

Improving Pulmonary Immunity to Bacterial Pathogens Through *Streptococcus pneumoniae* Colonisation of the Nasopharynx

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Streptococcus pneumoniae is a common cause of bacterial pneumonia especially in the elderly or those with significant comorbidities, and is also frequently associated with exacerbations of COPD (1, 2). Existing *S. pneumoniae* vaccines have partial strain coverage, may lack efficacy in high risk groups, and generally seem to have poorer efficacy against pulmonary compared to systemic infection (3,4). Hence alternative strategies to conventional vaccines maybe required to prevent the persisting high morbidity and mortality caused by *S. pneumoniae* lung infections.

Mitsi et al. present data obtained using the Experimental Human Pneumococcal Colonisation (EHPC) model that suggest one such alternative strategy for preventing pneumonia caused by multiple bacterial pathogens, including *S. pneumoniae*. Repeated episodes of *S. pneumoniae* colonisation throughout life induce and repeatedly boost protective antibody to both capsular and multiple protein antigens as well as poorly defined cellular immunity (5,6,7,8). Here, Mitsi et al. have now used the EHPC model to investigate the effects of *S. pneumoniae* colonisation on alveolar macrophage (AM) function in healthy volunteers and identified a novel mechanism by which successful colonisation improves lung immunity to multiple bacterial pathogens (9). *S. pneumoniae* AM (recovered by bronchoalveolar lavage) phagocytic capacity improved from 69% in uncolonized EHPC subjects to 80.4% for EHPC subjects that

were successfully colonised. This was a convincing change that was strengthened by a significant correlation to the density of *S. pneumoniae* colonisation of the nasopharynx. Matched pre- and post-colonisation data from each subject would clearly provide stronger evidence that successful *S. pneumoniae* nasopharyngeal colonisation was responsible for the differences in AM phenotypes, but is logistically difficult as it would require each volunteer to have two bronchoscopies and the first bronchoscopy could also affect the function of AMs recovered by a second bronchoscopy.

AM phagocytosis of invading pathogens is a major component of pulmonary innate immunity (10,11,12). However, whether a 16% relative increase in AM phagocytic capacity translates into improved protection against pneumonia is not at all clear – we simply do not know what degree of improvement in AM phagocytosis *in vitro* will result in a reduced risk of pneumonia. Furthermore, bacteria were opsonised with 1/16 pooled human IgG as well as complement and these conditions may not accurately represent the situation in epithelial lining fluid. Under alternative opsonising conditions the strength of the differences in AMs from colonised or uncolonized individuals may alter. However, whether bacteria reaching the lung establish active infection depends on the balance between host clearance mechanisms (comprising of mucociliary clearance, and epithelial and AM mediated killing mechanisms) versus pathogen virulence (a combination of replication rate and efficacy at evading pulmonary immunity) (Fig. 1) (10). It is therefore feasible that even a 16% relative improvement in AM phagocytosis could tip the balance in favour of the host for a substantial proportion of bacterial invasion events, and importantly the duration of the effect was surprisingly long at up to 120 days. However, it will require carefully designed animal experimentation and eventually clinical trials to demonstrate whether this improvement

in AM function translates to improved protection against infection. As well as their role as phagocytes AM act as sentinel cells that initiate inflammation (11), and it will be important to assess whether the macrophage inflammatory response to bacterial pathogens are affected by prior *S. pneumoniae* colonisation as this may also alter susceptibility to pneumonia.

Another novel observation made by Mitsi et al. was the detection of *S. pneumoniae* in BAL by PCR and culture in 41% of successfully colonised subjects, but at a time when they had already been treated with amoxicillin and had no detectable nasopharyngeal colonisation with *S. pneumoniae*. Previously it was thought *S. pneumoniae* reaching the lungs by microaspiration from the nasopharynx were rapidly cleared or occasionally resulted in pneumonia. These data show that *S. pneumoniae* can persist within the lung even after colonisation has been cleared, creating a reservoir of bacteria that could cause ongoing immune stimulation or even develop into pneumonia at a later stage. *S. pneumoniae* lung persistence could occur due to colonisation of the bronchial tree, becoming part of the respiratory microbiome, but another possibility is bacterial survival within AMs similar to the approach taken by *Mycobacterium tuberculosis*. *S. pneumoniae* is classically considered a purely extracellular pathogen, yet recent data suggest this is too simplistic. Some *S. pneumoniae* can persist within macrophages for many hours (12), and more recently *S. pneumoniae* have even been shown to replicate within a specific subset of marginal zone splenic macrophages (13). Intriguingly, Mitsi et al. identified *S. pneumoniae* internalised by AMs, an observation that needs further investigation to characterise which cellular compartment contains the bacteria, bacterial viability, and whether a particular sub-type of AM is involved.

What is the mechanism for improved AM phagocytic capacity after successful *S. pneumoniae* nasopharyngeal colonisation? The authors suggest two plausible mechanisms, either a manifestation of trained immunity with exposure to *S. pneumoniae* stimulating epigenetic changes in AMs or alternatively interferon gamma release from antigen-stimulated Th1 CD4 cells could improved AM function. A Th1 mechanism was supported by the association of successful colonisation with increased numbers of BAL Th1 CD4 cells, and by the positive correlation of AM phagocytic function with interferon gamma expression by lung CD4 cells after restimulation with *S. pneumoniae*. In addition, nanostring PCR showed colonisation was associated with a shift in AM phenotype towards a Th1-activated pattern, and this also showed some correlation with improved phagocytosis. Clarifying which mechanism(s) is/are involved is important as this may identify how the findings by Mitsi et al. can be exploited to prevent lung infections. Possible strategies include nasal administration of live virulence attenuated *S. pneumoniae*, *S. pneumoniae* Th1 antigens, or bacterial components that stimulate trained immunity in AMs.

The data presented by Mitsi et al. both challenges our pre-conceptions about *S. pneumoniae* biology and describes a novel mechanism that may improve lung immunity to bacterial pathogens. The results show that the interactions between bacterial colonisation of the respiratory tract and host immunity are highly complex, further investigation of which could lead to novel strategies for preventing bacterial lung infections.

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Legends:

Figure 1: Mechanisms by which nasopharyngeal colonisation by *S. pneumoniae* may improve protection against pneumonia. (A) Colonisation boosts pre-existing cellular (protein antigen-dependent Th1, Th2, Th17 CD4) and humoral (antibody to both protein and capsular antigens) adaptive immunity to *S. pneumoniae* (5,6,7,8). Mitsi *et al* (9) show colonisation leads to improved AM phagocytic capacity (B), potentially mediated by Th1 cellular immune responses (C) or by an antigen-independent trained immunity response (D). In addition, improved antibody responses could increase AM phagocytic capacity by improving *S. pneumoniae* opsonisation (E). Improved phagocytic capacity increases the clearance of bacterial pathogens reaching the lung (F), tilting the outcome of early bacterial / host lungs in the lung towards prevention of pneumonia. Mitsi *et al* (9) also show *S. pneumoniae* persistence within the lungs, which could contribute to improved immune responses (G) or could be a source of bacteria that develop into active infection (H) if bacterial numbers are poorly controlled.

Fig. 1

