

improved mucociliary clearance and mucous viscosity induced by losartan will enhance infection resolution.

The question remains: is blocking CFTR function the perfect setting for the preclinical development of losartan? If not, what model really reproduces a reasonable, cost-effective means by which to explore specific therapies in the context of consistent and reproducible complexities of CF lung disease? Kim and colleagues' manuscript introduces the potential of losartan as a CF therapeutic and also highlights innovations in model development used for open-minded investigations and for the systematic development of novel therapeutics for CF. Innovation here is the key. ■

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Improving Pulmonary Immunity to Bacterial Pathogens through *Streptococcus pneumoniae* Colonization of the Nasopharynx

Streptococcus pneumoniae is a common cause of bacterial pneumonia, especially in the elderly and patients with significant comorbidities, and is also frequently associated with exacerbations of chronic obstructive pulmonary disease (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 infection than against systemic infection (3, 4). Hence, alternative strategies to conventional vaccines may be required to prevent the persistent high morbidity and mortality caused by *S. pneumoniae* lung infections.

Mitsi and colleagues present data obtained using the experimental human pneumococcal colonization (EHPC) model that suggest one such alternative strategy for preventing pneumonia caused by multiple bacterial pathogens, including *S. pneumoniae*. Repeated episodes of *S. pneumoniae* colonization throughout life induce and repeatedly boost protective antibody to both capsular and multiple protein antigens, as well as poorly defined cellular immunity (5–8). In a study presented in this issue of the *Journal*, Mitsi and colleagues (pp. 335–347) used the EHPC model to investigate the effects of *S. pneumoniae* colonization on alveolar macrophage (AM) function in healthy volunteers and identified a novel mechanism by which successful colonization improves lung immunity to multiple bacterial pathogens (9). The phagocytic capacity of *S. pneumoniae* AMs (recovered by BAL) improved from 69% in uncolonized EHPC subjects to 80.4% in EHPC subjects who were successfully colonized. This was a convincing change that was strengthened by a significant correlation to the density of *S. pneumoniae* colonization of the nasopharynx. Matched pre- and

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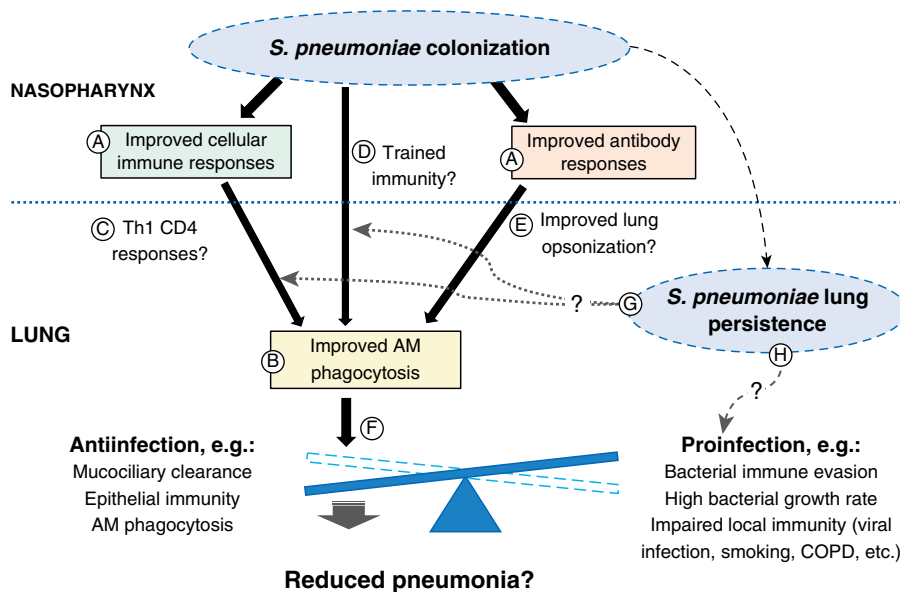


Figure 1. Mechanisms by which nasopharyngeal colonization by *Streptococcus pneumoniae* may improve protection against pneumonia. Colonization boosts preexisting cellular (protein antigen–dependent T-helper cell type 1 [Th1], Th2, and Th17 CD4) and humoral (antibody to both protein and capsular antigens) adaptive immunity to *S. pneumoniae* (A) (5–8). Mitsi and colleagues (9) show that colonization leads to improved alveolar macrophage (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* opsonization (E). Improved phagocytic capacity increases the clearance of bacterial pathogens that reach the lung, potentially shifting the outcome of early bacterial/host interactions toward prevention of pneumonia (F). Mitsi and colleagues 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. COPD = chronic obstructive pulmonary disease.

postcolonization data from each subject would clearly provide stronger evidence that successful *S. pneumoniae* nasopharyngeal colonization was responsible for the differences in AM phenotypes; however, obtaining such data would be logistically difficult because it would require each volunteer to undergo two bronchoscopies, and the first bronchoscopy could also affect the function of AMs recovered by the second bronchoscopy.

AM phagocytosis of invading pathogens is a major component of pulmonary innate immunity (10–12). 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 opsonized 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 opsonizing conditions, the strength of the difference between AMs obtained from colonized and uncolonized individuals may vary. However, whether bacteria that reach the lung establish active infection depends on a balance between host clearance mechanisms (i.e., mucociliary clearance and epithelial cell- and AM-mediated killing mechanisms) and pathogen virulence (a combination of replication rate and efficacy in evading pulmonary immunity) (Figure 1) (10). It is therefore feasible that even a 16% relative improvement in AM phagocytosis could tip the balance in favor of the host in a

substantial proportion of bacterial invasion events, and importantly, the duration of this effect was surprisingly long (up to 120 days). However, it will require carefully designed animal experiments and eventually clinical trials to demonstrate whether this improvement in AM function translates to improved protection against infection. In addition to their role as phagocytes, AMs act as sentinel cells that initiate inflammation (11), and it will be important to assess whether the macrophage inflammatory response to bacterial pathogens is affected by prior *S. pneumoniae* colonization, as this may also alter susceptibility to pneumonia.

Another novel observation made by Mitsi and colleagues was the detection of *S. pneumoniae* in BAL by PCR and culture in 41% of successfully colonized subjects, at a time when they had already been treated with amoxicillin and had no detectable nasopharyngeal colonization with *S. pneumoniae*. Previously it was believed that *S. pneumoniae* that reached 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 colonization 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* could persist in the lung due to colonization of the bronchial tree, becoming part of the respiratory microbiome; however, it is also possible that they survive within AMs in a manner similar to that observed for *Mycobacterium tuberculosis*. *S. pneumoniae* is classically

considered a purely extracellular pathogen, yet recent data suggest that this view is too simplistic. Some *S. pneumoniae* can persist within macrophages for many hours (12), and *S. pneumoniae* have even been shown to replicate within a specific subset of marginal zone splenic macrophages (13). Intriguingly, Mitsi and colleagues identified *S. pneumoniae* internalized by AMs, an observation that needs further investigation to characterize which cellular compartment contains the bacteria, the viability of the bacteria, and whether a particular subtype of AMs is involved.

What is the mechanism for improved AM phagocytic capacity after successful *S. pneumoniae* nasopharyngeal colonization? The authors suggest two plausible mechanisms: 1) trained immunity, with exposure to *S. pneumoniae* stimulating epigenetic changes in AMs, and 2) release of IFN- γ from antigen-stimulated T-helper cell type 1 (Th1) CD4 cells, resulting in improved AM function. A Th1 mechanism is supported by the association of successful colonization with increased numbers of BAL Th1 CD4 cells, and by the positive correlation between AM phagocytic function and IFN- γ expression by lung CD4 cells after restimulation with *S. pneumoniae*. In addition, NanoString PCR showed that colonization was associated with a shift in the AM phenotype toward a Th1-activated pattern, and this also showed some correlation with improved phagocytosis. It is important to clarify which mechanism(s) is involved because this may identify how the findings by Mitsi and colleagues can be exploited to prevent lung infections. Possible strategies include nasal administration of live virulence-attenuated *S. pneumoniae*, *S. pneumoniae* Th1 antigens, and bacterial components that stimulate trained immunity in AMs.

The data presented by Mitsi and colleagues both challenge our preconceptions about *S. pneumoniae* biology and describe a novel mechanism that may improve lung immunity to bacterial pathogens. The results show that the interactions between bacterial colonization of the respiratory tract and host immunity are highly complex, and further investigation of these interactions could lead to novel strategies for preventing bacterial lung infections. ■

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