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REVIEW

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

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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*,

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Hemophilus influenzae, *Neisseria meningitidis, Moraxella catarrhalis*, and *Staphylococcus aureus*.[1] The nasopharynx provides a relatively stable environment in which the commensal flora can flourish and be transmitted to other hosts.[2,3] 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.[4] 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 *S. pneumoniae* protects against *S. aureus* carriage,[5] and conversely reduction in *S. pneumoniae* carriage by vaccination could lead to increased carriage of *S. aureus*. Similarly, in animal models, bacterial species compete in establishing colonization.[6]

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[7–9] and chronic obstructive pulmonary disease[10,11] is related to the diversity of colonizing organisms in the nasal flora. In mice, lack of microbial colonization increases allergic airway inflammation,[12,13] and colonization with multiple species protects against airway inflammation.[14] 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.[15–21] 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 *S. pneumoniae* and *N. meningitidis* infections, which are used as case studies that illustrate the benefits and potential drawbacks of this approach.

Colonization and existing vaccines for *S. pneumoniae* and *N. meningitidis*

S. pneumoniae

Colonization with *S. 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,[22–27] sometimes with multiple strains.[28] Peak carriage rates occur at 3–5 years of age and then wane to ~10% in adult life.[6,29] Carriage prevalence depends on geographical location, and is generally higher in the developing world.[30–32] While initial colonization events may persist for up to 4 months, duration appears to shorten with increasing age to 2–4 weeks in adults.[33] The proportion of *S. pneumoniae* colonization events associated with disease is low in healthy adults. However, as colonization is very common, *S. pneumoniae* is a leading cause of acute otitis media (OM), pneumonia, sepsis, and meningitis globally[34–36] causing an estimated 2,858,000 severe pneumonia episodes and 411,000 deaths annually worldwide in infants.[37] The use of vaccines targeting polysaccharide capsule antigen in children has reduced the overall incidence of pneumococcal disease.[38,39] However, the adult vaccine fails to protect against pneumonia,[40] 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+ *S. pneumoniae* capsular serotypes. This restricted serotype coverage has led to the replacement of *S. pneumoniae* vaccine serotypes by non-vaccine serotypes as both colonizers of the nasopharynx and causes of disease.[31,41–43] Hence, there is a strong interest in alternative vaccine strategies that target all *S. pneumoniae* strains and could also prevent lung infection.

In addition to the profound effect on the relative prevalence of vaccine and non-vaccine *S. pneumoniae* serotypes, the introduction of the pneumococcal conjugate vaccine to infant immunization schedules has also disrupted the ecology of the nasopharyngeal flora in general.[44,45] 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 *S. pneumoniae* from the nasopharynx may remove the competition that *S. pneumoniae* exerts on other potentially pathogenic organisms, perhaps allowing their overgrowth. This could in turn lead to a greater incidence of disease caused by *H. influenzae, N. meningitidis, S. aureus,* or *M. catarrhalis*.[1] This disruption to the nasal flora will likely have implications on the incidence of disease and antibiotic strategies in the future.[5,46]

N. meningitidis

N. meningitidis is a pathogenic member of the Neisseria family, several of which are common human nasopharyngeal commensal species.[47] *N. meningitidis* is a major cause of rapidly progressive meningitis and septicemia, which, although rare, often result in death or permanent disability. Nevertheless, *N. meningitidis* is an asymptomatic nasopharyngeal colonizer in ~10% of healthy individuals at any

time.[48,49] 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.[49,50] Similar to *S. pneumoniae*, carriage rates are higher in smokers and after viral respiratory tract infections.[48,51]

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.[52] 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.*[53,54]

Regulation by commensal species

The nasal flora is acquired shortly after birth and is influenced by the environment, including contact with other persons.[55] 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,[56] 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 *Streptococcaceae* and *Corynebacteriaceae* commensal species in the nasopharynx.[57] 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,*[58,59] 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,*[60,61] and in the gut enterohemorrhagic *E. coli* is inhibited by bacteriocins produced by other *E. coli* strains.[62] On the skin, the commensal species, *Staphylococcus epidermidis*, produces antimicrobial proteins that prevent *S. aureus* growth.[63] Another mechanism that directly inhibits other bacteria species is the production of hydrogen peroxide (H_2O_2). *S. pneumoniae* is remarkably tolerant to H_2O_2 and although potential pathogens such as *S. aureus* and *H. influenzae* produce a catalase to neutralize H_2O_2, the concentrations produced by *S. pneumoniae* overwhelm these catalases without killing the *S. pneumoniae* itself.[46,64] 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.[65] Commensal nasopharyngeal flora can inhibit the growth of group A *Streptococcus*, although the mechanisms are not clear.[66] Finally, the impact of nasopharyngeal bacteria and viruses on other species can also be mediated via the modulation of the host's immune response.[4] 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.[67]

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,[68] although the strength of the immune response depends on the infecting serotype.[69,70] Exposure to a greater number of serotypes also enhances immune responses.[71] 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.[6,72] Unlike anti-capsular responses, anti-protein responses are rapidly detectable in the first year of life.[68,73,74] However, an epidemiological study in infants did not find evidence that anti-protein antibodies protected against subsequent colonization.[75] Colonization of adult volunteers with serotype 23F or 6B strains of *S. pneumoniae* led to the production of serum IgG to the protein antigens PspC and PspA and salivary IgA to PspA[76] and preexisting serum anti-PspA IgG levels correlating with protection against experimental colonization.[77] In another experimental human challenge model, nasopharyngeal colonization with a serotype 6B strain of *S. pneumoniae* was established in healthy adults. Rechallenge failed to result in a second carriage event by the same strain, with protection persisting for up to 1 year.[21] 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[16,18,19] that enhance phagocyte recruitment to the nasopharynx,[18,78] 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.*[79] A murine model of group A *Streptococcus* nasopharyngeal infection has also been established.[80] In this model, rapid clearance of recolonization was also dependent on an antigen-specific Th17-cell response.[81] These data raise the possibility that Th17-cell mechanisms may be broadly important in the control of bacterial colonization of the nasopharynx.

Human colonization also leads to serum antibody responses that are able to protect against sepsis caused by heterologous strains in mouse challenge models,[76] indicating that colonization-induced anti-protein responses are sufficient to enable protection. In addition to Th17-cell responses, the antibody response to colonization is also important for protection against *S. pneumoniae* lung infection in a mouse model.[19] In the human experimental challenge model, colonization augmented *S. pneumoniae*-specific IL-17-secreting CD4+ T-cells in the human lung,[20] suggesting that there may also be a role for colonization-induced Th17 cells in limiting lung infection in humans.

Overall, these observations demonstrate that colonization of healthy humans induces a mucosal and systemic immune response that protects against further colonization (in humans) or sepsis (in mice), and support data from animal models show similar findings.[15,16,18,19] Importantly, intranasal administration of the 6B strain without successful colonization also augmented local mucosal serological responses,[82] showing that exposure of the nasopharynx to the organism can stimulate an immunological response even if colonization was not detected.

Colonization of the upper airways with *N. meningitidis* is also an immunizing event that induces an antibody response persisting several weeks following acquisition.[83,84] Interestingly, childhood nasopharyngeal exposure to commensal strains of *Neisseria* increases antibody levels which is largely strain-specific but has some degree of cross-reactivity with *N. meningitidis*, and which perseveres for several months.[85–87]

Overall, there is now an abundance of data indicating that nasopharyngeal colonization with potentially pathogenic bacteria elicits both humoral and cellular protective adaptive immune responses in humans and mouse models. These data support colonization as a novel alternative vaccine strategy to induce protection against bacterial pathogens.[88,89]

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.[90] 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.[91] 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.[92] 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*.[93,94] These studies delivered the commensal organisms to the gut where they might enhance general mucosal immunity through interactions with the gut-associated immune system.[92]

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.[95] 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.[96] 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[97,98] and inhibition of *S. pneumoniae* infection in mouse models.[99,100] 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.[101] 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*.[102] 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 immunological response is not restricted to selected antigens and will induce redundant responses to multiple antigens, improving cross-reactivity between strains and reducing the potential for naturally occurring vaccine escape mutations. 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.

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,[103] and human carriage of *N. lactamica* results in a high titer of antibodies to *N. meningitidis*.[104] Outer membrane proteins and lipooligosaccharide structures common to both species are the major antigenic sources of cross-protection.[105] *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, caxP/mgtA*	Metabolic component deletion	111
	S. pneumoniae	*pabB*	Auxotroph	112
	S. pyogenes	*speB* and *gidA* mutation	Impaired tRNA modification	113
	Salmonella enterica serovar Typhimurium	*gidA* mutation	Impaired tRNA modification	114
Heterologous antigen expression	*S. pneumoniae*	PspA expression by *Lactobacillus casei*	Protective antigen expression	118
	S. pneumoniae	PspA, PpmA, PsaA, PppA, and SlrA expression by *L. lactis*	Protective antigen expression	119
	S. pneumoniae	Cps expression by *L. lactis*	Protective antigen expression	120, 121

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

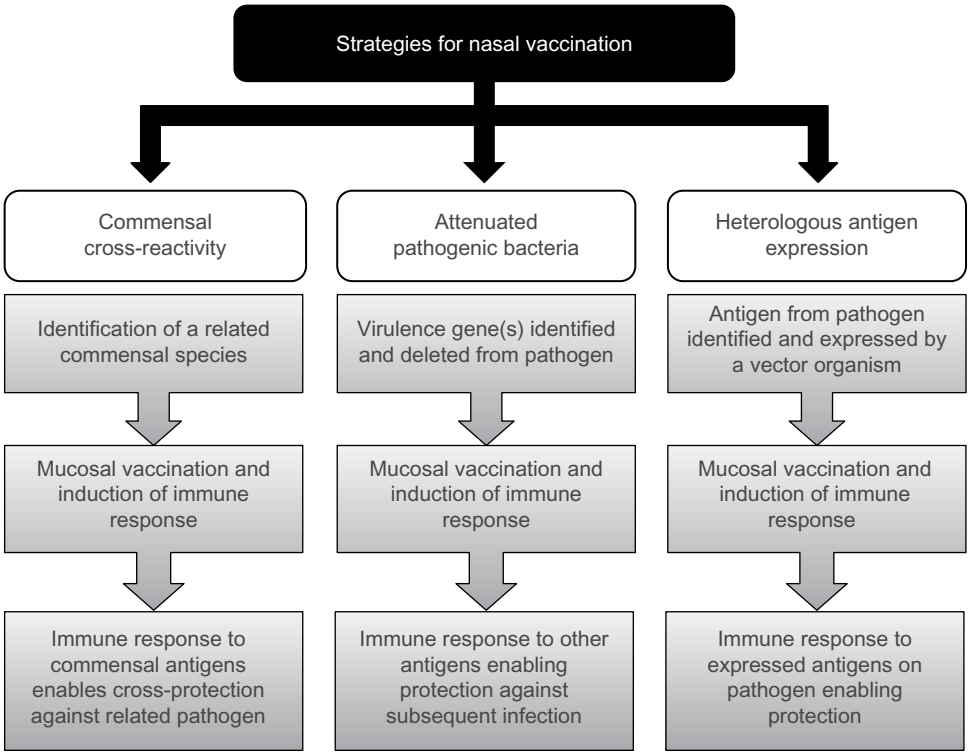

Figure 1 Summary of the principles employed in nasal vaccination strategies.

serum bactericidal effect. Furthermore, experimental *N. lactamica* colonization did not protect against subsequent natural *N. meningitidis* carriage acquisition.[104] 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.[106] 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.[107] 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.[108,109] 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[110] 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.[111] 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

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.[112] 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.[113] Mutation of *gidA* in *Salmonella enterica* serovar Typhimurium also lends itself to a novel vaccine strategy for this bacterium.[114]

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.[82,111,115] In mice, attenuated strains often have a reduced duration of colonization compared to wild-type bacteria,[107,111,115] 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.[112] Repeated dosing of poorly colonizing strains may overcome this issue.[82,107,110,115]

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 either were not or only weakly cross-protective against heterologous strains in mouse models.[112,115] 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.[116] 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.[117] 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.[118] Another LAB, *Lactococcus lactis*, has also been used to express *S. pneumoniae* protein antigens including PspA, PpmA, PsaA, PppA, and SlrA[119] or serotype 3[120] and serotype 14[121] capsular polysaccharides. Colonization with the *L. lactis* strains expressing *S. pneumoniae* capsules led to the induction of specific IgG and IgM antibodies.[120,121] The oral commensal species *Streptococcus gordonii*, which also stimulates mucosal immunity,[122] has been engineered to express protective antigens from *S. pyogenes* which are immunogenic in mouse models when inoculated intranasally and orally.[123] *S. gordonii* was also investigated as a means to express *N. meningitidis* antigens which induced bactericidal antibodies in intranasally immunized mice[124] and *Bordetella pertussis* antigens which when used in oral colonization induced systemic and mucosal antibodies.[122,125] 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.[126] 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 carriage[104,127–129] now 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.

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