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-infection. 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 (CFB) and Serine-asperate 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 mutant strains, We have found that SdrD decreases the recruitment of neutrophils whereas CFB 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

- Infection with secondary bacterial pneumonia (super-infection) leads to higher rates of hospitalization and death than influenza alone.
- 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.
- Little is known about what staphylococcal factors in the bacterial pneumonia and whether these factors play a role in susceptibility to co-infection with influenza.
- 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 to the bacterial super-infection. Neutrophils, macrophages, eosinophils, and lymphocytes are important in super-infection.
- WBC can be stained and differentiated by cell morphology (Figure 1).
- We hypothesize that MSCRAMM proteins affect the WBC populations during super-infection.

Methods

A. Schematic of methods. A. For MRSA pneumonia and influenza MRSA infected (350×10^6 cfu/ml) C57BL/6J mice were inoculated intranasally with phosphate buffered saline (PBS) or 100 plaque forming units (PFU) of influenza A/PR/8/34 (H1N1) virus (PR8). After 24 h the mice were infected with SdrD colony forming units (CFU) of S. aureus strain USA300 F2249 or corresponding mutant for twenty-four hours then euthanized. Bronchial lavages were collected from the right and left lung with 1 ml of PBS. Total cells in the BAL were counted and we plated onto plates using a cytospin. Slides were stained with hematoxylin and eosin and cells were counted on a microscope. The total number and percentages of immune cells were calculated from slide cell counts and total cell count. B. Immunophenotyping of lung macrophages and neutrophils. Figure 1. Methodology for studies. A. For MRSA pneumonia and influenza MRSA infected (350×10^6 cfu/ml) C57BL/6J mice were inoculated intranasally with phosphate buffered saline (PBS) or 100 plaque forming units (PFU) of influenza A/PR/8/34 (H1N1) virus (PR8). After 24 h the mice were infected with SdrD colony forming units (CFU) of S. aureus strain USA300 F2249 or corresponding mutant for twenty-four hours then euthanized. Bronchial lavages were collected from the right and left lung with 1 ml of PBS. Total cells in the BAL were counted and we plated onto plates using a cytospin. Slides were stained with hematoxylin and eosin and cells were counted on a microscope. The total number and percentages of immune cells were calculated from slide cell counts and total cell count. B. Immunophenotyping of lung macrophages and neutrophils.

Immune Infiltrate in Pneumonia and Super-infection

WT vs. AcdFB

WT vs. AsdrD

SdrD Alters Macrophage and Neutrophil Chemokines

Discussion

- MSCRAMMs proteins affects the immune response in super-infection.
- Mice infected with MRSA have increased macrophages and neutrophils in super-infection. This corresponds to changes in macrophage chemokines.
- Mice infected with CFB 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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References