The new first-line defense: the potential of nasopharyngeal colonization in vaccine strategies

Win-Yan Chan
Jonathan M Cohen
Jeremy S Brown

Centre for Inflammation and Tissue Repair, UCL Respiratory, Division of Medicine, University College London, London, UK

Abstract: Pathogens that can colonize the upper respiratory tract include *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Neisseria meningitidis*, *Moraxella catarrhalis*, and *Staphylococcus aureus*. While these pathogens commonly asymptomatically colonize the nasopharynx of healthy adults, disease progression may occur in some individuals. In addition to these respiratory pathogens, there are a large number of commensal species also found in the upper respiratory tract which only very rarely cause disease, creating a complex community of bacterial species in the nasopharynx. This review addresses the novel, potential strategies that utilize the interactions between both homologous and heterologous species in the nasopharynx to vaccinate individuals against pathogenic bacteria. These strategies include the mechanisms employed by colonizing bacteria to regulate the presence of other species in the nasopharynx and the effect that colonization of the nasopharynx has on the host immune response. Interventional strategies investigated so far include the introduction of nonpathogenic bacteria to the nasopharynx to immunize against a closely related species, controlled colonization using both wild-type and attenuated species, and the use of other nonpathogenic colonizers to express antigens from potential pathogens. All these approaches harness the ability of the colonization to induce a mucosal immune response that can protect against future infection. In this review, *S. pneumoniae* and *N. meningitidis* colonization are used as case studies for this approach as the immunological effects of colonization have been widely studied in animal and human models. Colonization-based strategies have great potential, and, in particular, the attenuated strain approach has produced some encouraging data in animal models. However, the strategy for attenuating virulence must be stringent and caused by highly stable mutations that are unlikely to revert. In addition, the consequences of artificial administration of genetically modified bacteria to the nasopharynx on the usual host microbiome are unknown and would need to be monitored carefully.

Keywords: *Streptococcus pneumoniae*, colonization, adaptive immunity, antibody, protein antigen, capsular antigen, *Neisseria* sp.

Introduction

Nasopharyngeal commensal species

The upper respiratory tract is a host to many commensal bacterial species that create a complex community of microbes. These commensal species include a number of potentially pathogenic bacteria that usually colonize the nasopharynx without further progression to disease, but occasionally can spread from the nasopharynx to the lungs or blood to cause serious infections such as pneumonia, septicemia, and meningitis. Potential pathogens found in the nasopharynx include *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Neisseria meningitidis*, *Moraxella catarrhalis*, and *Staphylococcus aureus*. While these pathogens commonly asymptomatically colonize the nasopharynx of healthy adults, disease progression may occur in some individuals. In addition to these respiratory pathogens, there are a large number of commensal species also found in the upper respiratory tract which only very rarely cause disease, creating a complex community of bacterial species in the nasopharynx. This review addresses the novel, potential strategies that utilize the interactions between both homologous and heterologous species in the nasopharynx to vaccinate individuals against pathogenic bacteria. These strategies include the mechanisms employed by colonizing bacteria to regulate the presence of other species in the nasopharynx and the effect that colonization of the nasopharynx has on the host immune response. Interventional strategies investigated so far include the introduction of nonpathogenic bacteria to the nasopharynx to immunize against a closely related species, controlled colonization using both wild-type and attenuated species, and the use of other nonpathogenic colonizers to express antigens from potential pathogens. All these approaches harness the ability of the colonization to induce a mucosal immune response that can protect against future infection. In this review, *S. pneumoniae* and *N. meningitidis* colonization are used as case studies for this approach as the immunological effects of colonization have been widely studied in animal and human models. Colonization-based strategies have great potential, and, in particular, the attenuated strain approach has produced some encouraging data in animal models. However, the strategy for attenuating virulence must be stringent and caused by highly stable mutations that are unlikely to revert. In addition, the consequences of artificial administration of genetically modified bacteria to the nasopharynx on the usual host microbiome are unknown and would need to be monitored carefully.

Keywords: *Streptococcus pneumoniae*, colonization, adaptive immunity, antibody, protein antigen, capsular antigen, *Neisseria* sp.
Hemophilus influenzae, Neisseria meningitidis, Moraxella catarrhalis, and Staphylococcus aureus. The nasopharynx provides a relatively stable environment in which the commensal flora can flourish and be transmitted to other hosts. The duration of carriage varies for each species and also between the different strains of the same species, from weeks to months and likely to be years for some species. The dynamics of nasopharyngeal colonization by different species are delicately balanced, and disturbances to this ecology may allow pathogenic organisms to cause disease. Factors that affect this balance include the acquisition of different species, interspecies interactions, bacterial interactions with the host, and interference by environmental factors. For instance, nasopharyngeal colonization by Staphylococcus pneumoniae protects against Staphylococcus aureus carriage, and conversely reduction in Staphylococcus pneumoniae carriage by vaccination could lead to increased carriage of Staphylococcus aureus. Similarly, in animal models, bacterial species compete in establishing colonization.

Colonization of the nasopharynx by bacteria can influence the host immune system even in the absence of overt disease. Epidemiologic studies have shown that the development of asthma and chronic obstructive pulmonary disease is related to the diversity of colonizing organisms in the nasal flora. In mice, lack of microbial colonization increases allergic airway inflammation, and colonization with multiple species protects against airway inflammation. Importantly, nasopharyngeal colonization by bacterial pathogens can be an immunizing event, stimulating both humoral and cellular adaptive immune responses that protect against either re-colonization or subsequent invasive disease. These observations suggest that novel vaccine strategies could harness the immunizing effects of nasopharyngeal colonization to prevent serious infections, and this is the subject addressed in this review with a particular focus on the potential of nasopharyngeal colonization for the prevention of Staphylococcus pneumoniae and Neisseria meningitidis infections, which are used as case studies that illustrate the benefits and potential drawbacks of this approach.

Colonization and existing vaccines for Staphylococcus pneumoniae and Neisseria meningitidis

Staphylococcus pneumoniae

Colonization with Staphylococcus pneumoniae is universal in the first few months of life, with between 50% and 90% of children aged under 2 years colonized at any one time, sometimes with multiple strains. Peak carriage rates occur at 3–5 years of age and then wane to ~10% in adult life. Carriage prevalence depends on geographical location, and is generally higher in the developing world. While initial colonization events may persist for up to 4 months, duration appears to shorten with increasing age to 2–4 weeks. The proportion of Staphylococcus pneumoniae colonization events associated with disease is low in healthy adults. However, as colonization is very common, Staphylococcus pneumoniae is a leading cause of acute otitis media (OM), pneumonia, sepsis, and meningitis globally causing an estimated 2,858,000 severe pneumonia episodes and 411,000 deaths annually worldwide in infants. The use of vaccines targeting polysaccharide capsule antigen in children has reduced the overall incidence of pneumococcal disease. However, the adult vaccine fails to protect against pneumonia, a key cause of respiratory morbidity and mortality in elderly subjects with comorbidities. In addition, the existing vaccines have a high cost of manufacture and have major limitations in strain coverage, only protecting against between seven and 23 of the 93+ Staphylococcus pneumoniae capsular serotypes. This restricted serotype coverage has led to the replacement of Staphylococcus pneumoniae vaccine serotypes by non-vaccine serotypes as both colonizers of the nasopharynx and causes of disease. Hence, there is a strong interest in alternative vaccine strategies that target all Staphylococcus pneumoniae strains and could also prevent lung infection.

In addition to the profound effect on the relative prevalence of vaccine and non-vaccine Staphylococcus pneumoniae serotypes, the introduction of the pneumococcal conjugate vaccine to infant immunization schedules has also disrupted the ecology of the nasopharyngeal flora in general. Although the short- and long-term consequences of these changes are not yet clear, they still raise some potential concerns about the effects of vaccination. The complete eradication of all Staphylococcus pneumoniae from the nasopharynx may remove the competition that Staphylococcus pneumoniae exerts on other potentially pathogenic organisms, perhaps allowing their overgrowth. This could in turn lead to a greater incidence of disease caused by Hemophilus influenzae, Neisseria meningitidis, Staphylococcus aureus, or Moraxella catarrhalis. This disruption to the nasal flora will likely have implications on the incidence of disease and antibiotic strategies in the future.
time. The carriage rate is the lowest in young children and highest in young adults, with the UK rates of 3% of children under 4 years, 24%–37% of the age group of 15–24 years, and <10% in older age groups. Similar to S. pneumoniae, carriage rates are higher in smokers and after viral respiratory tract infections.

Twelve different meningococcal serogroups have been defined based on capsular polysaccharide structure, of which A, B, C, W135, and Y are responsible for the majority of the disease. In addition, serogroup X has more recently been identified as the cause of sepsis and meningitis in Africa. Group A causes large-scale epidemics mainly in Africa but also in Asia, whereas the majority of cases of N. meningitidis disease in Europe and America are caused by serogroups B and C strains. As with S. pneumoniae, vaccines are currently polysaccharide based with newer vaccines utilizing protein conjugation to offer improved protection in children. In the UK, a conjugate vaccine to protect against serotype C has been in use since 1999 in infants, and also for teenagers and young adults. Unlike the serotype replacement seen with the introduction of the pneumococcal conjugate vaccine, the introduction of a vaccine for meningococcus C has not seen a rise in carriage or disease by meningococcus B. A conjugate vaccine targeting groups A, C, W135, and Y has been administered to adolescents since early 2016. Serogroup B has a capsule that is particularly poorly immunogenic, and the meningococcus B vaccine is based on outer membrane vesicles and protein antigens rather than capsular polysaccharide. This vaccine was added to the UK infant immunization schedule in 2015. Vaccine-related reduction in carriage may have contributed to herd immunity as was seen for S. pneumoniae.

Regulation by commensal species
The nasal flora is acquired shortly after birth and is influenced by the environment, including contact with other persons. The competition between potentially pathogenic bacteria and commensal species in the nasopharynx contributes to the regulation of pathogenic species, which are also influenced by host responses. Environmental changes such as the season will influence the makeup of the nasal flora, as will therapeutic interventions such as vaccination or antimicrobials. For example, children with pneumococcal OM treated with antibiotics, or those who were immunized with the pneumococcal conjugate vaccine, had a decrease in the prevalence of Streptococaceae and Corynebacteriaceae commensal species in the nasopharynx. Nasopharyngeal commensal species could prevent respiratory and invasive disease caused by pathogenic commensal species through a number of different mechanisms. These include the inhibition of colonization by potential pathogens by competition, either passively (occupying the same ecological niche) or actively (via direct growth inhibition or killing of competitor species). For example, in the gut, competition for nutrients causes a process referred to as colonization resistance, which is integral to controlling pathogenic bacteria such as enterohemorrhagic Escherichia coli, or Clostridium difficile, and similar processes are likely in the nasopharynx. Commensal species can also produce antimicrobial peptides that directly affect pathogen growth or survival. For example, the poorly pathogenic commensal species, Streptococcus salivarius, produces bacteriocins that inhibit S. pneumoniae, and in the gut enterohemorrhagic E. coli is inhibited by bacteriocins produced by other E. coli strains. On the skin, the commensal species, Staphylococcus epidermidis, produces antimicrobial proteins that prevent S. aureus growth. Another mechanism that directly inhibits other bacteria species is the production of hydrogen peroxide (H₂O₂). S. pneumoniae is remarkably tolerant to H₂O₂ and although potential pathogens such as S. aureus and H. influenzae produce a catalase to neutralize H₂O₂, the concentrations produced by S. pneumoniae overwhelm these catalases without killing the S. pneumoniae itself. Another strategy employed by S. pneumoniae is the production of neuraminidase, an enzyme that degrades H. influenzae cell-surface sialic acids, impairing the ability of H. influenzae to colonize the host. Commensal nasopharyngeal flora can inhibit the growth of group A Streptococcus, although the mechanisms are not clear. Finally, the impact of nasopharyngeal bacteria and viruses on other species can also be mediated via the modulation of the host’s immune response. For example, in a mouse model, initial colonization with H. influenzae stimulated an innate immune response via immune recognition of cell wall components that enhanced phagocytosis of S. pneumoniae and inhibited colonization.

Immunizing effect of colonization
Both human and animal data demonstrate that colonization is an immunizing event that prevents subsequent S. pneumoniae infection by both homologous and heterologous strains. Antibodies targeting capsular polysaccharides are detected in the serum of children following colonization, although the strength of the immune response depends on the infecting serotype. Exposure to a greater number of serotypes also enhances immune responses. In addition to anti-capsular antibodies, colonization in humans and in
animal models induces antibodies to surface and intracellular *S. pneumoniae* protein antigens, many of which are protective. Unlike anti-capsular responses, anti-protein responses are rapidly detectable in the first year of life. However, an epidemiological study in infants did not find evidence that anti-protein antibodies protected against subsequent colonization. Colonization of adult volunteers with serotype that anti-protein antibodies protected against subsequent colonization. Colonization of adult volunteers with serotype 18,78 and are critical for both the initial colonization and subsequent protection. In addition to Th17-cell responses, colonization-induced anti-protein responses are sufficient to enable protection. In this model, nasopharyngeal colonization with a serotype 6B strain of *S. pneumoniae* was established in healthy adults. Rechallenge failed to result in a second colonization event by the same strain, with protection persisting for up to 1 year. In these challenge studies, colonization resulted in cellular and humoral immune responses to *S. pneumoniae*. Data from mouse colonization models indicate that nasopharyngeal colonization leads to Th17-cell responses that enhance phagocyte recruitment to the nasopharynx, and are critical for both the initial clearance of the colonizing strain and subsequent protection against recolonization. The impact of these phagocytic responses is enhanced by the effects of specific antibody via opsonization and agglutination of *S. pneumoniae*. A murine model of group A *Streptococcus* nasopharyngeal infection has also been established. In this model, rapid clearance of recolonization was also dependent on an antigen-specific Th17-cell response. These data raise the possibility that the Th17-cell mechanisms may be broadly important in the control of bacterial colonization of the nasopharynx.

Colonization as a vaccine strategy

There are two potential strategies by which colonization could be used to prevent disease. The first is through harnessing the regulatory effects of commensal species on colonization by potential pathogens through competition for resources, immune modulatory effects, the secretion of bacteriocins, or other direct inhibitory mechanisms. The second is by stimulating a protective adaptive immune response, which unlike the first strategy requires colonization by an organism with significant antigenic overlap to the target pathogen.

Prevention of colonization using commensal species

The delivery of nonpathogenic commensal species as “probiotics” has been investigated for the prevention of OM. In some cases, the impact on bacterial nasopharyngeal colonization has also been assessed. These avirulent organisms were administered orally or via nasal spray, and the preliminary results suggest that they can reduce the incidence of upper respiratory infections. In a study of adults given an oral mixture of organisms (containing *Lactobacillus rhamnosus* GG, *Bifidobacterium*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus*), there was a significant reduction in nasal colonization with potential pathogens, including *S. aureus*, *S. pneumoniae*, and β-hemolytic streptococci. Studies in children given milk supplemented with *Lactobacillus rhamnosus* GG tend to show a reduction in respiratory infections, including OM, but have produced mixed results in the impact on the carriage of pathogens such as *S. pneumoniae*.
and *H. influenzae*. These studies delivered the commensal organisms to the gut where they might enhance general mucosal immunity through interactions with the gut-associated immune system.

Children who suffer from repeated episodes of OM are less likely to carry the oropharyngeal commensal alpha-hemolytic streptococci (AHS), whereas *H. influenzae* is more prevalent. This observation perhaps suggests that AHS have inhibitory effect on *H. influenzae* growth, similar to the ability of *Streptococcus oralis* to inhibit *S. pneumoniae* growth. This potential effect has been exploited by the oral administration of another AHS species *S. salivarius*, which was associated with a reduction in *S. pyogenes* infections in humans and inhibition of *S. pneumoniae* infection in mouse models. Furthermore, nasal spray administration of five strains of AHS from three species (*Streptococcus sanguis*, *Streptococcus mitis*, and *S. oralis*) reduced the incidence of recurrent OM and secretory OM in children. However, in another study, there was no significant change in the incidence of OM although there was a trend toward reduced carriage of *H. influenzae*. It is possible that in these studies, the administration of antibiotics prior to the bacterial nasal spray may have enabled stable colonization with AHS strains and therefore may make a positive result more likely.

**Colonization to induce adaptive immunity**

The remainder of this review focuses on using intranasal administration of bacteria as an immunizing event; these approaches are summarized in Table 1. This approach has a number of potential advantages to using conventional vaccines. A whole bacterial cell approach means that the reduced carriage of *H. influenzae* in the incidence of OM although there was a trend toward reduced carriage of *H. influenzae*. It is possible that in these studies, the administration of antibiotics prior to the bacterial nasal spray may have enabled stable colonization with AHS strains and therefore may make a positive result more likely.

Cross-reactive protection between commensal and potentially pathogenic species

Colonization with a commensal species could potentially enhance the clearance of a closely related potential pathogen if there are shared antigens between the species. This has been explored for the closely related species *Neisseria lactamica* and *N. meningitidis*. *N. lactamica* expresses antigens similar to those expressed by *N. meningitidis*, and sera from mice immunized with *N. lactamica* enhance *N. meningitidis* killing, and human carriage of *N. lactamica* results in a high titer of antibodies to *N. meningitidis*. Outer membrane proteins and lipooligosaccharide structures common to both species are the major antigenic sources of cross-protection. *N. lactamica* colonization has been studied as a vaccination strategy to prevent *N. meningitidis* disease. In one study of colonization of healthy volunteers, the mucosal and systemic antibody response against *N. lactamica* was cross-reactive against *N. meningitidis*. However, while these antibodies were opsonophagocytic in vitro, they had poor

**Table 1 Examples and potential mechanisms for inducing adaptive immunity to bacterial pathogens by nasopharyngeal colonization with live bacteria**

| Type of approach          | Target pathogen | Species/mutation(s) | Description                        | References |
|---------------------------|-----------------|---------------------|------------------------------------|------------|
| Commensal cross-reactivity| *N. meningitidis* | *N. lactamica*      | Induction of cross-reactive antibody | 103, 104   |
| Attenuated pathogenic bacteria | *S. pneumoniae* | cps, ply, and pspA | Virulence factor deletion           | 107        |
|                           | *S. pneumoniae* | cps, teichoic acids, ply | Virulence factor deletion           | 108, 109   |
|                           | *S. pneumoniae* | pep27               | Capsule reduction                  | 110        |
|                           | *S. pneumoniae* | ftsY, caaPingtA     | Metabolic component deletion        | 111        |
|                           | *S. pneumoniae* | pabB                | Auxotroph                          | 112        |
|                           | *S. pyogenes*   | speB and gidA mutation | Impaired tRNA modification         | 113        |
|                           | *Salmonella enterica serovar* | gidA mutation | Impaired tRNA modification         | 114        |
|                           | *Typhimurium*   |                     |                                    |            |
| Heterologous antigen expression | *S. pneumoniae* | PspA expression by Lactobacillus casei | Protective antigen expression    | 118        |
|                           | *S. pneumoniae* | PspA, PpmA, PsA, PppA, and SrrA expression by *L. lactis* | Protective antigen expression  | 119        |
|                           | *S. pneumoniae* | Cps expression by *L. lactis* | Protective antigen expression      | 120, 121   |

**Abbreviations:** *L. lactis*, *Lactobacillus lactis*; *N. lactamica*, *Neisseria lactamica*; *N. meningitidis*, *Neisseria meningitidis*; *S. pneumoniae*, *Streptococcus pneumoniae*; *S. pyogenes*, *Streptococcus pyogenes*.

In addition, the vaccine would be inexpensive to manufacture and would not need an adjuvant as antigens are presented in an immunostimulatory context of the whole bacterium. Nasal administration also offers the advantages over parenteral administration of higher safety levels, needleless delivery, and improved immunity at the mucosal surface which may be more likely to prevent respiratory tract infections. The key principles of colonization-induced immunity (Figure 1) are detailed in the following sections.
serum bactericidal effect. Furthermore, experimental *N. lactamica* colonization did not protect against subsequent natural *N. meningitidis* carriage acquisition. In fact, there has been some evidence to suggest that *N. lactamica* even protects *N. meningitidis* during colonization by triggering antibody-independent responses that do not induce a memory response. Nonetheless, the immunological response to colonization with *N. lactamica* could potentially protect against systemic infection with *N. meningitidis*, and further investigation of this strategy is ongoing. A similar strategy, in theory, could be applicable to other related pairs of pathogenic and nonpathogenic species, such as *S. pneumoniae* and *S. mitis*.

**Attenuated pathogenic bacteria**

Observations that natural mucosal exposure induces antibody and cellular immune responses to a range of bacterial antigens suggest that an alternative to current vaccine strategies could be the colonization of the nasopharynx with whole bacteria. This would reflect a more natural situation than subunit vaccines. To avoid the potential for causing active invasive infection, vaccination by artificial colonization of the nasopharynx would need to use attenuated strains unable to cause serious infection. This can now be achieved by targeted mutation of important virulence determinants, although these mutations could reduce the antigenicity of the attenuated strain. A critical aspect in the design of a live attenuated mucosal vaccine is achieving the balance between virulence attenuation for safety while retaining immunogenicity.

The use of attenuated *S. pneumoniae* as vaccines has been explored by several groups in animal models. In one example, genes encoding the capsule, Ply, and PspA were deleted rendering these strains avirulent yet still able to colonize and induce both systemic and mucosal antibodies that protected against disease in mice. A similar approach was the SPY1 mutant strain, where the capsule, teichoic acids, and Ply were deleted from a D39 *S. pneumoniae* strain and used for intranasal immunization. This protected against colonization and invasive disease caused by heterologous strains of *S. pneumoniae* in a T-cell- and B-cell-dependent manner. Further examples include the deletion of pep27 leading to an avirulent strain with reduced capsule expression which when used to immunize mice intranasally protected against colonization and systemic infection and double mutation of the signal recognition pathway component *ftsY* and the calcium/magnesium transporter *caxP/mgtA* which when administered to the nasopharynx induces heterologous protection against OM, pneumonia, and invasive disease in a CD4+ cell-dependent manner. Another strategy for generating live vaccines is by creating auxotrophic organisms. In this way, key protective surface antigens such as PspA and the polysaccharide capsule for *S. pneumoniae* can be retained. For example, the deletion of the *pabB* gene

---

**Figure 1** Summary of the principles employed in nasal vaccination strategies.
creates an *S. pneumoniae* mutant strain auxotrophic for para-aminobenzoic acid and unable to replicate in the mammalian host. Systemic vaccination with this mutant was able to protect from homologous challenge in mouse models of sepsis and pneumonia. The attenuated mutant approach has been investigated for other bacteria. For example, in *S. pyogenes*, the deletion of the SpeB protease creates a mutant that is impaired in tRNA modification and has the potential to be used in vaccination strategies. Mutation of gidA in *Salmonella enterica* serovar Typhimurium also lends itself to a novel vaccine strategy for this bacterium.

A potentially significant problem is that mouse and human data indicate that a reduced duration of nasopharyngeal colonization by *S. pneumoniae* significantly weakens the induced adaptive immune response. In mice, attenuated strains often have a reduced duration of colonization compared to wild-type bacteria, and this may therefore affect the efficacy of using attenuated mutants for preventing *S. pneumoniae* infections. For example, the *pabB* deletion strain is very rapidly cleared from the mouse nasopharynx and was only weakly immunogenic after nasopharyngeal administration. Repeated dosing of poorly colonizing strains may overcome this issue.

Another potential problem is the surprising lack of cross-protection induced by attenuated strains in some of these studies. For example, although the protein antigens targeted by protective responses are largely conserved between *S. pneumoniae* strains, adaptive responses to one episode of colonization or systemic vaccination with a live attenuated vaccine were not or only weakly cross-protective against heterologous strains in mouse models. The reasons for this poor cross-protective immunity are not clear and require further investigation to ensure that nasopharyngeal administration with a single strain can provide the broad heterologous protection required for an effective vaccine.

Reversion to wild type with loss of the attenuating mutation is a significant safety risk in the use of live attenuated bacteria as vaccines. This is a particular concern for pathogens such as *S. pneumoniae* that are naturally competent and are known to undergo recombination events during colonization. To avoid this, attenuated strains would need to contain at least two independent mutations. Another strategy to mitigate this risk would be to delete the competence machinery, rendering the strain unable to uptake foreign DNA and thereby preventing recombination events with the resident nasal flora which may possess similar virulence factors. In addition, the effect of administration of genetically modified bacteria to the nasopharynx on the existing nasal flora is not known, and will need to be evaluated carefully to ensure there are no unforeseen deleterious consequences.

**Heterologous expression of protective antigens**

An alternative approach has been to express recombinant protein in nonpathogenic species. Such strategies provide effective protection at the mucosal surface and during invasive disease. Lactic acid bacteria (LAB) are commonly used to manufacture foodstuffs and are therefore a safe alternative which are also known to elicit systemic and mucosal responses. The LAB, *Lactobacillus casei*, has been developed as an intranasal vaccine which expresses the *S. pneumoniae* protein antigen PspA and induces antibodies that protect mice from a systemic challenge. Another LAB, *Lactococcus lactis*, has also been used to express *S. pneumoniae* protein antigens including PspA, PpmA, PsaA, PppA, and SlrA or serotype 3 and serotype 14 capsular polysaccharides. Colonization with the *L. lactis* strains expressing *S. pneumoniae* capsules led to the induction of specific IgG and IgM antibodies. The oral commensal species *Streptococcus gordonii*, which also stimulates mucosal immunity, has been engineered to express protective antigens from *S. pyogenes* which are immunogenic in mouse models when inoculated intranasally and orally. *S. gordonii* was also investigated as a means to express *N. meningitidis* antigens which induced bactericidal antibodies in intranasally immunized mice and *Bordetella pertussis* antigens which when used in oral colonization induced systemic and mucosal antibodies. These animal models and early studies in humans indicate that *S. gordonii* is a suitable vector for presenting heterologous antigens for a colonization approach vaccination strategy. However, a limitation of this strategy is the use of a limited number of antigens, which could restrict the range and strength of any protective immune response. Nevertheless, these early results indicate that this may be an area of potential future development applicable to a number of bacteria species, perhaps in combination with the use of closely related nonpathogenic species discussed earlier. For example, the expression of important *N. meningitidis* antigens in *N. lactamica* could increase the strength of cross-protective immunity induced by colonization with the modified *N. lactamica* strain.

**Overview and future directions**

Colonization of the nasopharynx is central to disease development and adaptive immune responses to potentially pathogenic organisms. Modulation of host–pathogen interac-
tions at this site could be a powerful method of preventing serious bacterial infections for a range of common pathogens. There are several important characteristics which an attenuated microorganism must have to serve as a potential live human vaccine: 1) mutations must be stable and severely attenuate virulence to prevent the strain from causing lung or systemic infection; 2) two or more virulence genes should be mutated to minimize the chance of revertants developing; 3) the attenuated strain should retain the ability to stimulate significant increases in adaptive immune responses after nasopharyngeal administration, including mucosal immune responses that prevent lung infection; and 4) adaptive immunity to the mutant strain should result in cross-strain protection. Currently, most data showing the utility of nasopharyngeal colonization with attenuated or nonpathogenic organisms as a vaccination strategy have been obtained using animal models. However, the development of human models of nasopharyngeal carriage104,127–129 pow allows the strategies using colonization to prevent infection to be tested in humans and to assess whether their potential can be fulfilled.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Watson K, Carville K, Bowman J, et al. Upper respiratory tract bacterial carriage in Aboriginal and non-Aboriginal children in a semi-arid area of Western Australia. Pediatr Infect Dis J. 2006;25(9):782–790.
2. Kadioglu A, Weiser JN, Paton JC, Andrew PW. The role of Streptococcus pneumoniae virulence factors in host respiratory colonization and disease. Nat Rev Microbiol. 2008;6(4):288–301.
3. Simell B, Auranen K, Käyhty H, et al; Pneumococcal Carriage Group. The fundamental link between pneumococcal carriage and disease. Expert Rev Vaccines. 2012;11(7):841–855.
4. Blaser MJ, Falkow S. What are the consequences of the disappearing human microbota? Nat Rev Microbiol. 2009;7(12):887–894.
5. Regev-Yochay G, Dagan R, Raz M, et al. Association between carriage of Streptococcus pneumoniae and Staphylococcus aureus in Children. JAMA. 2004;292(6):716–720.
6. Bogaert D, De Groot R, Hermans PWM. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. Lancet Infect Dis. 2004;4(3):144–154.
7. Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. N Engl J Med. 2007;357(15):1487–1495.
8. Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in astmatic airways. PLoS One. 2010;5(1):e8578.
9. Marri PR, Stern DA, Wright AL, Billheimer D, Martinez FD. Asthma-associated differences in microbial composition of induced sputum. J Allergy Clin Immunol. 2013;131(2):346–52.e1–3.
10. Erb-Downward JR, Thompson DL, Han MK, et al. Analysis of the lung microbiome in the “healthy” smoker and in COPD. PLoS One. 2011;6(2):e16384.
11. Sze MA, Dimitriu PA, Hayashi S, et al. The lung tissue microbiome in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2012;185(10):1073–1080.
12. Herbter T, Sichelstiel A, Schär C, et al. Dysregulation of allergic airway inflammation in the absence of microbial colonization. Am J Respir Crit Care Med. 2011;184(2):198–205.
13. Hagner S, Harb H, Zhao M, et al. Farm-derived Gram-positive bacterium Staphylococcus scouri W620 prevents asthma phenotype in HDI- and OVA-exposed mice. Allergy. 2013;68(3):322–329.
14. Coughlin J, Koller Y, Wyss M, Geuking MB, McCoy KD. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. Cell Host Microbe. 2013;14(5):559–570.
15. Richards L, Ferreira DM, Miyaji EN, Andrew PW, Kadioglu A. The immunising effect of pneumococcal nasopharyngeal colonisation; protection against future colonisation and fatal invasive disease. Immunology. 2010;215(4):251–263.
16. Cohen JM, Khandavilli S, Camberlein E, Hynans C, Baxendale HE, Brown JS. Protective contributions against invasive Streptococcus pneumoniae pneumonia of antibody and Th17-cell responses to nasopharyngeal colonisation. PLoS One. 2011;6(10):e25558.
17. Cohen JM, Chimalalapati S, de Vogel C, van Belkum A, Baxendale HE, Brown JS. Contributions of capsule, lipoproteins and duration of colonisation towards the protective immunity of Streptococcus pneumoniae nasopharyngeal colonisation. Vaccine. 2012;30(30):4453–4459.
18. Zhang Z, Clarke TB, Weiser JN. Cellular effectors mediating Th17-dependent clearance of pneumococcal colonization in mice. J Clin Invest. 2009;119(7):1899–1909.
19. Wilson R, Cohen JM, José RJ, de Vogel C, Baxendale H, Brown JS. Protection against Streptococcus pneumoniae lung infection after nasopharyngeal colonisation requires both humoral and cellular immune responses. Mucosal Immunol. 2015;8(3):627–639.
20. Wright AKA, Bangert M, Gritzfeld JF, et al. Experimental human pneumococcal carriage augmentes IL-17A-dependent T-cell defence of the lung. PLoS Pathog. 2013;9(3):e1003274.
21. Ferreira DM, Neill DR, Bangert M, et al. Controlled human infection and rechallenge with Streptococcus pneumoniae reveals the protective efficacy of carriage in healthy adults. Am J Respir Crit Care Med. 2013;187(8):855–864.
22. Gray BM, Dillon HC Jr. Epidemiological studies of Streptococcus pneumoniae in infants: antibody to types 3, 6, 14, and 23 in the first two years of life. J Infect Dis. 1988;158(5):948–955.
23. Loda FA, Collier AM, Glezen WP, Strangert K, Clyde WA Jr, Denny FW. Occurrence of Diplococcus pneumoniae in the upper respiratory tract of children. J Pediatr. 1975;87(6 pt 2):1087–1093.
24. Syrjänen RK, Kilpi TM, Kajialainen TH, Herva E, Takala AK. Nasopharyngeal carriage of Streptococcus pneumoniae in Finnish children younger than 2 years old. J Infect Dis. 2001;184(4):451–459.
25. Regev-Yochay G, Dagan R, Raz M, et al. Association between carriage of Streptococcus pneumoniae and Staphylococcus aureus in Children. JAMA. 2004;292(6):716–720.
26. Bogaert D, De Groot R, Hermans PWM. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. Lancet Infect Dis. 2004;4(3):144–154.
27. Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. N Engl J Med. 2007;357(15):1487–1495.
28. Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. PLoS One. 2010;5(1):e8578.
29. Marri PR, Stern DA, Wright AL, Billheimer D, Martinez FD. Asthma-associated differences in microbial composition of induced sputum. J Allergy Clin Immunol. 2013;131(2):346–52.e1–3.
30. Erb-Downward JR, Thompson DL, Han MK, et al. Analysis of the lung microbiome in the “healthy” smoker and in COPD. PLoS One. 2011;6(2):e16384.
31. Sze MA, Dimitriu PA, Hayashi S, et al. The lung tissue microbiome in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2012;185(10):1073–1080.
31. Kandassamy R, Gurung M, Thapa A, et al. Multi-serotype pneumococcal nasopharyngeal carriage prevalence in vaccine naïve Nepalese children, assessed using molecular serotyping. *PLoS One.* 2015;10(2):e0114286.

32. Kamg'ona AW, Hinds J, Bar-Zeev N, et al. High multiple carriage and emergence of *Streptococcus pneumoniae* vaccine serotype variants in Malawian children. *BMC Infect Dis.* 2015;15:234.

33. Ekdahl K, Ahlinder I, Hansson HB, et al. Duration of nasopharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae* experiences from the South Swedish Meningococcal Intervention Project. *Clin Infect Dis.* 1997;25(5):1113–1117.

34. Sleeman KL, Griffiths D, Shackley F, et al. Capsular serotype-specific attack rates and duration of carriage of *Streptococcus pneumoniae* in a population of children. *J Infect Dis.* 2006;194(5):682–688.

35. O’Brien KL, Wolfson LJ, Watt JP, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet.* 2007;369(9563):893–902.

36. Black RE, Cousens S, Johnson HL, et al. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet.* 2010;375(9730):1969–1987.

37. Walker CLF, Rudan I, Liu L, et al. Global burden of childhood pneumonia and diarrhoea. *Lancet.* 2013;381(9875):1405–1416.

38. Isaacman DJ, McNeil ED, Reinert RR. Burden of invasive pneumococcal disease and serotype distribution among *Streptococcus pneumoniae* isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. *Int J Infect Dis.* 2010;14(3):e197–e209.

39. Davis SM, Deloria-Knoll M, Kassa HT, O’Brien KL. Impact of pneumococcal conjugate vaccines on nasopharyngeal carriage and invasive disease among unvaccinated people: review of evidence on indirect effects. *Vaccine.* 2013;32(1):133–145.

40. Moherley S, Holden J, Tatham DP, Andrews RM. Vaccines for preventing pneumococcal infection in adults. *Cochrane Database Syst Rev.* 2013;1:CD000422.

41. Yildirim I, Hanage WP, Lipsitch M, et al. Serotype specific invasive capacity and persistent reduction in invasive pneumococcal disease. *Vaccine.* 2010;28(2):283–288.

42. Hanage WP, Finkelstein JA, Huang SS, et al. Evidence that pneumococcal serotype replacement in Massachusetts following conjugate vaccination is now complete. *Epidemiol. 2010;2(2):80–84.

43. Flasche S, Van Hoek AJ, Sheasby E, et al. Effect of pneumococcal conjugate vaccination on serotype-specific invasive disease and carriage in England: a cross-sectional study. *PLoS Med.* 2011;8(4):e1001017.

44. Biesbroek G, Wang X, Keijser BJF, et al. Seven-valent pneumococcal conjugate vaccine and nasopharyngeal microbiota in healthy children. *Emerg Infect Dis.* 2014;20(2):201–210.

45. Spijkerman J, Prevaea SMPJ, van Gils EJM, et al. Long-term effects of pneumococcal conjugate vaccination on nasopharyngeal carriage of *S. pneumoniae*, *S. aureus*, *H. influenzae* and *M. catarrhalis*. *PLoS One.* 2012;7(6):e39730.

46. Pericone CD, Overweg K, Hermans PW, Weiser JN. Inhibitory and bactericidal effects of hydrogen peroxide production by *Streptococcus pneumoniae* on other inhabitants of the upper respiratory tract. *Infect Immun.* 2000;68(7):3990–3997.

47. Johnson AP. The pathogenic potential of commensal species of *Neisseria*. *J Clin Pathol.* 1983;36(2):213–223.

48. Stephens DS. Unclouking the meningococcus: dynamics of carriage and disease. *Lancet.* 1999;353(9157):941–942.

49. Cartwright KAV, Stuart JM, Jones DM, Noah ND. The Stonehouse survey: nasopharyngeal carriage of meningococci and *Neisseria lactamica*. *Epidemiol Infect.* 1987;99(3):591–601.

50. Caugant DA, Hoiy E, Magnus P, et al. Asymptomatic carriage of *Neisseria meningitidis* in a randomly sampled population. *J Clin Microbiol.* 1994;32(2):323–330.

51. Davies AL, O’Flanagan D, Salmon RL, Coleman TJ. Risk factors for *Neisseria meningitidis* carriage in a school during a community outbreak of meningococcal infection. *Epidemiol Infect.* 1996;117(2):259.

52. Boisier P, Nicolas P, Djibo S, et al. Meningococcal meningitis: unprecedented incidence of serogroup X-related cases in 2006 in Niger. *Clin Infect Dis.* 2007;44(5):657–663.

53. Trotter CL, Maiden MCJ. Meningococcal vaccines and herd immunity: lessons learned from serogroup C conjugate vaccination programs. *Expert Rev Vaccines.* 2009;8(7):851–861.

54. Høiby EA, Magnus P, et al. Asymptomatic carriage of *Neisseria meningitidis* in children younger than 5 years: global estimates. *Lancet.* 2007;369(9563):893–902.

55. Kononen E. Anaerobes in the upper respiratory tract in infancy. *Anaerobe.* 2005;11(3):131–136.

56. Bogaert D, Keijser B, Huse S, et al. Variability and diversity of nasopharyngeal microbiota in children: a metagenomic analysis. *PLoS One.* 2011;6(2):e17035.

57. Hilty M, Qi W, Brugger SD, et al. Nasopharyngeal microbiota in infants with acute otitis media. *J Infect Dis.* 2012;205(7):1048–1055.

58. van der Waaaij D, Berghuis-de Vries JM. Lekkerkerk Lekkerkerk-v. Colony resistance of the digestive tract in conventional and antibiotic-treated mice. *J Hyg (Lond).* 1971;69(3):405–411.

59. Kamada N, Chen GY, Inohara N, Núñez G. Control of pathogens and pathobionts by the gut microbiota. *Nat Immunol.* 2013;14(7):685–690.

60. Cardoso PA, Heng NC, Burton JP, Chilcott CN, Tagg JR. Streptococcal bacteriocins and the case for *Streptococcus salivarius* as model oral probiotics. *Future Microbiol.* 2009;4(7):819–835.

61. Santagati M, Scillato M, Patane F, Aiello C, Stefanelli S. Bac teriocin-producing oral streptococci and inhibition of respiratory pathogens. *FEMS Microbiol Med Microbiol.* 2012;65(1):23–31.

62. Hamman R, Fernandez B, Lacroix C, Fliss I. Anti-infective properties of bacteriocins: an update. *Cell Mol Life Sci.* 2013;70(16):2947–2967.

63. Iwase T, Uehara Y, Shinji H, et al. *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature.* 2010;465(7296):346–349.

64. Regev-Yochay G, Trzcinski K, Thompson CM, Lipstick M, Malley R. *SpbX* is a suicide gene of *Streptococcus pneumoniae* and confers a selective advantage in an in vivo competitive colonization model. *J Bacteriol.* 2007;189(18):6532–6539.

65. Shakhnovich EA, King SJ, Weiser JN. Neuraminidase expressed by *Streptococcus pneumoniae* desialylates the lipopolysaccharide of *Neisseria meningitidis* and *Haemophilus influenzae*: a paradigm for interbacterial competition among pathogens of the human respiratory tract. *Infect Immun.* 2002;70(12):7161–7164.

66. Crowe CC, Sanders WE, Longley S. Bacterial interference. II. Role of the normal throat flora in prevention of colonization by group A *Streptococcus*. *J Infect Dis.* 1973;128(4):527–532.

67. Lysenko ES, Ratner AJ, Nelson AL, Weiser JN. The role of innate immune responses in the outcome of interspecies competition for colonization of mucosal surfaces. *PLoS Pathog.* 2005;1(1):e1.

68. Turner P, Turner C, Green N, et al. Serum antibody responses to pneumococcal colonization in the first 2 years of life: results from an SE Asian longitudinal cohort study. *Clin Microbiol Infect.* 2013;19(12):E551–E558.

69. Sohrin A, Pursiainen H, Kilpi T, Käyhty H. Natural development of pneumococcal侵袭性 proteins in children during the pre-infancy enhances antibody response to a pneumococcal conjugate vaccine. *Clin Microbiol Immunol.* 2003;19(12):e0114286.
73. Rapola S, Jäntti V, Haikara R, et al. Natural development of antibodies to pneumococcal surface protein A, pneumococcal surface adhesin A, and pneumococcal surface protein CbpA and Phd in children. Vaccine. 2009;27(34):4615–4621.

74. Prevaes SMPJ, van Wamel WJB, de Vogel CP, et al. Nasopharyngeal colonization elicits antibody responses to staphylococcal and pneumococcal proteins that are not associated with a reduced risk of subsequent carriage. Infect Immun. 2012;80(6):2186–2193.

75. Roche A, Richard AL, Rahkola JT, Janoff EN, Weiser JN. Antibody blocks acquisition of bacterial colonization through agglutination. Mucosal Immunol. 2015;8(1):176–185.

76. Park H-S, Francis KP, Yue J, Cleary PP. Membranous cells in nasal-associated lymphoid tissue: a portal for entry of the respiratory mucosal pathogen group A Streptococcus. J Immunol. 2003;171(5):2532–2537.

77. Wáng B, Díepean T, Bricco S, et al. Induction of TGF-beta1 and TGF-beta1-dependent predominant Th17 differentiation by group A streptococcal infection. Proc Natl Acad Sci U S A. 2010;107(13):5937–5942.

78. Wright AKA, Ferreira DM, Griffield JF, et al. Human nasal challenge with Streptococcus pneumoniae is immunising in the absence of carriage. PLoS Pathog. 2012;8(4):e1002622.

79. Kremistinou J, Tzianakaki G, Pagalis A, Theodorou M, Weir DM, Blackwell CC. Detection of IgG and IgM to meningooccal outer membrane proteins in relation to carriage of Neisseria meningitidis or Neisseria lactamica. FEMS Immunol Med Microbiol. 1999;24(1):73–78.

80. Jones GR, Christodoulides M, Brooks JL, Miller AR, Cartwright KAV, Heckels JE. Dynamics of carriage of Neisseria meningitidis in a group of military recruits: subtype stability and specificity of the immune response following colonization. J Infect Dis. 1998;178(2):451–459.

81. Robinson K, Neal KR, Howard C. Characterization of humoral and cellular immune responses elicited by meningococcal carriage. Infect Immun. 2002;70(3):1301–1309.

82. Sánchez S, Troncoso G, Ferreirós C, Criado M. Evaluation of cross-reactive antigens as determinants of cross-bacterial activity in pathogenic and commensal Neisseria. Vaccine. 2001;19(25–26):3390–3398.

83. Sánchez S, Troncoso G, Criado MT, Ferreirós C. In vitro induction of memory-driven responses against Neisseria meningitidis by priming with Neisseria lactamica. Vaccine. 2002;20(23–24):2957–2963.

84. Frey SE, Lottenbach KR, Hill H, et al. A Phase I, dose-escalation trial in adults of three recombinant attenuated Salmonella Typhi vaccine vectors producing Streptococcus pneumoniae surface protein antigen PspA. Vaccine. 2013;31(42):4874–4880.

85. Tennant SM, Levine MM. Live attenuated vaccines for invasive Salmonella infections. Vaccine. 2015;33(suppl 3):C36–C41.

86. Alvarez-Olmos MI, Oberhelman RA. Probiotic agents and infectious diseases: a modern perspective on a traditional therapy. Clin Infect Dis. 2001;32(11):1567–1576.

87. Niitnynen L, Pitkäkanta A, Korpela R. Probiotics and otitis media in children. Int J Pediatr Otorhinolaryngol. 2012;76(4):465–470.

88. Glück U, Gebbers J-O. Ingested probiotics reduce nasal colonization with pathogenic bacteria (Staphylococcus aureus, Streptococcus pneumoniae, and beta-hemolytic streptococci). Am J Clin Nutr. 2003;77(2):517–520.
111. Rosch JW, Iversion AR, Humann J, et al. A live-attenuated pneumococcal vaccine elicits CD4+ T-cell dependent class switching and provides serotype independent protection against acute otitis media. *EMBO Mol Med.* 2014;6(1):141–154.

112. Chimalapati S, Cohen J, Camberlein E, et al. Infection with conditionally virulent *Streptococcus pneumoniae* Δpab strains induces antibody to conserved protein antigens but does not protect against systemic infection with heterologous strains. *Infect Immun.* 2011;79(12):4965–4976.

113. Cho KH, Caparon MG. tRNA modification by GidA/MmrE is necessary for *Streptococcus pyogenes* virulence: a new strategy to make live attenuated strains. *Infect Immun.* 2008;76(7):3176–3186.

114. Shippy DC, Fadl AA. Immunological characterization of a gidA mutant strain of *Salmonella* for potential use in a live-attenuated vaccine. *BMC Microbiol.* 2012;12:286.

115. Cohen JM, Wilson R, Shah P, Baxendale HE, Brown JS. Lack of cross-protection against invasive pneumonia caused by heterologous strains following murine *Streptococcus pneumoniae* nasopharyngeal colonization despite whole cell ELISAs showing significant cross-reactive IgG. *Vaccine.* 2013;31(19):2328–2332.

116. Hannifey SB, Carter AT, Hitchin E, Wells JM. Mucosal delivery of a pneumococcal vaccine using *Lactococcus lactis* affords protection against respiratory infection. *J Infect Dis.* 2007;195(2):185–193.

117. Wells JM, Mercenier A. Mucosal delivery of therapeutic and prophylactic molecules using lactic acid bacteria. *Nat Rev Microbiol.* 2008;6(5):349–362.

118. Campos IB, Darrieux M, Ferreira DM, et al. Nasal immunization of *Haemophilus influenzae* in young adults: a controlled human infection study. *Clin Infect Dis.* 2015;60(10):1512–1520.

119. Medina MS, Vintiño EO, Villena J, Raya RR, Alvarez SG. *Lactococcus lactis* as an adjuvant and delivery vehicle of antigens against pneumococcal respiratory infections. *Bioeng Bugs.* 2014;1(5):313–325.

120. Gilbert C, Robinson K, Le Page RW, Wells JM. Heterologous expression of an immunogenic pneumococcal type 3 capsular polysaccharide in *Lactococcus lactis*. *Infect Immun.* 2000;68(6):3251–3260.

121. Nierop Groot MN, Godfrooij J, Kleerebezem M. Heterologous expression of the pneumococcal serotype 14 polysaccharide in *Lactococcus lactis* requires lacticoccal epsABC regulatory genes. *Appl Environ Microbiol.* 2008;74(3):912–915.

122. Lee SF, Halperin SA, Wang H, MacArthur A. Oral colonization and immune responses to *Streptococcus gordonii* expressing a pertussis toxin S1 fragment in mice. *FEMS Microbiol Lett.* 2002;208(2):175–178.

123. Medaglini D, Pozzi G, King TP, Fischetti VA. Mucosal and systemic immune responses to a recombinant protein expressed on the surface of the oral commensal bacterium *Streptococcus gordonii* after oral colonization. *Proc Natl Acad Sci USA.* 1995;92(15):6868–6872.

124. Ciabattini A, Giomarelli B, Parigi R, et al. Intranasal immunization of mice with recombinant *Streptococcus gordonii* expressing NAdA of *Neisseria meningitidis* induces systemic bactericidal antibodies and local IgA. *Vaccine.* 2008;26(33):4244–4250.

125. Lee SF, Halperin SA, Knight JB, Tait A. Purification and immunogenicity of a recombinant Bordetella pertussis S1S3FHA fusion protein expressed by *Streptococcus gordonii*. *Appl Environ Microbiol.* 2002;68(9):4253–4258.

126. Kotloff KL, Wasserman SS, Jones KF, et al. Clinical and microbiological responses of volunteers to combined intranasal and oral inoculation with a *Streptococcus gordonii* carrier strain intended for future use as a group A *Streptococcus* vaccine. *Infect Immun.* 2005;73(4):2360–2366.

127. Ferreira DM, Jambo KC, Gordon SB. Experimental human pneumococcal carriage models for vaccine research. *Trends Microbiol.* 2011;19(9):464–470.

128. Deasy AM, Guccione E, Dale AP, et al. Nasal inoculation of the commensal *Neisseria lactamica* inhibits carriage of *Neisseria meningitidis* by young adults: a controlled human infection study. *Clin Infect Dis.* 2015;60(10):1512–1520.

129. Winokur PL, Chaloner K, Doern GV, Ferreira J, Apicella MA. Safety and immunological outcomes following human inoculation with nontypeable *Haemophilus influenzae*. *J Infect Dis.* 2013;208(5):728–738.