin et al, 2018.)

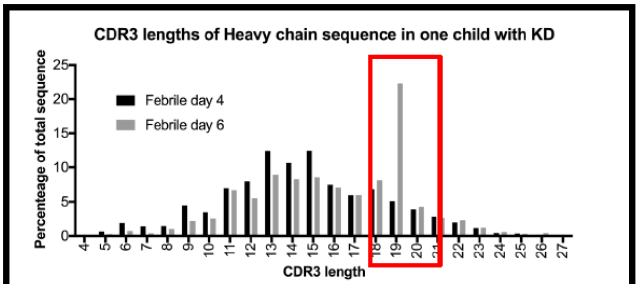


Figure 2: CDR3 analysis shows massive clonal expansion in subject 24's repertoire from day 4 to 6. RNA was extracted from total PBMCs and heavy chain primers were used on the Illumina MiSeq platform per the methods section. The IMGT HighV-QUEST Database matches sequences to the closest V-, D-, and J-gene families and allele and provided output of 265000 day 4 (black) and 190024 day 6 (gray) analyzed sequences. Sequences analyzed by CDR3 length are shown as percentage of total. Strikingly, there is a massive expansion of B cell sequences with CDR3 length of 19 on day 6 of fever (red box). This child's circulating PBs as a percentage of B cells for day 4 and 6 were 13.5% and 7.0% respectively.

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407. The Effect of *Streptococcus pneumoniae* Pneumonia on Atherosclerosis

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Thursday, October 3, 2019: 12:15 PM

Background. Clinical studies consistently find an increase in the risk of acute coronary syndrome (ACS) in the weeks following pneumonia, although the mechanisms underlying this finding are unknown. ACS most commonly occurs as a result of thrombosis at the site of ruptured atherosclerotic plaques. We hypothesized that the systemic inflammatory response to pneumococcal pneumonia leads to acute localized inflammatory changes within established atherosclerotic plaques, favoring plaque instability and rupture, thereby resulting in ACS.

Methods. Male ApoE-/- mice, a well-established model of atherosclerosis, were fed an atherogenic diet for 7–8 weeks before intranasal infection with *Streptococcus pneumoniae* or mock infection. Mice were sacrificed 2 or 8 weeks post-infection. Formalin-fixed, paraffin-embedded aortic sinus plaque sections were analyzed to assess markers of plaque vulnerability to rupture. To characterise post-pneumonic plaque macrophage phenotype, aortic sinus plaque cryosections 2 weeks post pneumonia/mock infection were immunostained for MAC-3 to identify macrophage-rich areas. These plaque regions were collected using laser capture microdissection and RNA extracted for microarray analysis.

Results. *S. pneumoniae* infection was associated with increased aortic sinus atherosclerotic plaque macrophage content (18.1 vs. 8.0%; $P < 0.05$) at 2 weeks post infection, but no significant difference in aortic sinus plaque burden, plaque smooth muscle or collagen content. There was no significant difference in any of these plaque vulnerability markers at 8 weeks post infection. Microarray analysis of laser capture micro-dissected plaque macrophages identified downregulation of the expression of three genes coding for specific E3 ubiquitin ligases following pneumonia. Pathway analysis identified a significant perturbation in the ubiquitin proteasome system pathway as a result.

Conclusion. In this murine model, pneumococcal pneumonia resulted in increased atherosclerotic plaque macrophage content, a marker of plaque instability, at 2 weeks post infection. Pneumonia may therefore lead to an increased propensity for atherosclerotic plaques to rupture soon after pneumonia, due to infiltration of macrophages into the plaque.

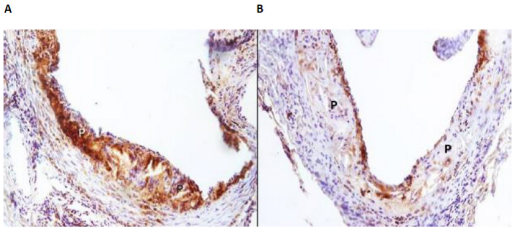

Figure 1. Representative images of MAC 3 immunostained aortic sinus plaque sections from *S. pneumoniae* infected (A) and mock infected (B) mice (x100 magnification, p= plaque area), demonstrating increased macrophage content 2 weeks post infection. Positive stained cells appear brown in colour.

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

408. Single-cell Sequencing Identifies Variability in Host Response Among Different Genera of Influenza Viruses

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Background. Seroprevalence and surveillance studies indicate that influenza C virus (ICV) infection is common among humans, and initial exposure occurs early in life. ICV often causes milder disease than influenza A and B viruses, but the mechanisms underlying differences in pathogenicity remain poorly understood.

Methods. To compare early events of infection in natural target sites, we cultured primary human tracheal/bronchial epithelial cells under air-liquid interface conditions to allow differentiation. We-infected these cells with human strains of influenza A, B or C virus. Cells were infected at low MOI (0.1) to ensure populations of directly infected cells and uninfected neighboring cells. To compare the early immune response and cell tropism among these viruses, we performed single-cell RNA sequencing of mock- and influenza-infected cells. In parallel, we infected cells pretreated with interferon to mimic later rounds of infection after an early immune response is initiated.

Results. Infection of primary cells by all three viruses was confirmed by RT-qPCR of bulk cell lysates. As expected, prior exposure to interferon β results resulted in reduced levels of viral transcripts. At the single-cell level, we identified expression of genes associated with specific cell types, including basal, ciliated and secretory cells. We also identified expression of interferon stimulated genes, but these genes were not homogeneously expressed among all cell subpopulations and varied among cultures infected with different influenza viruses. We also found different patterns in gene expression in cells previously exposed to interferon, suggesting that host environment varies over subsequent rounds of infection.

Conclusion. Single-cell sequencing is an important tool for studying the host response to influenza infection in complex cellular environments such as the respiratory tract, in which cells vary in their susceptibility to infection and antiviral response. Further analysis will characterize differences among directly infected vs. neighboring cells and correlate responses with pathogenicity.

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

409. Using the Host Response to Reduce Unnecessary Antibiotic Use in Outpatient Acute Respiratory Infections

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Background. Acute respiratory tract infections (ARI) often resolve without antibiotics. Yet, antibiotics are prescribed in 60–98% of cases despite lack of confirmed bacterial etiology. Antigen, culture and molecular testing identify pathogens; however, do not differentiate colonization from invasive infection. Since antibiotics are often prescribed despite the low prevalence of confirmed bacterial infection in patients with ARI, we analyzed the impact of adding host response biomarkers to the clinical and microbiological evaluation of outpatients with ARI.

Methods. A secondary analysis was performed using data from su suspected ARI cohorts derived from two clinical studies. A clinical reference algorithm, which included bacterial culture, respiratory PCR panels for viral and atypical pathogens, procalcitonin, CBC, serology, and Myxovirus resistance protein A (MxA), was used to define invasive infection based on pathogen detection plus host response and classify infections that may benefit from antibiotics. Antibiotics were considered "warranted" if patients exhibited a bacterial-specific host response, with or without bacterial pathogen detection, and a detected bacterial pathogen without a host response was deemed to be colonization and "at risk for antibiotics." The percentage requiring antibiotics was calculated by dividing the number of patients with a host response for bacteria by the total number of patients at risk for receiving antibiotics (warranted + at risk). A Chi-square test was performed to determine the difference between patients likely to be treated with antibiotics, bacteria detected with or without host response and bacteria detected with a host response.

Results. Each dataset (Self, n = 205) and (Shapiro, n = 220) was analyzed separately and pooled (n = 445). Upon enrollment, 15% (Self) and 55% (Shapiro) were febrile. A pathogen was detected in 67% (Self) vs. 82% (Shapiro) subjects. Reduction in antibiotic prescription was calculated to be 35–44%, ($P < 0.001$–0.004), when host response was evaluated in addition to bacterial pathogen detection. Results presented in Table 1.

Conclusion. Host response may aid in differentiating viral infection and bacterial colonization from invasive bacterial infections requiring antibiotics.

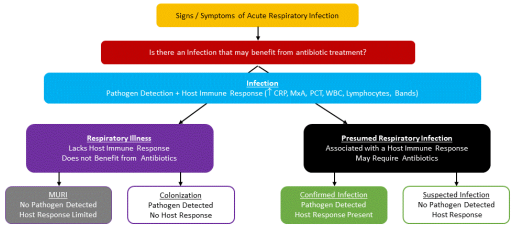

Figure 1. Definition of Infection: Pathogen Detection and Host Response