MSCRAMM Proteins Linked to Immune Cell Recruitment in Influenza and Bacterial Super-Infection

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Abstract

Influenza is one of the most common human respiratory illnesses, but when paired with secondary bacterial pneumonia (super-infection), it increases rates of hospitalization and death. Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common secondary bacterial pneumonia following influenza. The increase in prevalence of community acquired MRSA (CA-MRSA) has led to increasing rates of MRSA pneumonia and influenza MRSA super-infections[9]. MRSA is a gram-positive bacterium that is resistant to many antibiotics, leading to limited clinical interventions for pneumonia and super-infection. Attachment to host cells is controlled by staphylococcal surface proteins called MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix Molecules). Clumping Factor B (ClfB) and Serine-aspartate repeat-containing protein D (SdrD) are two MSCRAMM family members that have known colonization roles in the nose. We hypothesize that these proteins have roles in causing infection in the lung as well. In comparing wild type (WT) MRSA and mutated strains, We have found that SdrD decreases the recruitment of neutrophils whereas ClfB increases recruitment of lymphocytes and eosinophils in the lung during super-infection. This suggests that MSCRAMMs play a role in immune cell recruitment into the lung during infection.

Background

- Influenza with secondary bacterial pneumonia (super-infection) lead to higher rates of hospitalization and death than influenza alone[1].
- Methicillin-resistant *S. aureus* (MRSA) is more virulent and harder to treat than *S. aureus*
- Due to the increase in community acquired MRSA (CA-MRSA), *S. aureus* is the most common secondary infecting bacteria[7]
- Little is known about what staphylococcal factors in the bacterial pneumonia and whether these factors play a role in susceptibility of co-infection with influenza[9]
- Adhesion to host cells is important in bacterial infection. MSCRAMMs (microbial surface component recognizing adhesive matrix molecules) help MRSA bind to host proteins and receptors
- The immune response to MRSA is led by white blood cells (WBC). These cells play a leading role in inflammatory response of the bacterial super-infection. Neutrophils, macrophages, eosinophils, and lymphocytes are important in super-infection[8]
- WBC can be stained and differentiated by cell morphology (**Figure 1**).
- We hypothesize that MSCRAMM proteins affect the WBC populations during super-infection

Methods

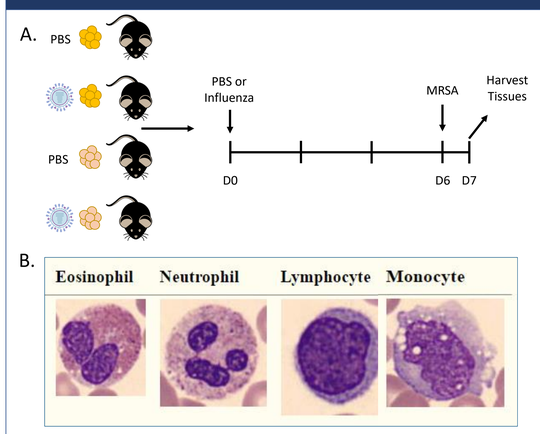

Figure 1. Methodology for studies. A. For MRSA pneumonia and influenza MRSA super-infection, mice were inoculated with phosphate buffered saline (PBS) or 100 plaque forming units (PFU) of mouse adapted influenza A/PR/8/34 H1N1 (PR8). After six days the mice were infected with $1x10^8$ colony forming units (CFU) of *S. aureus* MRSA strain USA300 JE2 or corresponding mutant for twenty-four hours then euthanized. Bronchoalveolar lavage (BAL) was collected by lavaging the lungs with 1 ml of PBS. Total cells in the BAL were counted and were placed onto slides using a cytospin. Slides were stained with Hema-3 and 200 cells were differentially counted per slide based on cellular morphology of immune cells. The total number and percentages of immune cells were calculated from slide cell counts and total BAL cells. **B.** Morphology of the four types of WBC important in super-infection.

Immune Infiltrate in Pneumonia and Super-infection

WT vs. *ΔsdrD*

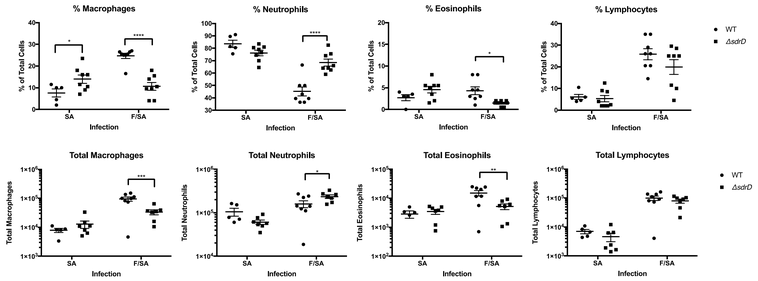

Figure 2. Percent and total number of immune cells in mice infected with WT or *ΔsdrD* MRSA. Mice were infected as described in methods section. Immune cells in BAL were plated on slides using a cytospin. Cells were counted to determine the percent of each WBC and the approximate total number was calculated based on total cell count. Statistics were performed using a Two-way ANOVA, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. n=8

WT vs. *ΔclfB*

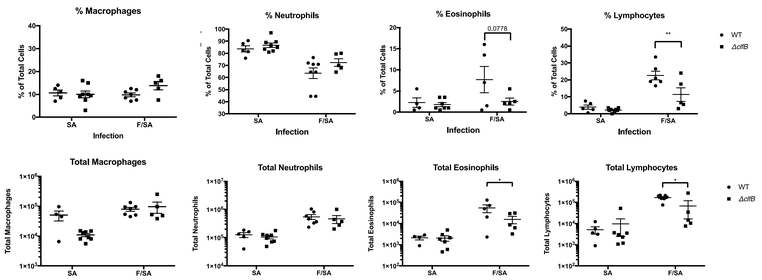

Figure 3. Percent and total number of immune cells in mice infected with WT or *ΔclfB* MRSA. Mice were infected as described in methods section. Immune cells in BAL were plated on slides using a cytospin. Cells were counted to determine the percent of each WBC and the approximate total number was calculated based on total cell count. Statistics were performed using a Two-way ANOVA, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. n=8

SdrD Alters Macrophage and Neutrophil Chemokines

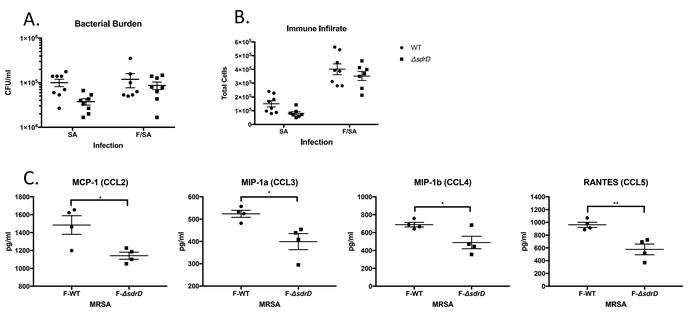

Figure 4. SdrD alters macrophages and neutrophil chemokines. While there is no change in overall bacterial burden (**A**) or immune infiltrate (**B**), SdrD induces changes to chemokines responsible for recruiting macrophages and neutrophils[2] (**C**). **B.** Bacterial burden was determined by homogenizing the right upper lung lobe in 1ml of PBS and plating serial dilutions. **C.** Chemokines[3,6] were measured in homogenized lung using a Procarta 36plex kit. Statistics were performed using a T test, Two-way ANOVA, * p<0.05, ** p<0.01. n=4-8

ClfB Induces Immune Inflammation in Super-infection

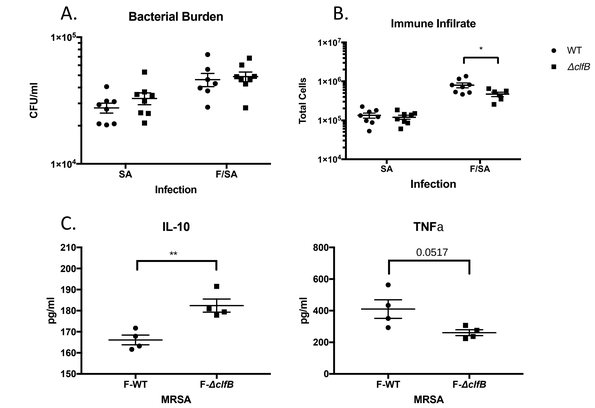

Figure 5. ClfB induces inflammation during super-infection. While there is no change in overall bacterial burden (**A**), ClfB induces a higher immune infiltrate during super-infection (**B**). **C.** ClfB decreases the anti-inflammatory cytokine IL-10[5] while increasing the pro-inflammatory cytokine TNFa[4] . **B.** Bacterial burden was determined by homogenizing the right upper lung lobe in 1ml of PBS and plating serial dilutions. **C.** Chemokines were measured in homogenized lung using a Procarta 36plex kit. Statistics were performed using a T test, Two-way ANOVA, * p<0.05, ** p<0.01. n=4-8

Discussion

- MSCRAMM proteins affects the immune response in super-infection
- Mice infected with *ΔsdrD* have decreased macrophages and increased neutrophils in super-infection. This corresponds to changes in macrophage chemokines.
- Mice infected with *ΔclfB* have less inflammation in super-infection as measured by immune infiltrate, specifically due to decreases in eosinophils and lymphocytes. There are also changes in pro- and anti-inflammatory cytokines

Future Directions

- Investigate function of WBCs influenza MRSA super-infection
  - Bacterial killing, cytokine/chemokine production
- Investigate the role of other MSCRAMMS in the lung during super-infection

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