EXPERT REVIEWS

Mucosal vaccination against bacterial respiratory infections

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Mucosal vaccination offers attractive advantages to conventional systemic vaccination, such as higher levels of antibodies and protection at the airway surface. This review gives an overview of recent experimental and clinical data on nasal, oral and sublingual vaccines against bacterial respiratory pathogens, such as *Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, Moraxella catarrhalis, Bordetella pertussis*, *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*. Subsequently, we discuss further vaccine development that opens the focus to clinical use.

Keywords: adjuvant • *Bordetella pertussis* • *Haemophilus influenzae* • *Moraxella catarrhalis* • mucosa-associated lymphoid tissue • mucosal vaccination • *Mycobacterium tuberculosis* • nasal vaccination • oral vaccination • *Pseudomonas aeroginosa* • respiratory infection • *Streptococcus pneumoniae*

The majority of human pathogens invade the body via the mucosal membranes, predominantly the respiratory mucosa. The number of bacteria causing respiratory diseases poses a considerable burden of disease for both children and adults. Antibiotic treatment and vaccination programs have greatly reduced this burden (Table 1). Pertussis is largely prevented with more tolerable acellular vaccines. The introduction of conjugated vaccines afforded a long-lasting protection against encapsulated bacteria, such as *Haemophilus influenzae* type b (HIB), and, more recently, *Streptococcus pneumoniae*. Importantly, conjugated vaccines allow the successful immunization of infants that have only weak immune responses to the polysaccharide surface of these pathogens. Moreover, the last few years have seen an unexpected rise in herd immunity against *S. pneumoniae* following the implementation of a general vaccination program of infants and preschool children. Conjugate vaccines against an even broader spectrum of serotypes of *S. pneumoniae* await licensing in the near future.

Despite this substantial progress made in the prevention of bacterial respiratory infections, there is still an urgent need for better protection. Common conditions, such as acute otitis media (AOM) and sinusitis, are only partly met by the available vaccines. These conditions are frequently caused by pathogens that are not targeted by the licensed vaccines (Table 2). Nonvaccine-type serotypes of *S. pneumoniae*, nontypeable *H. influenzae* (NTHi), *Staphylococcus aureus* and *Moraxella catarrhalis* cause endemic infections of the upper and lower airways. No licensed vaccine is available against *Pseudomonas aeruginosa*, a leading cause of death in several conditions, including cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD). Finally, more effective vaccines are urgently required against *Mycobacterium tuberculosis,* one of the major health threats worldwide due to growing multiple-antibiotic resistances and the detrimental conjunction with the HIV epidemic.

All vaccines presently available against bacterial respiratory pathogens are used as conventional systemic vaccines that are administered intramuscularly and elicit a systemic immune response. This review summarizes the success and limitations of the available systemic vaccines. We then focus on the basic principles, evidence and potential of nasal and oral vaccination as key strategies to optimize future vaccines that have to meet the challenges still posed by bacterial respiratory pathogens.

Success & limitations of licensed vaccines against bacterial respiratory infections

Streptococcus pneumoniae

Invasive pneumococcal disease causes an estimated 1.6million deaths per year worldwide, the majority of which could be prevented by vaccination with available vaccines [1]. The pneumococcal polysaccharide 23-valent vaccine (PPV23) is immunogenic against approximately 80% of the more than 90 serotypes that cause

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Table 1. Licensed vaccines against bacterial respiratory pathogens.

Pathogen	Related conditions	Available vaccines	Acronyms	Application	Protection rate*	Ref.
Streptococcus pneumoniae	Invasive disease (pneumonia, septicemia) AOM	Pneumococcal polysaccharide vaccine (23 valent) Pneumococcal conjugate vaccine (7 valent)	PPV23 PCV7	Systemic	Invasive disease: 60–70% (PPV23); 89% (PCV7) AOM: None (PPV23); 6% (51% of vaccine type infection [PCV7])	[2,3,7]
Haemophilus influenzae type b	Invasive disease (meningitis pneumonia, septicemia)	*Haemophilus influenzae* type b conjugate vaccine		Systemic	Invasive disease: 84% Pneumonia: 69%	[8]
Neisseria meningtidis	Invasive disease (meningitis)	*N. meningitidis* polysaccharide AC/ACWY vaccines MenC conjugate vaccines MenBC outer membrane vesicle vaccines	MenAC MenACWY MenC MenB: VA-MENGOC-BC MenB OMV	Systemic	90–10% (MenC, year 1–3 after vaccination) 40–90% (MenB)	[9–13]
Bordetella pertussis	Pertussis	Acellular pertussis vaccine	aP	Systemic	92%	[1]
Mycobacterium tuberculosis	Tuberculosis	Bacillus Calmette–Guerin live vaccine	BCG	Intradermal	15–46% (tuberculous meningitis)	[24]

*Protection rates can vary with the setting of the studies.
AOM: Acute otitis media; OMV: Outer membrane vesicle.

invasive disease and reduces invasive pneumococcal disease by 36–52% [2]. Its drawbacks are a weak immunogenicity in children below 2 years of age, a limited duration of protection and no effect on AOM. The pneumococcal conjugate vaccine (PCV)7 covers seven serotypes. The PCV7 vaccine is reported to reduce invasive disease by 97% of vaccine-type infections or by 89% of infections with all serotypes [3]. PCV7 also reduces nasal colonization and confers herd immunity leading to protection of nonimmunized individuals [4]. However, vaccine efficacy depends on the serotype prevalence that changes with location and time. Moreover, serotype replacement by nonvaccine serotypes may particularly jeopardize the beneficial effects of the PCV7 vaccine [5]. New conjugate vaccines covering an extended range of serotypes aim to account for serotype replacement and to potentially broaden protection. Two extended PCV formulations presently await licensure. Protein-based vaccines may improve vaccine coverage more efficiently as they target highly conserved epitopes [6]. However, the PCV7 vaccine is less efficient in preventing mucosal infection, such as AOM, with a prevention rate of 51% for vaccine-type infections [7]. Since nonencapsulated serotypes of pneumococcus and other bacteria are responsible for the great majority of AOM episodes, PCV7 prevents only 6% of all AOM episodes.

Haemophilus influenzae

Vaccination against HIB as part of routine vaccination successfully prevents invasive illnesses attributed to these bacteria, such as meningitis, epiglottitis and septicemia. Vaccine efficacy conferred by the presently used conjugate vaccine is estimated to be 84% for invasive disease and 69% for pneumonia [8]. However, close to 400,000 annual deaths are attributed to HIB in countries without effective vaccination programs [1]. Moreover, HIB accounts only for a minority of episodes of infections that are limited to the airway mucosa. NTHi, which is not covered by a polysaccharide capsule, is the second most common bacterial pathogen recovered in AOM and lower respiratory tract infections, in particular in preschool-aged children. Experimental vaccines directed against NTHi are discussed in the section on mucosal vaccines.

Neisseria meningitidis

Neisseria meningitidis also frequently colonizes the nasopharynx. The pathogen is classified in 13 serogroups, based on differences in antigenicity of the capsular polysaccharides. The serotypes A, C, Y and W135 account for approximately 90% of invasive meningococcal disease worldwide. However, serotype B has caused local epidemics, contributing for up to 50% of invasive infections. Overall, invasive meningococcal infection causes 26,000 deaths per year worldwide. Several polysaccharide and conjugate vaccines are available, covering serotypes A, C, Y and W135. The protection rate for a conjugate vaccine against serotype C is 99% of seroconverters [9]. Similar to the polysaccharide vaccine against pneumococcus, the polysaccharide vaccine against *N. meningitidis* has a limited duration of protection with the rate dropping from 90 to 10% within 3 years [10]. Capsular polysaccharide vaccines against serotype B are not efficient due to antigenic similarity to host glycoproteins. Vaccine development against serotype B, therefore, employed outer membrane vesicles conferring protection rates of 40–90% [11–13]. However, protection afforded by the

various serotype B vaccines appears to be strain specific [14]. New serotype B vaccines containing either more or better conserved epitopes are under development [15].

Moraxella catarrhalis

M. catarrhalis is part of the normal flora of the human upper-respiratory tract. However, it is also isolated in up to a quarter of children with AOM. Resistance to ampicillin and other β-lactam antibiotics poses an increasing risk of a complicated course of disease. No vaccine directed against *M. catarrhalis* is available.

Bordetella pertussis

The introduction of a vaccine against *Bordetella pertussis* is a major success story of vaccine development and mass-vaccination programs. Vaccine-induced protection from pertussis is 90% or more, limiting this condition to sporadic cases. Susceptibility to pertussis despite vaccination is relatively high in infants who have not completed the vaccination schedule and in adolescents who have a weaning immunity [1]. Resurgence of pertussis cases in countries with a high vaccine coverage prompted discussions on efficacy of the present cellular and acellular vaccines. Although better diagnostic tools may have contributed to a rising number of cases, the small epidemics argued for an improvement of the vaccine design. A proposed strategy is to include new antigens that account for the changing molecular epidemiology of the pathogen [16]. Again, public-health policies aim for a high vaccination coverage and an extension up to the age of adulthood to achieve herd immunity and protect infants that are particularly susceptible to complicated disease [17,201,202]. Despite these efforts, pertussis is still one of the ten most frequent causes of death in childhood worldwide, accounting for 300,000 children per year [1].

Pseudomonas aeruginosa

P. aeruginosa is one of the most frequently isolated nosocomial pathogens, the second most common pathogen for patients with COPD who require intensive care, and the most frequent cause of death for patients with CF [18–20]. Intrinsic and acquired antibiotic resistance complicate treatment of acute and chronic *P. aeruginosa* infection [21]. Prevention of *P. aeruginosa* by vaccines, therefore, is particularly desirable. A bivalent flagella vaccine was effective in preventing vaccine-type *P. aeruginosa* lung infection in CF. However, a substantial number of vaccinees revealed only a partial protection [22]. A retrospective case–control study with 30 CF patients immunized with an octavalent antipseudomonal conjugate vaccine suggested a protection rate of 50% [23]. No licensed antipseudomonal vaccine is available.

Mycobacterium tuberculosis

TB causes 1.7 million deaths each year with an estimated 9.2 million new cases [202]. The deleterious effects of HIV coinfection and the spread of multidrug-resistant strains have even worsened the burden, making it one of the most significant global health problems [203]. Vaccination with bacillus Calmette–Guerin (BCG) live bacteria is effective to prevent between 15 and 46% of meningitis cases with *M. tuberculosis* in childhood, but protection is unreliable against pulmonary TB and for adults [24]. Revaccination with BCG confers no protective effects [25]. However, booster vaccination strategies for BCG-primed individuals employing recombinant and other technologies are currently being investigated in Phase I and II clinical trials [26,27].

Despite the broad variety of the pathogens and the related diseases, all conditions have in common the fact that the pathogen enters the body via the airway epithelium. Provided appropriate antigens are used, vaccines are highly effective in preventing systemic disease, but far less so in preventing infection at the airway mucosa. Optimization strategies of vaccines aiming to prevent airway disease, therefore, have to pursue the improvement of airway defense mechanisms.

Table 2. Bacterial respiratory pathogens against which no vaccine is available.

Pathogen	Related conditions	Comment
Nonencapsulated *Streptococcus pneumoniae*	AOM, sinusitis, bronchitis	
Nonvaccine-type *S. pneumoniae*	AOM, sinusitis, bronchitis	
Nontypeable *Haemophilus influenzae*	AOM, sinusitis, bronchitis	
Moraxella catarrhalis	AOM	β-lactam resistance
Pseudomonas aeruginosa	Pneumonia (COPD, ventilation) bronchitis/bronchiectasis (CF), sepsis (burn, intensive care and nosocomial)	

AOM: Acute otitis media; CF: Cystic fibrosis; COPD: Chronic obstructive pulmonary disease.

Mucosal adaptive immunity in mucosal antibacterial defense

Antibacterial defense mechanisms strongly rely on the innate immune system, including mechanical barriers, inhibition of growth by defensins, activation of effector cells by Toll-like and other pathogen-recognition receptors, bacterial killing by phagocytes and complement mediated lysis, and other mechanisms. In adaptive immunity, antibodies play an indispensable role for protection against most bacterial respiratory pathogens with the exception of intracellular pathogens, such as *M. tuberculosis,* as demonstrated in patients with primary antibody deficiency [28]. Indeed, bacterial respiratory infections of the upper and lower airways are the most frequent infections in this patient group, emphasizing the paramount importance of antibody-mediated protection against the persistent threat of invasion of the airway mucosa by respiratory pathogens. We can also learn from this 'experiment' of nature that antibody-mediated protection of the respiratory mucosa relies on more than one isotype. Although IgA is the predominant

antibody in the upper and bronchial airways, selected IgA deficiency does not necessarily lead to an increased rate of bacterial infections [29]. IgM appears to substitute, at least in part, the protection, since patients with common variable immunodeficiency who show activated IgM memory B cells upon antipneumococcal vaccination are largely protected from chronic airway disease due to chronic bacterial infection [30]. Another important observation drawn from these patients is the lack of protection conferred by IgG-replacement therapy from bacterial infections at the upper- and bronchial-airway surface. Despite regular systemic immunoglobulin replacement therapy, virtually all patients with X-linked agammaglobulinemia develop chronic lung disease and chronic sinusitis [28]. By contrast, systemically applied immunoglobulins effectively protect from pneumonia and invasive bacterial infections.

It is long known that IgG is the predominant isotype in the distal airways as shown in analysis of bronchoalveolar lavage fluid (BALF) in humans. Indeed, IgG is the second largest protein component after albumin in BALF. The distribution of IgG subclasses in BALF largely resembles the distribution in serum [31]. We, and others, demonstrated that specific IgG antibody levels in BALF correlate well with levels in serum [32]. This has led to the assumption that IgG in the distal airways largely derives from systemic IgG reaching the alveoli by passive diffusion.

IgG exerts its immunological function by a number of mechanisms, most importantly by opsonizing pathogens by binding to Fc γ-receptors of myeloid cells, such as alveolar macrophages and neutrophils. Activation of the Fc receptors FcγRI and FcγRIII also enhances the ability of the phagocytes to kill the pathogens in the phagolysosome [33]. Another important mechanism exerted by IgG complexes is the activation of the classical complement cascade that results not only in direct killing of some pathogens by the lytic complex assembled in the terminal pathway, but also in further opsonization of the pathogen by cleavage products of the early part of the pathway, C3b, and by recruiting neutrophils by the anaphylatoxins C3a and C5a. However, the contribution of the complement system to the protection against respiratory pathogens seems to be limited to protection against pneumonia. Patients with primary complement deficiencies are prone to pneumonia and invasive infections primarily due to *N. meningitis* and *S. pneumoniae*, but not to bacterial infection of the bronchi or the upper airways [34].

On the mucosal surface of the upper airways the concentration of IgA largely outweighs IgG. The composition of mucosal IgA is different to serum IgA in several important aspects. First, it is found at the respiratory airway surface, predominantly as dimers and assembled with a fragment of the polymeric immunoglobulin receptor during the transepithelial transport, thus termed 'secretory' (S)IgA; SIgA is less prone to enzymatic cleavage. Second, the mucosal surface has a much larger proportion of IgA_2 subclass molecules than the serum IgA. In serum, IgA prevails in its monomeric form, mostly as IgA_1. Specific IgA antibody levels in nasal wash do not correlate well to serum IgA antibody levels [35,36]. The differences in subclass composition and the differences of serum and nasal antibody responses argue for a local (i.e., mucosal) regulation of IgA transport and/or antibody production.

Secretory IgA differs also in its immune function from monomeric IgG and IgA, which are prevalent in serum. While monomeric IgA largely has the same abilities as IgG to activate phagocytes and the complement cascade, SIgA has noninflammatory functions as it binds and immobilizes pathogens or neutralizes bacterial toxins but does not fix complement. This prevents adherence of pathogens to the epithelium and the promotion of an innate immune response by epithelial cells. IgA may also neutralize intracellular pathogens or their exotoxins during the transepithelial transport. A similar role applies to the secretory IgM molecules [37].

The antibody composition at the bronchial surface is less well investigated. The ratio of IgG to IgA differs with the sampling method. IgG is the predominant isotype in BALF, particularly in the later fractions of the lavage procedure that are considered to be of alveolar origin. In expectorated sputum, however, the ratio changes in favor to IgA, suggesting a more proximal site of origin of the sputum samples. Again, specific IgA antibodies obtained from sputum do not correlate to serum antibodies, while sputum IgG antibodies do [32]. This argues for a mucosal regulation of IgA synthesis and/or transport at the bronchial airways.

Antibody-mediated protection may not be the only basis of adaptive immunity against extracellular bacteria in the respiratory tract. Recent studies with various pneumococcal protein and conjugate vaccines applied to B- and T-cell-deficient mice demonstrate protection against nasal challenge with *S. pneumoniae* in the absence of a humoral immune response, but not in the absence of $CD4^+$ T cells [38]. Antigen-specific $CD4^+$ T cells may exert their protective activity by recruitment and/or activation of phagocytes [39].

These, and other observations, have led to the concept of a mucosal-associated lymphoid tissue (MALT) that differs to the systemic immune system in several ways. Priming of specific T and B cells occurs in close proximity to the mucosal surface with no afferent lymphatic structure. Antigens reach instead the MALT directly after transepithelial transport via specialized microfold (M) cells or by dendritic cells that reach out to the mucosal surface. The most striking difference to the systemic immune system is the differential expression of mucosa-specific homing receptors that guide the effector cells back to their site of induction (inductive site) [40–42]. MALT is divided into different anatomical sites, the nasopharynx-associated lymphoid dissue (NALT) and bronchus-associated lymphoid tissue (BALT) and the gut-associated lymphoid tissue (GALT) [41]. Located in the lamina propria of the gut tissue, the Peyer's patches (PPs) are the most organized structure of the GALT. PPs have a comparatively complex structure with close proximity to naive $CD4^+$ T cells and an adjacent subepithelial dome that accommodates antigen-presenting cells (APCs) that collect antigens following M-cell mediated transepithelial transport. The notion that antigen processing, structure of the submucosal lymphatic tissue, and homing mechanisms of the MALT are largely comparable, together with the observation that plasma cell homing can occur also at distant sites of the MALT, has led to the concept of a common mucosal immune system.

Mucosal vaccination strategies

Vaccination at a mucosal site differs from systemic vaccination in that it directs the immune effector cells to the site of vaccination (Figure 1). Priming of naive T and B cells in the NALT induces the expression of $\alpha_4\beta_1$ integrin and other molecules that guides effector cells from the systemic circulation back to the respiratory mucosa and also to the bone marrow by interaction with the endothelial receptor VCAM-1 [43]. Priming in the GALT induces expression of $\alpha_4\beta_7$ integrin, the ligand of the mucosal addressin cell-adhesion molecule 1 and CC-chemokine receptor 9, the receptor for chemokine ligand 25, which is expressed in the small intestinal endothelium. These and other ligand–receptor interactions provide the molecular basis for a distinct pattern of nasal and oral (i.e., intestinal) vaccination strategies that both differ from conventional systemic immunization [40].

B cells homing to the lamina propria of the mucosa characteristically produce J-chains in addition to the immunoglobulin molecules. Although IgA-secreting plasma cells are the most prevalent cell type, there are also IgM- and IgG-producing mucosal plasma cells, all of which produce J-chains. However, only IgA and IgM are secreted as polymers and actively transported after coupling to the polymeric immunoglobulin receptor through the epithelial cells, reaching the mucosal surface as SIgA and SIgM. IgG is not polymerized by J-chains and is thought to reach the mucosal surface by paracellular leakage and by active transport mediated by the neonatal Fc receptor [44].

With approximately 80% of all plasma cells, the human gut hosts the majority of plasma cells of the body irrespective of the immunoglobulin isotype [45]. Here, 90% of the entire immunoglobulin production is secreted in the gut lumen as SIgA [41]. By contrast, the number of plasma cells in other mucosal tissues is relatively small [46]. Immunoglobulin-producing plasma cells in the bone marrow and peripheral lymph nodes are approximately a quarter of the gut plasma cells in number. They produce mostly monomeric IgG (~60%) and equal proportions of IgA and IgM [47].

Mucosal plasma and memory B cells promote a mucosa-specific antibody response in the proximity of the site where the pathogen is encountered. This allows the immune system not only to rapidly respond to an offensive antigen, it may also lead to higher local antibody levels than would be achieved with antibodies produced at distant sites and diluted by serum transport [48]. Together, the conception of the NALT and BALT provides the basis for strategies to specifically enhance mucosal immunity at the airway surface.

While this review focuses on protective mucosal vaccines, it has to be emphasized that the recognition of antigens by MALT most frequently induces tolerance rather than immunity [49]. This applies to both respiratory and gut mucosa, both of which are physiologically colonized with an extensive variety and number of bacteria, without causing inflammation. Dose, duration and frequency of antigen exposure, and the properties of antigens and adjuvants are known factors that impact the immune response towards tolerance or immunity.

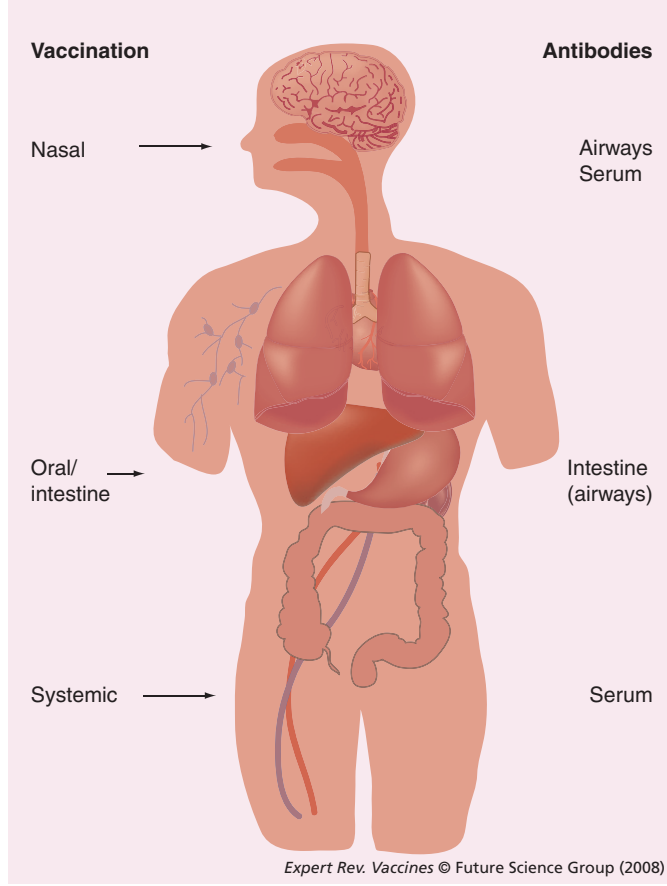

Figure 1. Influence of vaccination strategy on the site of antibody formation. Vaginal vaccination can also induce an antibody response at the airway site (not discussed in this review).

Nasal vaccination

Vaccination via the nasal mucosa appears to be a particularly suitable strategy for immunization against respiratory pathogens. An extensive body of literature demonstrates that nasal vaccination induces mucosal antibody formation on the mucosal surface of both upper and lower airways (Figure 1) [50]. Moreover, nasal vaccination leads also to the systemic immunity required to protect against pneumonia and invasive infection. The nasal mucosa is easily accessible for application of the vaccine. Antigens conveyed to the nasal mucosal surface are subjected far less to dilution and enzymatic degradation than occurs at the gut epithelium [41]. In contrast to systemic application, nasal vaccine application inevitably results in a more variable antigen dose that enters the body. Nasal delivery systems include spray, gel and powder formulations. With respect to mass vaccination, powder formulations offer the highest practicability as they are more easy to apply than gel and do not require cold-chain storage [51].

Two licensed nasal vaccines against influenza demonstrated strong immunogenicity and protection [52–54]. The fact that the innovative liposomal influenza vaccine was withdrawn from the market following concerns of toxin transport via the olfactory tract [55] illustrates that development of nasal vaccines has not only to prove efficacy but also to resolve important safety issues.

Oral vaccination

In order to activate the GALT, oral vaccination has to overcome several hurdles. It requires suitable delivery systems that resist the enzymatic degradation and the dilution exerted by the digestive enzymes. Importantly, oral vaccination induces antibody formation at the respiratory mucosa only weakly. Thus, additional strategies are required to customize oral vaccines for airway immunogenicity [56].

An oral vaccine, the oral polio live vaccine (OPV), is one of the most successful immunization strategies in the history of vaccination. The OPV is more effective than its systemic counterpart, the intramuscular polio vaccine (IPV), as it induces a considerably stronger protection at the gut mucosa, consistent with the principles of the mucosal immune system. Despite an excellent efficacy, the OPV was replaced in many countries by IPV at the time when the OPV vaccine had virtually eradicated poliomyelitis and the rare cases of vaccine-induced poliomyelitis became more frequent than infections with the wild-type virus.

Oral vaccination with attenuated pathogens, such as OPV or the oral *Salmonella* Typhi vaccine (Ty21a), generally confers the risk to cause vaccine disease in a susceptible host, such as patients with immunodeficiency. As with nasal vaccination, safety deserves particular attention in the development of oral live vaccines.

Sublingual vaccination

Sublingual vaccination is performed by administration of antigens to the oral mucosa and suits much more the term 'oral vaccination', which is commonly used for vaccines that are swallowed for uptake by the intestinal mucosa. A large body of literature exists on allergen-specific immunotherapy that attempts to induce tolerance against offending allergens by stimulating uncommitted T-helper cells (Th) towards Treg. More recently, an oral vaccine against influenza was tested in a mouse model. Similar to nasal vaccines the sublingual administration of virus antigen induced an antigen-specific humoral immune response at the respiratory mucosa, a specific T-cell response and conferred protection against experimental influenza infection. Importantly, the sublingual administration did not lead to transport of the vaccine into the CNS, as it was observed with nasal vaccination. Thus, sublingual vaccination may be an attractive alternative to nasal vaccination in terms of safety considerations [57].

Strategies of enhancing mucosal vaccines

Mucosal vaccination relies on transport of the antigen over the mucosal barrier. Dilution by mucus and digestive secretions, enzymatic degradation, rapid clearance by mucociliary transport or carriage by chyme moving through the gut by peristalsis and a limited diffusion of macromolecules across the mucosal barrier have to be considered. To overcome these problems, various strategies have been developed (Table 3). These strategies can broadly be separated into two approaches. Delivery systems (DSs) help the antigen to reach the mucosal surface, protect it from degradation and promote interaction with the MALT. Other substances, immune potentiators (IPs), have an intrinsic stimulatory effect on cells of the immune system that augments the immune response to the vaccine antigen. DSs and IPs are frequently called adjuvants, since they are coadministrated with a vaccine in order to enhance the immunogenicity of the vaccine antigens. Many substances used as adjuvants exert both DS and IP function.

Delivery systems

Live-attenuated pathogens that are able to persist and replicate after invasion can elicit a sustained immune response, but do not cause disease. This strategy is successfully applied for a live-attenuated nasal influenza vaccine. Experimental vaccines successfully employed nasal BCG and *B. pertussis* vaccination (Table 3) [58–60]. Recombinant lactobacilli expressing a heterologous antigen from *S. pneumoniae* persisted for 3 days on the nasal mucosa, conferring a prolonged antigen exposure [61]. Inactivated Gram-negative bacteria that retain the ability to adhere to the mucosa and induce mechanisms of uptake by the host, termed bacterial ghosts, have the advantage of lack of replication and can be used as IPs carrying heterologous antigens [62,63]. Other bacterial particles derive from acid-treated *Lactococcus lactis* and are termed Gram-positive enhancer matrix [64]. Carrying a heterologous antigen, these particles also exert an IP function. A potentially easy to produce, store and handle substance are bacterial spores. Recombinant technology allows to convey heterologous antigens, as has been shown for a nasal vaccine containing *Bacillus subtilis* spores used against *Bacillus anthracis* toxin [65]. Again, these particles also have immune potentiator properties.

Respiratory viruses possess numerous mechanisms to attach to the respiratory epithelium and to pass the cellular membrane. Adenoviruses and other viruses were successfully used as carriers for recombinant antigens from *M. tuberculosis* for use as a mucosal vaccine [66,67].

Microparticles are vaccine formulations of a size in the order of 1 µm. They appear to improve the immunogenicity of an antigen in that they facilitate transepithelial transport via the M cells and subsequently activate APCs of the NALT [68]. Thus, less antigen is required to achieve an immune response. Vaccine antigens can be covered by liposomes or coated with adjuvants, such as chitosan, allowing various ways for drug formulation aggregations, including suspension and dry powder. Liposomes have no adjuvant effect but they can carry the antigen together with adjuvants [69]. Chitosan is a polysaccharide that builds complexes with cholesterol and fatty acids. It has been shown to open tight junctions of epithelial cell layers and to reduce the rate of mucociliary clearance, thereby facilitating higher drug and carrier bioavailability. Although chitosan has immune stimulatory properties, chitosan microsphere vaccines were more effective in combination with other adjuvants [70,71].

Mucosal vaccination enjoys the reputation to be needle-free, but injection technique was also successfully applied for NALT immunization. Mice receiving an adjuvanted *M. catarrhalis* whole-cell vaccine by injection into the palatine showed a stronger mucosal immune response than mice receiving the suspension onto the nasal mucosa [72].

The simplest, and perhaps easiest, way in terms of licensing is to increase the amount of antigen applied to the nasal mucosa without use of DSs or IPs until the desired level of immunization is achieved.

Table 3. Delivery systems and immune potentiators (adjuvants) used with mucosal vaccines against bacterial respiratory pathogens.

Strategy	Type	Vaccine/antigen	Targeted pathogen	Vaccinated species	Ref.
Bacterial systems					
Live-attenuated bacterial accines	DS, IP	BCG *Bordetella pertussis* strain BPZE1 *Salmonella typhimurium* expressing OprF-OprI from *Pseudomonas aeroginosa*	*Mycobacterium tuberculosis* *B. pertussis* *P. aeruginosa*	Mouse	[56,58–60,109]
Bacterial ghosts and Gram-positive enhancer matrix	DS, IP	*Streptococcus pneumoniae* antigens (PpmA, SlrA, Ig1Ap) embedded in *Lactococcus lactis* particle	*S. pneumoniae*	Mouse	[122]
Bacterial spores	DS, IP	Protective antigen from anthrax with *Bacillus subtilis* spores	Anthrax	Mouse	[65]
Viral systems					
Recombinant viruses	DS, IP	Adenovirus, vaccinia virus expressing Ag85A from *M. tuberculosis*	*M. tuberculosis*	Mouse	[66,67]
Microparticles					
Liposomes	DS	OpaB, OpaJ or outer membrane vesicles from *Neisseria meningitidis*	*N. meningitidis*	Mouse, rabbit	[69,95]
Chitosan and *N*-trimethyl chitosan chloride microspheres	DS, IP	Group C meningococcal conjugated vaccine	*N. meningitidis* group C	Mouse	[71]
Others					
Microinjection	DS	*Moraxella catarrhalis* whole-cell vaccine	*M. catarrhalis*	Mouse	[72]
High-dose application	DS	OprF-OprI from *P. aeruginosa*	*P. aeruginosa* OprF-OprI vaccine	Human	[73]
Attenuated bacterial toxins					
Tetanus toxoid	IP	Lipooligosaccharide from nontypeable *Haemophilus influenza* tetanus toxoid conjugate, Arabinomannan–tetanus toxoid conjugate	NTHi, *M. tuberculosis*	Mouse	[85,111]
Monophosphoryl lipid A	IP	OpaB, OpaJ from *N. meningitidis* in liposomes Hexavalent group A streptococcal M-protein-based vaccine	*N. meningitidis* Group A *Streptococcus*	Mouse	[69,116]

BCG: Bacillus Calmette–Guerin; CRM: Nontoxic mutant of diphtheria toxin; CRM-MenC: Meningococcus group C vaccine, contains CRM_{197} and alum (licensed vaccine); DS: Delivery system; IP: Immune potentiator; LPS: Lipopolysaccharide; NTHi: Nontypeable *Haemophilus influenzae*; OMP: Outer membrane protein; PCV7: 7-valent conjugated *Streptococcus pneumoniae* vaccine, contains CRM_{197} and alum (licensed vaccine).

Table 3. Delivery systems and immune potentiators (adjuvants) used with mucosal vaccines against bacterial respiratory pathogens (cont.).

Strategy	Type	Vaccine/antigen	Targeted pathogen	Vaccinated species	Ref.
Cholera toxin and derivatives (CTA1-DD, CT-B, CT-E29H)	IP	Ag85B-ESAT-6 Hexavalent group A streptococcal M-protein-based vaccine Whole-cell *S. pneumoniae* vaccine Phosphorylcholine Recombinant lipidated P2086 protein	*M. tuberculosis* Group A streptococci *S. pneumoniae* *H. influenzae and S. pneumoniae* *N. meningitidis* group B	Mouse, rat	[84,94,116,123,124]
Attenuated bacterial toxins					
Proteosomes from *N. meningitidis* OMP complexed with LPS from *Shigella flexneri*	IP	Hexavalent group A streptococcal M-protein-based vaccine	Group A streptococci	Mouse	[116]
Escherichia coli heat-labile toxins (LTK63, LTR72 and others)	IP	CRM-MenC Surface proteins App, NhhA, and NadA from *N. meningitidis* Group B OpaB, OpaJ from *N. meningitidis* in liposomes Ag85A from *M. tuberculosis* Surface adhesin protein AgI/II of *Streptococcus mutans* BCG	*N. meningitidis* group C *N. meningitidis* group B *N. meningitidis* *M. tuberculosis* *S. mutans* *M. tuberculosis*	Mouse	[69,71,97,110,113,125]
Membrane bound diptheria toxin (CRM_{197})	IP	PCV7	*S. pneumoniae*	Mouse	[80,126]
LPS	IP	OpaB, OpaJ from *N. meningitidis*	*N. menigitidis*	Mouse	[69]
Other immune potentiators					
CpG oligodeoxynucleotide	IP	P6 protein of NTHi	NTHi	Mouse	[88]
Immune-stimulating complexes	IP	Ag85B-ESAT-6	*M. tuberculosis*	Mouse	[124]
Adamantylamide dipeptide	IP	OMP CD	*M. catarrhalis*	Mouse	[117]
Aminoalkyl glucosaminide-phosphate compound aquaeous formulation (RC529-AF)	IP	Recombinant lipidated P2086 protein	*N. meningitidis*	Mouse	[94]
Interleukins	IP	PCV7	*S. pneumoniae*	Mouse	[80]
Adenoviral vector	DS/IP	AdAg85A	*M. tuberculosis*	Mouse	[127]

BCG: Bacillus Calmette–Guerin; CRM: Nontoxic mutant of diphtheria toxin; CRM-MenC: Meningococcus group C vaccine, contains CRM_{197} and alum (licensed vaccine); DS: Delivery system; IP: Immune potentiator; LPS: Lipopolysaccharide; NTHi: Nontypeable *Haemophilus influenzae*; OMP: Outer membrane protein; PCV7: 7-valent conjugated *Streptococcus pneumoniae* vaccine, contains CRM_{197} and alum (licensed vaccine).

Proteins that can be taken up by dendritic cells are more suitable than polysaccharides for this strategy [73]. A potential drawback of this strategy is the cost of the increased amount of antigen.

Oral vaccines have to use particular strategies to avoid dilution and degradation in the intestinal lumen. Live-attenuated oral vaccines based on *Salmonella* expressing a heterologous protein were successfully used in experimental and clinical studies [74-77]. *Salmonella* actively invades the host mucosa via the M cells and survives intracellularly in the phagosome of the APCs during their migration to the lymphoid tissue. Expression of the recombinant vaccine antigen can lead to a prolonged presentation of the antigen directly at the immunological synapse and a sustained mucosal immunogenicity (Table 3) [56].

Mucosal immune potentiators

Many adjuvants promote an enhanced immune response by activating the immune cells via receptors of the innate immune system or by intrinsic effects. Most frequently, adjuvants are derivatives of bacterial toxins that have lost their toxicity but retained their immunostimulatory properties (Table 3). The licensed systemic conjugate vaccines successfully employ adjuvants, such as the CRM_{197}-protein, from *Corynebacterium diphtheriae*. They induce a long lasting T- and B-cell memory with polysaccharides that otherwise induce only a transient B-cell response.

Various adjuvants were also tested for use with nasal administration and were shown to enhance the mucosal immune response and protective immunity following airway challenge (Table 3). Beside the attenuated bacterial toxins, other stimulatory agents are also used, such as CpG oligodeoxynucleotide, interleukins and other chemical substances. Most nasal vaccines use a combination of DSs and mucosal IPs or a combination of several adjuvants [78,79]).

Experimental & clinical studies with mucosal vaccines

Development of vaccines aimed for immunization in humans is a long-lasting process, starting in animals with experimental toxicity and immunogenicity studies, followed by efficacy testing prior to the same course of studies in humans. To date, the majority of the mucosal vaccines are in the stage of animal testing, most based on mouse models. Fortunately, the mechanisms of NALT and BALT immune activation and homing appear to be reasonably comparable between mice and humans facilitating preclinical development [41]. Infection models to test the efficacy of antibacterial vaccination, however, are less well transferable, varying with the pathogen and the related clinical condition. Unless stated otherwise, data on immunogenicity and efficacy are obtained from mouse experiments.

Streptococcus pneumoniae

Licensed conjugate vaccines against *S. pneumoniae* afford high protection rates against invasive disease, but only partial protection against mucosal infection, such as AOM, even with vaccine-type serotypes. Variants of nasal mucosal antipneumococcal vaccines, therefore, aim to enhance mucosal host defense (Table 4). An example is the nasal vaccine based on the commercial PCV7 (Prevenar®) that uses the conjugated adjuvant CRM_{197} together with a host

Table 4. Mucosal vaccines against *Streptococcus pneumoniae* in animal and clinical studies.

Antigen/vaccine	Adjuvant/delivery system	Route	Vaccinated species	Immunogenicity	Protection	Ref.
Whole-cell/capsular polysaccharides	Cholera toxin B	Nasal	Mouse	Not assessed	Nasal colonization Septicemia Pneumonia	[38,123]
Recombinant fusion proteins	Gram-positive enhancer matrix	Nasal	Mouse	Mucosal plus systemic	Pneumonia	[122,128]
7-valent conjugate vaccine (Prevenar®)	IL-2, CRM_{197}, alum	Nasal	Mouse	Mucosal plus systemic	Acute otitis media Nasal colonization	[80–82]
Phosphorylcholine	Cholera toxin	Nasal	Mouse	Mucosal plus systemic	Nasal colonization	[84]
Pneumococcal surface antigen A and Pneumococcal surface protein A		Nasal	Mouse	NA	Nasal colonization	[129]
Pneumococcal surface antigen A (fusion protein)	Cholera toxin B (fused to pneumococcal surface antigen A)	Nasal	Mouse	Mucosal plus systemic	Nasal colonization	[130,131]
PsaA expressed in lactic acid live bacteria	Lactic acid bacterial compounds	Nasal	Mouse	Mucosal plus systemic	Nasal colonization	[61]
Pneumococcal surface protein A and C	Recombinant attenuated live *Salmonella*	Oral	Mouse	Mucosal and systemic	Pneumonia	[76]
Pneumococcal surface adhesin A	Microspheres, cholera toxin B	Oral	Mouse	Mucosal and systemic	Pneumonia	[132]

CRM: Nontoxic mutant of diphtheria toxin.

cytokine, IL-12 [80–82]. All nasal pneumococcal vaccines engender a strong immunogenicity at both mucosal and systemic sites. The availability of murine models of nasal colonization and of AOM allowed to testing the protective efficacy of antipneumococcal vaccines at the mucosal site. Nasal colonization, AOM and invasive *S. pneumoniae* infection were successfully prevented by the nasal pneumococcal vaccines summarized in Table 4. One of the first studies that directly compared immunogenicity and protection afforded by nasal and systemic (subcutaneous) vaccination using the same antigen, pneumococcal surface protein A demonstrated the superiority of the mucosal vaccination strategy. The nasal vaccine was more successful in preventing pneumococcal colonization on the nasal mucosa surface but also in attenuating experimental pneumonia and peritonitis [83]. Vaccination with a component of capsular polysaccharide and the cellular membrane, phosphorylcholine, which is present in a number of pathogens, including *S. pneumoniae* and *H. influenzae*, protected mice from nasal colonization with both pathogens [84]. Interestingly, nasal but not systemic (intramuscular), vaccination with Prevenar-protected mice from nasal colonization, supporting the concept of enhanced mucosal efficacy of mucosal vaccines [82]. To date, there are no clinical studies on nasal pneumococcal vaccination.

Non-typeable Haemophilus influenzae & Moraxella catarrhalis

Several nasal vaccines were developed against NTHi, which is not covered by the licensed conjugate vaccine, but causes a high proportion of AOM episodes (Table 5). As with *S. pneumoniae* vaccines, all reported nasal NTHi vaccines reduce nasal colonization in mice irrespective of the adjuvant [84–87], thus reducing the risk of AOM [88]. Some vaccines are directed against NTHi and other bacterial species that frequently cause AOM, such as *M. catarrhalis* or *S. pneumoniae* (Table 5) [84,89]. A successful nasal clearance of *M. catarrhalis* also followed nasal immunization with a mutant of diphtheria toxin (dLOS-CRM) which is crossreactive to *M. catarrhalis* and exerts IP function [90]. All vaccines are in the stage of animal studies.

Clincal studies with oral vaccination using an *H. influenzae* lysate date back to the early nineties, showing inconsistent results (Table 5) [91,92]. Similarly, only weak evidence for beneficial effects was found in controlled clinical trials using a bacterial lysate derived from a collection of bacteria, including *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* (Bronchovaxom®), which are widely used for oral vaccination in several European countries [93].

Table 5. Mucosal vaccines against *Haemophilus influenzae* and *Moraxella catarrhalis* in animal and clinical studies.

Antigen/vaccine	Adjuvant	Route	Vaccinated species	Immunogenicity	Protection	Ref.
NTHi	Detoxified lipooligosaccharide–tetanus toxoid	Nasal	Mouse	Mucosal plus systemic	NA	[85]
Phosphorylcholine of NTHi and *Streptococcus pneumoniae*	Cholera toxin	Nasal	Mouse	Mucosal plus systemic	Nasal clearance	[84]
P6 protein of NTHi	Cholera toxin or CpG ODN	Nasal	Mouse	Mucosal plus systemic	Nasal clearance, AOM	[86,88]
rLP4/rLP6/UspA2 of NTHi and *Moraxella catarrhalis*	Lipidation	Nasal	Mouse	Mucosal plus systemic	Pulmonary clearance of NTHi	[89]
P4 protein of NTHi	Cholera toxin	Nasal	Mouse	Mucosal plus systemic	Nasal clearance	[87]
Detoxified-lipooligosaccharide-cross-reactive mutant of diphtheria toxin (dLOS-CRM)	None	Nasal	Mouse	Mucosal plus systemic	Nasal clearance	[90]
Killed *Haemophilus influenzae*	None	Oral	Human	NA	Fewer antibiotic prescriptions in treatment group	[92]
Killed *H. influenzae* and *Staphylococcus aureus*	None	Oral	Human	Mucosal (saliva) plus systemic	NA	[91]
Lysate from *S. pneumoniae, H. influenzae, M. catarrhalis* and others (Bronchovaxom®)	None	Oral	Human	NA	Reduced rate of upper airway infections in some trials	[93]

AOM: Acute otitis media; NA: Not assessed; NTHi: Nontypeable *Haemophilus influenzae;* ODN: Oligodeoxynucleotide.

Table 6. Nasal vaccines against *Neisseria meningitidis* in animal and clinical studies.

Antigen/vaccine	Adjuvant/delivery systems	Route	Vaccinated species	Immunogenicity	Protection	Ref.
CRM-MenC	TMC, LTK63, SMBV, CRM_{197}	Nasal	Mouse	Mucosal and systemic	NA	[71,133]
Neisseria meningitidis group B antigens	LTR72	Nasal	Mouse	Mucosal and systemic	NA	[97]
OMP from *N. meningitidis*	CT-E29H, RC529-AF	Nasal	Mouse	Systemic	Nasal clearance	[94]
Opa from *N. meningitidis*	LPS, MPL, EtxB, liposomes	Nasal	Mouse	Mucosal and systemic	NA	[69]
CRM-MenC	CRM_{197}, chitosan	Nasal	Human	Mucosal and systemic	NA	[98]

CRM_{197}: Nontoxic mutant cross-reacting material 197 of diphtheria toxin; CRM-MenC: Meningococcal group C conjugated vaccine, contains CRM and alum; CT-E29H: Modified cholera toxin; EtxB: B-subunit of *Escherichia coli* heat-labile enterotoxin; LPS: Lipopolysaccharide; LTK63: Heat-labile enterotoxin 63 from *E.coli*; LTK72: Heat-labile enterotoxin 72 from *E. coli*; MPL: Monophosphoryl lipid A; NA: not assessed; OMP: Outer membrane protein; RC529-AF: Aminoalkyl glucosaminide-phosphate compound aquaeous formulation; SMBV: Supramolecular biovector system nanoparticles; TMC: Trimethyl chitosan chloride.

Neisseria meningitidis

N. meningitidis is both a commensal and a pathogen as it frequently colonizes the nasal mucosa but occasionally causes serious invasive disease. Nasal vaccination strategies aim to reduce the risk of invasion at the mucosal site by limiting nasal colonization. However, as all nasal vaccines also engender systemic immunity, protection is not limited to the mucosal surface (Table 6) [69,71,94,95]. Licensed vaccines do not cover serotype B capsular polysaccharide (α2–8-*N*-acetylneuraminic acid) because the structure is homologous to that of a nerve cell adhesion molecule that is present in developing neural tissue and in small amounts in adult tissues. Reverse vaccinology and screening antibodies induced by natural infection enabled to detect new epitopes for vaccination [96] and to develop new antiserotype B vaccines [97]. In a Phase I clinical trial, conjugated MenC vaccine mixed with chitosan powder insufflated into the nasal cavity elicited levels of bactericidal serum antibodies comparable to systemic vaccination, but was related to fewer local side effects and, importantly, induced higher levels of specific SIgA antibodies at the nasal mucosa (Table 6) [98].

Bordetella pertussis

B. pertussis antigens are repeatedly used as adjuvants for nasal vaccines and may confer specific pertussis immunity as an additional benefit [99]. Adjuvants may even mutually reinforce their immunogenicity. A divalent vaccine consisting of a fusion protein from pertussis toxin and cholera toxin A_2B afforded protection in murine *B. pertussis* lung infection [100]. Few vaccines have been developed to directly protect from pertussis (Table 7). A live attenuated *B. pertussis* vaccine showed a comparable immune response and protection to conventional systemic immunization with the acellular pertussis vaccine in mice [60]. A promising feature of this approach was the observation that infant mice were better protected with the nasal live vaccine [59]. Nasal vaccination of single pertussis antigens or whole-cell lysate elicited a broad cellular immune response in neonatal mice [101]. A nasal whole-cell lysate vaccination was already tested in human volunteers eliciting specific and crossreactive nasal IgA antibodies [102].

Pseudomonas aeruginosa

Nasal vaccination against *P. aeruginosa* appears a particularly promising strategy to induce an enhanced immunity at the respiratory site required in patients suffering from *P. aeruginosa* airway infection, such as CF and COPD. However, few studies are as yet reported (Table 7). Nasal immunization with an attenuated AroA-deficient mutant of *P. aeruginosa* afforded protection from fatal pneumonia and elicited bactericidal antibodies [103]. However protection was serotype dependent, suggesting limited crossreactivity of the epitopes of this strain. A nasal vaccine based on a recombinant fusion protein of the highly conserved outer membrane proteins OprF and OprI was immunogenic in rodents and humans [32,73,104]. In this approach, the vaccine antigen was formulated in an inert gel without IP properties. Clinical data available so far show an excellent safety profile and a strong immunogenicity in human airways [32,104].

Oral vaccination is known to elicit a sustained intestinal immune response but weaker reaction at distant sites, the respiratory mucosa and the systemic immune system. Oral vaccination strategies, therefore, are rarely used in conjunction with respiratory pathogens. Oral primary vaccination followed by a systemic booster, however, seems to be a promising strategy for airway immunization. This was shown with an attenuated *Salmonella* expressing the heterologous outer membrane proteins OprF and OprI from *P. aeruginosa*. Mice immunized with the *Salmonella enterica serovar* Typhi OprF-OprI vaccine exhibited high levels of IgA and IgG antibodies at the upper and lower airways as well as in the systemic circulation [56].

Mycobacterium tuberculosis

The induction of a cellular immune response is essential for the development of an effective vaccine [105]. However, experimental data suggest that mucosal antibodies may also contribute to the protective immune response to *M. tuberculosis*. Mice lacking the polymeric immunoglobulin receptor, essential for transepithelial transport of polymeric immunoglobulins, are more susceptible to pulmonary TB infection [106]. Passive vaccination with *M. tuberculosis*-specific antibodies attenuates the course of TB infection in mice [107]. Since in pulmonary TB, the pathogen is acquired

via the airways, a preventive vaccine that induces both, a strong cellular Th1 polarized systemic and mucosal immune response together with a mucosal antibody response, may be the most promising approach [108]. Experimental data using nasal vaccination strategies strongly support this concept (Table 8). Importantly, some studies directly show that nasal vaccination is superior to systemic vaccination. Multiple strategies successfully employed both nasal application of the conventional BCG live vaccine and a set of promising recombinant antigens. The simplest approach is to change the common subcutaneous application of BCG live bacteria to intranasal application. This change of strategy alone reduces intrapulmonary bacterial load more efficiently than subcutaneous vaccination in mice [58,109]. One of the most frequently used approaches in mouse models is the enhancement of primary live BCG vaccination with a nasal adjuvanted booster using heat-killed BCG [110,111]. Recombinant proteins from *M. tuberculosis* vaccines proved more protective as nasal than as systemic vaccines, in protein prime-booster schedules, or as a protein booster following BCG

Table 7. Mucosal vaccines against *Borella pertussis* and *Pseudomonas aeroginosa* in animal and clinical studies.

Antigen/vaccine	Adjuvant/delivery system	Route	Vaccinated species	Immunogenicity	Protection	Ref.
Bordetella pertussis						
Live-attenuated *Bordetella pertussis* BPZE1	None	Nasal	Mouse	Systemic	Pulmonary clearance	[59]
Peractin, whole-cell lysate	DT, TT, LT	Nasal	Mouse	Cellular (neonates)	NA	[101]
PT	CT $\alpha_2\beta$	Nasal	Mouse	Systemic plus mucosal	Pulmonary clearance	[100]
PT	CT and commercial DPT vaccine	Nasal	Mouse	Systemic plus mucosal	Pulmonary clearance	[134]
Filamentous hemagglutinin and recombinant pertussis toxin	Chitosan	Nasal	Mouse	Systemic plus mucosal	NA	[135]
PT live vaccine	Attenuated live *Salmonella typhimurium*	Oral	Mouse	Systemic plus mucosal	NA	[136]
Whole-cell pertussis vaccine	None	Nasal	Human	Systemic, mucosal plus cellular	NA	[102]
Pseudomonas aeruginosa						
AroA-deficient nasal live mutant *Pseudomonas aeruginosa* (nasal)	None	Nasal	Mouse	Systemic	Survival in lethal lung infection	[103]
S. typhimurium oral live vaccine expressing OprF-OprI	Attenuated *S. typhimurium*	Nasal	Mouse	Mucosal plus systemic	NA	[56]
OprF-OprI nasal gel	None	Nasal	Human, mouse, rat	Mucosal plus systemic (humans)	NA	[32,73,104]
Octavalent O-polysaccharide toxin A conjugate	CT, CpG oligonucleotide	Nasal	Mouse	Mucosal plus systemic	Survival in lethal lung infection	[137]
Purified proteins Pa13, azurin, acyl carrier protein, amidase, aminopeptidase, KatE	None	Nasal	Rat	NA	Pulmonary clearance	[138]
Killed *P. aeruginosa*	None	Subserosal injection in Peyer's patches	Rat	NA	Pulmonary clearance	[139]
Polysaccharide antigens	Liposomes	Nasal, oral	Rat	Mucosal plus systemic	Survival in lethal lung infection	[140]

CT: Cholera toxin; DPT: Diphtheria, pertussis, and tetanus vaccine, contains alum (licensed vaccine); DT: Diptheria toxoid; LT: *Escherichia coli* heat-labile enterotoxin; NA: Not assessed; OprF-OprI: Recombinant fusion protein of the outer membrane proteins F and I from *Pseudomoas aeruginosa*; PT: Pertussis toxin; TT: Tetanus toxoid.

primary vaccination [67,112,113]. Nasal application of live bacteria may raise safety issues for use in humans as bacterial invasion of the CNS cannot be excluded. Recombinant antigens applied nasally, therefore, are more likely to be used in future clinical trials.

Expert commentary

Despite unprecedented progress in the field of vaccine development and vaccination campaigns, bacterial respiratory infections cause a death toll of several millions each year. While invasive disease with potentially serious sequelae, such as pertussis and HIB, are rare in most countries, these diseases retain their threat in those countries that cannot afford appropriate routine vaccination programs. Vaccines that are cheaper in production than the relatively expensive conjugate vaccines or are more simple to apply are needed.

However, even with better resources in healthcare, protection against bacterial respiratory pathogens has many gaps. The presently available vaccines against *S. pneumoniae* and *N. meningitidis* do not cover all serotypes, necessitating optimization in antigen selection. Less well discussed, but even more frequently needed, is better protection against bacterial pathogens that cause AOM, sinusitis and other upper airway infections, against which the present vaccines afford only partial, if any, protection, even if the infection is caused by a vaccine-type pathogen. No licensed vaccines are available against NTHi, nontypeable *S. pneumoniae* or *M. catarrhalis*, which cause the majority of these common conditions. More rarely, but associated with a high mortality rates, are *P. aeruginosa* infections, causing an increasing burden with nosocomial infections and fatal pneumonia in patients with COPD and CF. Again, no *P. aeruginosa* vaccine is presently available.

The most urgent need for improved protection, however, is for an effective vaccine against TB, not only for developing countries. Despite intense research, the conventional BCG vaccine affords only limited and weaning protection and leaves adults essentially unprotected.

Although this review focuses on mucosal vaccines, mucosal vaccination is obviously not the only strategy to optimize vaccine-based prevention against bacterial respiratory pathogens. Rational antigen selection is an indispensable basis for optimal vaccines. However, the choice of the mucosal route for vaccination may contribute to a considerable extent in alleviating the global burden of these bacterial infections.

Nasal, oral and sublingual vaccinations involve a relatively simple administration technique that is suitable for mass vaccination as it does not require the strict adherence to sterile injection techniques in order to prevent transmission of HBV and HIV. Therefore, in countries with severely limited resources, campaigns for mucosal vaccinations would be more realistic to implement than systemic vaccination campaigns. This would improve worldwide coverage of vaccination with already available vaccines, such as pertussis, HIB and pneumococcus. Needless to say that not only healthcare policy makers, but vaccinees would welcome a needle-free vaccination.

The nasal vaccination strategy is superior to conventional systemic vaccination in that it induces both a strong mucosal and systemic immune response. A large body of literature using multiple experimental settings and vaccines demonstrates higher antibody levels at the upper and lower airways engendered by mucosal vaccination in comparison with systemic immunization (Table 9). Concordantly, nasal vaccines engender better rates of protection at the respiratory mucosa in a number of animal models, including bacterial respiratory infection. Several licensed vaccines that have shown limited protection against respiratory infections, such as AOM, would greatly benefit from employing a nasal immunization strategy. This applies particularly to the polysaccharide and conjugated pneumococcal vaccines. Importantly, nasal vaccines also reliably induce systemic immunity at a comparable level to systemic vaccination. A change from systemic to nasal application would, therefore, not jeopardize the required protection against invasive disease.

Despite their well-documented conceptual advantages, no licensed nasal antibacterial vaccine is available at present. Why have nasal vaccines still not reached the stage of a commercial product?

Among the reasons why mucosal respiratory vaccines have not received much attention are the difficulties of assessing the mucosal immunogenicity in humans. The immunogenicity is commonly assessed in serum, occasionally in saliva, but rarely at the airways. Assessment of antibodies at the nasal mucosa is not well standardized. To determine antibodies in the lower airways is expensive due to the invasive nature of bronchoalveolar lavage [32,114], preventing application of this technique in larger cohorts. Recovery of bronchial antibodies with induced sputum may facilitate future studies [32].

It has yet to be demonstrated that nasal vaccines are generally immunogenic and effective in young children. Nasal vaccination of children aged 6–59 months with the cold-adapted live influenza vaccine resulted in a superior relative efficacy compared

Table 8. Nasal vaccines against *Mycobacterium tuberculosis*.

Antigen/vaccine	Adjuvants	Immunogenicity	Protection	Ref.
BCG		NA	Pulmonary clearance	[58,109]
Killed BCG	LT	Cellular response	Pulmonary clearance	[110]
Killed BCG	AM-TT	NA	Systemic clearance, pulmonary granuloma	[111]
BCG/Ag85B-ESAT-6	CTA1-DD/ ISCOM	Cellular	Pulmonary clearance	[124]
BCG/Ag85B-ESAT-6	LTK63	Cellular response	Pulmonary clearance	[113]
Ag85A from MTB	Adenovirus vector	Cellular response in lung	Pulmonary clearance	[67,112]

BCG: Bacillus Calmette–Guerin, CT: Cholera toxin; ISCOM: Immunostimulating complex; LT: *Escherichia coli* heat-labile enterotoxin; MTB: *Mycobacterium tuberculosis*, NA: Not assessed, TT: Tetanus toxoid.

Table 9. Animal and clinical studies directly comparing mucosal and systemic vaccination against bacterial respiratory pathogens.

Antigen/vaccine	Vaccination routes	Vaccination with best airway immunogenicity	Vaccination with best protection	Ref.
Animal studies				
PspA	Nasal/sc.	Nasal	Nasal	[83]
Pneumococcus (PCV7)	Nasal/im.	Nasal	Nasal	[82]
Pertussis	Nasal (live)/im. (aPV)	NA	Nasal	[59]
Meningococcus	Nasal/im.	Nasal	NA	[71,133]
Moraxella	Nasal/sc.	Nasal	Nasal	[90]
Pseudomonas	Oral (live)/im. (protein)	Oral	NA	[56]
BCG	Nasal/sc.	Nasal	Nasal	[109]
BCG	Nasal/sc.	Nasal	Nasal	[58]
AdAg85A/BCG	Nasal/im./sc.	Nasal	Nasal	[67]
Clinical studies				
Meningococcus	Nasal/im.	Nasal		[98]

aPV: Acellular pertussis vaccine (licensed vaccine, contains alum); BCG: Bacillus Calmette–Guerin; im.: Intramuscular; NA: Not assessed; PCV7: 7-valent conjugated *Streptococcus pneumoniae* vaccine, contains CRM_{197} and alum (licensed vaccine); PspA: Pneumococcal surface protein A; sc.: Subcutaneous.

with systemic vaccination with an inactivated trivalent influenza vaccine in a large controlled clinical trial [53]. Neonatal mice mount a protective antibody response following various nasal vaccines against pneumococcus [81] or *B. pertussis* [59,101]. These data do not support the assumption that young age contradicts nasal immunization.

The most important obstacle for commercial development of nasal vaccines, however, is the lack of licensed nasal adjuvants. Multiple experimental studies demonstrated the potential of adjuvants to significantly enhance the mucosal and systemic immunogenicity of nasally applied antigens (Table 3). The impact on the Th1-Th2 balance is well described, allowing to tailor the immune response into a desired direction [115]. The multitude of available substances, however, requires systematic research on the optimal combination of antigen and adjuvant [116].

With a host of adjuvants well characterized in experimental studies, safety for use in humans is a central issue for further vaccine development. A nasal liposomal influenza vaccine with an enterotoxin from *E. coli* was withdrawn from the market due to an association with Bell's palsy [55]. The observation that substances applied nasally can reach the CNS via the olfactory tract makes it particularly important to develop less-toxic substances. This does not mean that every particle applied through the nose is necessarily toxic. The nasal live vaccine with attenuated influenza viruses has an excellent safety profile despite its ability for viral replication. Several nasal vaccine adjuvants, including the extensively used adjuvant CRM_{197}, highly attenuated enterotoxin, bacterial outer membrane proteins and nanoparticles were successfully used in clinical trials exhibiting good tolerability (Table 10). Newly developed synthetic adjuvants have an attractive immunologic and safety profile and may further reduce the risk of central nervous toxicity [117–120]. Some nasal vaccines may even be used without adjuvants. Highly immunogenic proteins, such as recombinant outer membrane proteins from *P. aeruginosa* were successfully used for nasal vaccination in experimental and clinical studies [32,104,121].

Five-year view

The coming years will see important steps in the development of nasal vaccines. New and well-characterized adjuvants will allow tailoring of the immune response to nasal vaccines into a desired direction, such as a Th1-type response to *M. tuberculosis*, in conjunction with an acceptable safety profile. Nasal booster vaccination using recombinant antigens from *M. tuberculosis* promises to expand the efficacy of the presently used BCG vaccination to a better protection against pulmonary TB. The implementation of a booster strategy also allows maintaining the proven, albeit limited, protection offered by BCG vaccination while the potential benefit of the nasal recombinant booster can be assessed in clinical trials.

The introduction of PCVs with an extended spectrum of serotypes may delay the problem of serotype replacement, but it will not extensively change the overall prevalence of bacterial upper airway infections, including AOM and bacterial sinusitis. Therefore, nasal vaccines against the nontypeable variants of *H. influenzae* and other pathogens will be attractive for commercial development as they offer to be successful in a mass market.

Table 10. Nasal adjuvants used in clinical trials.

Adjuvant	Vaccine against	Ref.
Heat-labile toxins from *Escherichia coli* (LTK63 and others)	Influenza	[54,141]
SMBV	Influenza	[142]
Proteosome (Outer membrane proteins of *Neisseria meningitidis)*	Influenza, *Shigella*	[143–145]
Adenovirus	Influenza	[146]
CRM_{197}	*N. meningitidis*	[98]
Chitosan	*N. meningitidis,* diphtheria	[98,147]

CRM197: Nontoxic mutant crossreacting material 197 of diphtheria toxin; LTK63: Heat-labile enterotoxin 63 from *Escherichia coli*; SMBV: Supramolecular biovector system nanoparticles.

Antipseudomonal vaccines are less frequently, but more urgently, needed for selected patient groups. Recombinant protein vaccines will evolve as an important strategy to prevent fatal septicemia and pneumonia with *P. aeruginosa* for patients with burns or receiving artificial ventilation. The already clinically tested nasal OprF-OprI vaccine holds promise to be particularly effective in patients with pulmonary conditions, such as CF and COPD.

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Key issues

- Presently available vaccines against *Streptococcus pneumoniae, Haemophilus influenzae* type b and *Neisseria meningitidis* have limited efficacy against noninvasive mucosal infection, such as acute otitis media and bacterial sinusitis.
- No vaccines are available against nontypeable *H. influenzae* and *Moraxella catarrhalis*.
- A better vaccine against pulmonary tuberculosis is urgently needed.
- Nasal vaccination enhances the antibody formation at the airway mucosa, inducing both secretory IgA and IgG antibodies.
- Nasal vaccination also induces systemic immunity at a comparable level to systemic immunization.
- The superiority of nasal over systemic vaccination to engender antibodies and protection at the airways is shown in experimental studies for *S. pneumoniae, H. influenzae, N. meningitidis, Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*.
- Adjuvants can significantly enhance the immune response, reduce the required amount of antigen and direct the Th1–Th2 balance into a desired direction. However, adjuvants also have to prove their safety in clinical trials.
- Nasal vaccination allows a needle-free application, suitable for mass vaccination.
- Nasal vaccination is a key strategy for optimization of future vaccines.

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