The Effect of PspA and PspC on Complement Deposition on Pneumococci and Their Immune Adherence to Erythrocytes through the CR1 Receptor

David E. Briles[1], Jie Li[1], Jennifer Wang[2], Haley Echlin[1], Anna Cerney[2], Robert Finberg[2], and Alex Szalai[3]

Departments of [1]Microbiology and [3]Medicine at the University of Alabama at Birmingham, USA and the [2]Department of Medicine at the University of Massachusetts Medical School, Worcester, Massachusetts, USA

Streptococcus pneumoniae (pneumococci) are major causes of pneumonia, meningitis, and otitis media in humans. In the majority of cases of pneumococcal pneumonia the pneumococci are largely confined to the lung and upper airways. In those patients the pneumococci that do enter the blood are rapidly cleared. However, in other patients pneumococci in the blood are able to survive well enough to increase in numbers. Once this happens the patient can become septic and have a poor prognosis. Pneumococcal sepsis occurs often enough that over 1 million children die each year of pneumococcal pneumonia worldwide and over 10,000 elderly individuals die in the US each year of pneumococcal pneumonia. Thus, the ability of pneumococci to be cleared from the blood is critical to the survival of the infected patient.

Data from animals and disease rates in complement deficient humans make it clear that the clearance of pneumococci from the blood requires their opsonization with complement. Antibody and C-reactive protein are able to enhance the opsonization of pneumococci with complement.

Pneumococci are protected from complement-mediated clearance from the blood by the pneumococcal capsule, and the pneumococcal virulence proteins PspA, PspC, pneumolysin, and Pht. In the absence of significant concentrations of specific antibody in the serum the complement deposition on pneumococci is dependent on the classical pathway. PspA interferes with C1q binding to the pneumococcal surface and thus blocks the classical pathway of complement activation. In the absence of PspA the complement activation triggered by the classical pathway is amplified by the alternative pathway. PspC interferes with alternative pathway amplification of the complement activation that is triggered by the absence of PspA. Thus, in the absence of both PspA and PspC significant amounts of C3 are deposited on pneumococci and they are quite avirulent in mice.

Once complement C3 is deposited on pneumococci, either through the absence of PspA and PspC or through the presence of antibody to capsule, the pneumococci are able to show immune adherence to the CR1 receptor of human red blood cells (RBC). Once bound to the human RBC through C3b and the CR1 receptor, pneumococci can be readily transferred to macrophages where they can be killed. This process requires C3

since neither the immune adherence nor the transfer of pneumococci from RBC to macrophages occurs in C3-depleted sera. However, if purified C3 is use to restore C3 activity to the original C3-depleted system then immune adherence and transfer of pneumococci from RBC to macrophages is restored.

The role of the CRIII complement receptor on the macrophages was shown by the fact that pre-treatment of the macrophages with antibody to CRIII reduced transfer of the pneumococci from RBC to macrophages by over 50%. In the case where the pneumococci were opsonized with both antibody and complement, we observed that prior treatment of the macrophages with antibody to the Fc-gammaRIII/II receptor also caused about 50% inhibition of transfer of the opsonized pneumococci from RBC to macrophages

The role of the CR1 receptor on the red blood cells was demonstrated using mice that were transgenic for the human CR1 receptor. Unlike humans, mice lack CR1 on their RBC. Measurement of immune adherence and the transfer of pneumococci from RBC to macrophages was carried out using wild-type mouse RBC and transgenic mouse RBC expressing human CR1. These studies demonstrated that CR1 is necessary for immune adherence and transfer of opsonized pneumococci from RBC to macrophages.

The significance of this process to in vivo clearance of pneumococci was determined by making use of the transgenic mice that express human CR1 on their red blood cells. In one study pneumococci opsonized with complement were mixed with RBC from CR1-transgenic mice or RBC from wild-type mice. When the mixtures were injected intravenously into wild-type mice it was observed that the opsonized pneumococci that had been pre-incubated with CR1-expressing RBC were cleared from the blood more rapidly than those pre-incubated with wild-type RBC. In other studies wild-type or CR1-transgenic mice were infected with pneumococci intravenously. As expected, we observed better clearance from the blood of pneumococci injected into the CR1-transgenic mice versus the pneumococci injected into the wild-type mice. These studies make it clear that CR1 expression on RBC has an actual, not just a theoretical effect on the clearance of pneumococci from the blood. The studies also indicate that vaccine antigens that elicit antibody-mediated blood clearance of pneumococci may work even better in humans than they do in mice.