O105

2-hour Oral Session

Mechanisms of action of human IVIG for treatment of community-associated methicillin-resistant *Staphylococcus aureus* necrotizing pneumonia

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Background: Necrotizing pneumonia caused by community-associated MRSA strains is associated clinically with a rapid and high mortality rate, despite appropriate antibiotic treatment. There is an urgent need for new therapeutic approaches that would supplement current antibiotic therapy to improve survival during the acute phase of the infection. *In vitro* data showing that intravenous immunoglobulin (IVIG) contains antibodies against Panton-Valentine leukocidin (PVL) and alpha-toxin (Hla), and a limited number of case reports have documented use of IVIG as an effective adjunct to antimicrobial therapy to treat severe cases of PVL-associated staphylococcal pneumonia.

Methods: A rabbit model of necrotizing pneumonia was used to identify toxins critical for the pathogenesis of necrotizing pneumonia using major epidemic clones of CA-MRSA. Isogenic mutants containing in-frame deletions of 17 different toxin genes at 8 chromosomal loci were constructed and compared to a USA300 parent strain for their capacity to cause acute lung injury in the rabbit model. Survival of rabbits infected with a lethal dose of a PVL-producing USA300 CA-MRSA strain after treatment of 200 mg/kg once with IVIG, anti-Hla IgG and anti-PVL IgG affinity purified from IVIG, or IVIG depleted for anti-PVL and anti-Hla IgG, or saline were also compared.

Results: Only *Δpvl*, *Δhla* and to a lesser extent *Δpsm-α* exhibited reduced capacity to cause the disease in term of survival, acute lung injury and acute lung inflammation. Other major CA-MRSA clones (ST1, ST30, ST59, and ST80) also demonstrated a similar capacity to cause rapidly lethal necrotizing pneumonia compared to USA300, and PVL and Hla produced at toxic concentrations in the lungs of rabbits infected with these five hypervirulent strains. IVIG administered at 24 h and 1.5 h before infection and 1.5 h after infection resulted in 50% reduction in mortality compared to saline. Treatment with IVIG and vancomycin resulted in 70% reduction in mortality compared to treatment with vancomycin alone or saline. Treatment with 1 mg/kg of anti-LukS/F-PV IgG or anti-HLA IgG were similar to 200 mg/kg IVIG in reducing mortality, whereas treatment with IVIG depleted of anti-LukS/F-PV IgG or anti-HLA IgG did not result in significant reduction in mortality when compared to saline.

Conclusions: Our results indicate that PVL and Hla are the major virulence factor in necrotizing pneumonia and that neutralization of these toxins by specific antibodies in IVIG confers protection. These data strongly support the use of IVIG as adjunctive therapy for the treatment of necrotizing pneumonia.