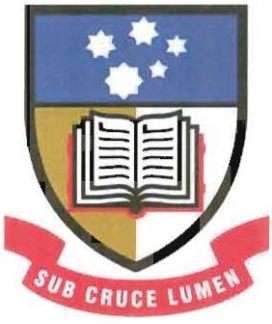
CXC Chemokine Responses of Respiratory Epithelial Cells to *Streptococcus pneumoniae*



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Abstract

Streptococcus pneumoniae (the pneumococcus) remains a major cause of morbidity and mortality worldwide, particularly in young children and the elderly. It is responsible for a spectrum of diseases ranging from otitis media, to potentially fatal conditions such as pneumonia and meningitis, and is estimated to cost health services billions of dollars each year. The interaction of *S. pneumoniae* with the host generally begins in the nasopharynx, and invasive disease is almost invariably preceded by nasopharyngeal colonisation. In some circumstances, *S. pneumoniae* may translocate from the nasopharynx to the lungs where pneumonia can develop, and inflammation is believed to play a role in this process. The presence of pneumococci in the lungs also triggers an inflammatory response, which is important for clearance of the bacteria. However, a prolonged inflammatory response leads to tissue damage, and is linked with a poor prognosis of disease. It has been shown that respiratory epithelial cells are able to play an active part in the response to respiratory pathogens by releasing chemokines that are responsible for neutrophil recruitment, and it has recently been shown that infection of type II pneumocytes with *S. pneumoniae* leads to the release of interleukin (IL)-8. In order to determine the role of specific pneumococcal factors in eliciting a CXC chemokine response from type II pneumocytes (A549) and nasopharyngeal cells (Detroit-562), monolayers of these cells were infected with wild type (WT) *S. pneumoniae* D39, or mutants deficient in choline binding protein A (CbpA), pneumococcal surface protein A (PspA), or pneumolysin (Ply), and the CXC chemokine mRNA response was measured by real-time RT-PCR. Release of IL-8 was also measured by ELISA. In response to WT D39, both A549 and Detroit-562 cells showed a significant increase in CXC chemokine mRNA, and IL-8 protein. This response was increased 2-fold when a CbpA-negative (ΔCbpA) mutant was used to infect cells, suggesting that CbpA may have an

inhibitory effect on the CXC chemokine response of these cells. Further investigation demonstrated that this activity is dependent on the N-terminal region of CbpA and that all three N-terminal domains are required for this effect, as deletion of any one of these domains had the same effect on the CXC chemokine response as removing CbpA altogether.

Infection with a PspA-negative mutant (ΔPspA) led to a 2-fold decrease in the CXC chemokine response of A549 cells, compared to infection with WT D39 at 2 h, but no difference was seen in the response of Detroit-562 cells to this mutant compared to WT D39. Thus, PspA appears to have the ability to stimulate an early CXC chemokine release from A549 cells. Deletion of the first of 2 regions of the N-terminal α-helical domain of PspA reduced the ability of *S. pneumoniae* to elicit a chemokine response to the same degree as removing PspA altogether, indicating that it is this region that is responsible for the chemokine inducing ability of PspA.

Ply appeared to have no effect on the CXC chemokine response of A549 cells with no obvious difference seen in the response of these cells to ΔPly compared to WT D39. However, infection of Detroit-562 cells with ΔPly led to a 2-fold decrease in IL-8 mRNA and protein release compared to WT D39. Using D39 strains producing mutant forms of Ply with reduced cytotoxicity and/or complement activating abilities, the role of the cytotoxic activity of Ply was demonstrated to be important in generation of a chemokine response from both cell lines. Infection of A549 or Detroit-562 cells with mutants producing Ply with only 0.02% or 0.1% haemolytic activity led to a 2-fold decrease in IL-8 release compared to that elicited by WT D39. The complement activating ability of Ply also appeared to be important in the generation of a CXC chemokine response from A549 cells. Cells infected with a mutant that produced Ply with no complement activating ability released significantly less IL-8 than cells infected with WT D39. This activity of Ply did not appear to have an effect on the CXC chemokine release of Detroit-562 cells. Thus all three virulence factors investigated had some role in the ability of *S. pneumoniae* to generate a CXC chemokine response from

respiratory epithelial cells, although their roles and the cell lines that were affected differed.