Mucosal immunization against respiratory bacterial pathogens

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Bacterial respiratory diseases remain a major cause of morbidity and mortality throughout the world. The young and the elderly are particularly susceptible to the pathogens that cause these diseases. Therapeutic approaches remain dependent upon antibiotics contributing to the persistent increases in antibiotic resistance. The main causes of respiratory disease discussed in this review are Mycobacterium tuberculosis, Corynebacterium diphteriae, Bordetella pertussis, Streptococcus pneumoniae, nontypeable Haemophilus influenzae, Moraxella catarrhalis and Pseudomonas aeruginosa. All these organisms initiate disease at the mucosal surface of the respiratory tract and thus the efficacy of the host’s response to infection needs to be optimal at this site. Vaccines available for diseases caused by many of these pathogens have limitations in accessibility or efficacy, highlighting the need for improvements in approaches and products. The most significant challenges in both therapy and prevention of disease induced by bacteria in the respiratory tract remain the development of noninjectable vaccines and delivery systems/immunization regimens that improve mucosal immunity.


Respiratory disease due to bacteria remains a major cause of morbidity and mortality in both developed and developing communities. The major groups affected by bacterial respiratory illness are: the young, with a high incidence of otitis media (OM) and lower incidence of pneumonia and whooping cough; all age groups, with tuberculosis or with conditions that compromise the immune system; and the elderly, with a susceptibility for chronic bronchitis, chronic obstructive pulmonary disease (COPD) and pneumonia. Bacterial pathogens that colonize or invade through mucosal surfaces are a major cause of disease in all these groups. Prevention of disease should therefore be targeted at prevention of invasion or disturbance of these mucosal membranes.

This review will briefly summarize the epidemiology, including burden of disease, of the bacterial respiratory pathogens causing greatest human and economic impact; comment on optimal available therapy and its ‘limitations’; provide a synopsis of the ‘state of the art’ of mucosal vaccines against bacterial respiratory pathogens; and present key issues for future research and clinical strategies.

Respiratory disease & bacterial pathogens
Epidemiology & burden of disease

Respiratory infections account for 50 million deaths worldwide annually [1], with 3 million of these deaths being caused by a single microbial agent, Mycobacterium tuberculosis [2]. Children of less than 5 years of age in developing countries are at greatest risk of mortality from respiratory tract infections, with 2 million deaths in this cohort in 2002 [3]. Over the last decade mortality from respiratory tract infections has tended to decrease due to broader access to antibiotics. However, with increasing antibiotic resistance, for example, as seen with Streptococcus pneumoniae [4,5] and both resistance and disease resurgence, as seen with M. tuberculosis [6], this trend is already reversing. Vaccine-preventable illnesses that have high priority with the World Health Organization include tuberculosis, diphtheria and whooping cough.
There is a significant human and economic burden resulting from bacterial respiratory disease morbidity. While the use of antibiotics has reduced the mortality associated with bacterial diseases, such as pneumonia, it has had little overall effect on the morbidity [7]. Other lung-based bacterial-associated conditions, such as chronic bronchitis and COPD, have been estimated to cost up to US$3000 per clinical case per year, almost twice that reported for asthma [8]. The sleeping giant of lung morbidity is tuberculosis with an estimated 2 billion individuals infected worldwide. The majority of these individuals do not exhibit overt signs of disease but are at increased risk of reactivating tuberculosis should their immune system become compromised, as it does with age or through contracting other infectious agents, such as HIV [9].

Respiratory tract infections also include infections of the middle ear cavity. OM is the most common reason for a child under 3 years to visit a general practitioner in the USA and most developed countries. In the USA, it is estimated that more than 20 million episodes of acute OM occur annually [9], resulting in a cost of $4–5 billion per year [10]. Developing communities, as well as certain ethnic groups, such as the Aboriginals in Australia, have higher rates of OM at younger ages and longer term morbidity [11]. In developed countries, extensive usage of antibiotics in the treatment for OM remains a controversial issue amongst specialists in the field. Complications are increasing due to drug resistance and sequelae, such as mastoiditis, meningitis and chronic supplicative OM [12].

**Bacterial respiratory pathogens**

Seven organisms are responsible for the majority of bacterial respiratory infections, *S. pneumoniae*, nontypeable *Haemophilus influenzae* (NTHi), *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *M. tuberculosis*, *Corynebacterium diphtheriae* and Bordetella pertussis. *S. pneumoniae*, NTHi and *M. catarrhalis* are often found as part of the normal microbiota of the upper airways and as such, can be considered ‘commensal pathogens’.

*S. pneumoniae*, a commensal of the upper respiratory tract, is carried in 95% of healthy children less than 3 years of age and 40% of adults [13]. Infection often follows acquisition of a new serotype and results in a variety of mucosal infections, such as OM and pneumonia and/or leads to invasive cases of septicaemia and meningitis. The morbidity associated with this micro-organism is high and it remains a leading cause of mortality in young children [14]. NTHi and, to a lesser extent, *M. catarrhalis*, follow similar patterns of colonization and disease to that seen by *S. pneumoniae* [15,16]. The organisms that commonly colonize the nasopharynx and their links to the disease state are well reviewed in a recent publication by Garcia-Rodriguez and Martinez [17]. The impact that an effective vaccine can have is demonstrated by the marked decrease in pneumonia and other debilitating infections associated with *H. influenzae* type b since the vaccine was introduced in 1988 [18]. Thus, it is a priority to develop effective and preferably multivalent vaccines for these pathogens.

*M. tuberculosis* is a complex pathogen, capable of establishing persistent infection in the host despite the development of an appropriate cell-mediated immune response [19]. Recent evidence would suggest that secretory immunoglobulin (Ig)A (sIgA) is also part of the effector mechanisms in disease prevention, however, more studies need to be performed to establish its role as a protective agent against primary disease or disease reactivation [20]. The bacterium has been estimated to infect one in three people worldwide and although many people survive the acute phase, 2–23% will develop disease symptoms in later life with approximately 90 million expected to die of tuberculosis within the next 30 years [1]. Targeted research to develop better preventative therapies to this microorganism is still required.

*C. diphtheriae*, like *H. influenzae* type b, was a cause of serious disease prior to an effective vaccine. Death as a result of infection has been reported to be as high as 10% [21]. Vaccine efficacy has been high and Europe was considered almost ‘diphtheria free’ until a lack of vaccine compliance resulted in an outbreak in the newly formed Independent States of the former Soviet Union between 1990 and 1999, resulting in more than 150,000 cases and 5000 deaths [4,22]. The vaccine, active against a toxin produced by the bacteria, does not affect the bacteria itself and the molecular characterization of isolates from the European outbreak identified the strain as being part of the endemic reservoir that existed in Russia prior to the epidemic [22]. The general community has become complacent over time and there is a clear need to continually remind the public, through active media programs, of the need to maintain vaccination against bacteria such as *C. diphtheriae*.

*B. pertussis*, the cause of whooping cough, remains circulating in the population despite the introduction of community-wide vaccination programs. Officially reported statistics of incidence and complications in whooping cough are grossly underestimated. Of the 330/100,000 cases identified in a study in Birmingham, England, only 4/100,000 were officially recorded in the statutory notifications for England and Wales [23]. In areas of good vaccine compliance, such as in England and France, there has been an age shift in organism colonization and a subsequent disease shift from the young to older age groups [24]. Hence, this highlights the issue of duration of immunological memory for some vaccines. Whooping cough can be a severe illness which can lead to brain damage and death and, therefore, it has been suggested that immunization coverage be extended from infants to include adolescent and adult populations [24]. The reduction in side effects following the introduction of the acellular pertussis vaccine will increase acceptability in all population age groups.

**Disease prevention**

*S. pneumoniae*. NTHi, *M. catarrhalis* and *C. diphtheriae* are constantly circulating between individuals in the community and colonize the mucosal epithelium of the upper respiratory tract. In the majority of individuals the acquisition of a new bacterial strain is harmless – a neutral phenomenon to the
Table 1. Vaccine delivery technology and application for mucosal delivery of respiratory bacterial antigens.

<table>
<thead>
<tr>
<th>Adjuvants</th>
<th>Advantage</th>
<th>Antigens against infection</th>
<th>Mucosal application</th>
</tr>
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<tbody>
<tr>
<td><strong>Endotoxins</strong></td>
<td>CT more potent mucosal adjuvant either conjugated or mixed. CTB not as effective orally.</td>
<td>Extensive number of studies for many microbial and dietary antigens.</td>
<td>Yes as well as transcutaneously for mucosal protection.</td>
</tr>
<tr>
<td><strong>Monophosphoryl lipid A (MPL)</strong></td>
<td>Potent adjuvant + low toxicity (1/4)</td>
<td>T1, HbsAg, FluShield® (65), (75) Pneumovax-CRM197 (44)</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Oligodeoxynucleotides containing CpG</strong></td>
<td>Immunosstimulatory sequences, synthetic</td>
<td><strong>Hemophilus influenzae</strong> type b-CRM197 (47)</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Lipid core peptide (LCP)</strong></td>
<td>Synthetic</td>
<td>Chlamydia trachomatis (76), Group A Streptococci (49)</td>
<td>Not yet reported</td>
</tr>
</tbody>
</table>

**Bacterial systems**

<table>
<thead>
<tr>
<th>Salmonella strains</th>
<th>Attenuated live carrier expressing foreign antigens</th>
<th>Targets antigen to mucosal lymphoid tissues and can deliver improved antigen load (74)</th>
<th>Many antigens in addition to model antigens and includes M. tuberculosis and pneumococcal antigens as well as DNA with eukaryotic expression systems (52,77–80).</th>
<th>Yes</th>
</tr>
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<tbody>
<tr>
<td>Mycobacterium strains</td>
<td>Attenuated strain and novel recombinant technology incorporated</td>
<td>Licensed vaccine, although current BCG vaccine is questionable for effective protection against TB.</td>
<td>Against tuberculosis plus carrier of foreign antigens and immunomodulators (60,61)</td>
<td>Yes and was used as an oral vaccine until the 1940s</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>Expression of antigens in Lactococcus and Lactobacillus (81)</td>
<td>Lactic acid are well tolerated as food microbes and have been used as probiotics</td>
<td>Ti, pneumococcal polysaccharide, H. influenzae antigen (82–85)</td>
<td></td>
</tr>
<tr>
<td>Bacterial ghosts</td>
<td>Recombinantly engineered bacteria</td>
<td>Potential for the bacterial envelope to be both carrier and vaccine</td>
<td>Mostly as homologous vaccine, but some recent advances as a carrier as well (55–55,86,87)</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis spores</td>
<td>Recombinant spores with chimeric antigen expression</td>
<td>Recombinant system, organism used as probiotic</td>
<td>Ti delivered orally and nasally (88)</td>
<td></td>
</tr>
</tbody>
</table>

**Microparticles**

<table>
<thead>
<tr>
<th>ISCOMs</th>
<th>Saponin mixture Quil A, cholesteryl and phospholipids</th>
<th>Potent adjuvant and available in veterinary vaccines</th>
<th>Influenza virus, hepatitis A, diphtheria and tetanus toxoids, but mostly model antigens for mucosal applications (65)</th>
<th>Yes but not extensively and new formulations being investigated (64,89,90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomes</td>
<td>These are particles with a bilayer structure with different phospholipid-based compositions (63)</td>
<td>Potency depends on formulation, antigen and route of immunization as does type of response, Th1 and/or Th2</td>
<td>Range of antigens including DNA and influenza (66,67,91–93)</td>
<td>Yes, especially for oral delivery</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>Cationic cross-linked polysaccharides enclosed in lipid bilayer</td>
<td>Designed for nasal delivery</td>
<td>DNA and bacterial antigens (69–71)</td>
<td>Yes, nasal</td>
</tr>
</tbody>
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**Plants**

| Edible plants | Recombinant technology in potato, tomato, cereals, alfalfa, legumes, banana | Simplicity of delivery in a food, but technology still needs further development | Diarrheal antigens, Hepatitis B antigens in human trials (72) | Yes |

RFF: Bacillus Calmette-Guérin; CT: Cholera toxin; CTB: Cholera toxin B subunit; E.coli: Escherichia Coli; HbsAg: Hepatitis B surface antigen; ISCOM: Immunostimulatory complex; LPS: Lipopolysaccharide; TB: Tuberculosis; Th: T helper cell; TT: Tetanus toxoid; V. Cholerae: Vibrio Cholerae.
host. Infections occur when the host–commensal balance is disturbed for some reason or in individuals more susceptible due to age, genotype, current immune status and other similar factors. Hence, these bacteria are part of the normal microflora, so the question that must be asked is whether a vaccine that creates sterile immunity would open a niche for new pathogens to colonize the nasopharynx. Is it even necessary that vaccines against ‘commensal pathogens’ induce sterile immunity? A number of studies have suggested that it is high bacterial load that predisposes to disease, so the ideal vaccine may be one that controls bacterial load sufficiently such that disease is prevented. Clearly a better understanding of immune responses that enable control of the micro-organisms at the mucosal surface as well as an understanding of the microbial ecology of the upper airways is essential to optimize therapeutic initiatives.

P. aeruginosa is ubiquitous within the environment and tends to rely on an underlying disease-state or structural damage to tissue before the host becomes susceptible to infection. As such, it is a significant cause of acute and chronic disease in specific population groups. It is these groups that need to be targeted for more effective prophylactic and therapeutic approaches, rather than the general population.

In the case of C. diphtheriae, it is the immunity induced by vaccination against the toxin that prevents disease and not immunity preventing bacterial colonization and, as stated above, vaccine programs must be maintained. M. tuberculosis and B. pertussis are specific human pathogens that are not normal commensals and thus prevention of initial colonization on the mucosal epithelium is essential.

Ideal outcome for protection
The question as to whether elimination, from the host, of the bacteria that are responsible for the different respiratory diseases is the optimal outcome for therapy or if control of a potential disease state is more desirable still needs to be addressed.

In the case of absolute pathogens, such as M. tuberculosis and B. pertussis, bacterial elimination from the host is essential. This is also probably the most desirable outcome for C. diphtheriae, however, the current toxoid vaccine is safe and effective and, despite research into changes in the delivery mode (Table 1), it is unlikely that the injectable vaccine will be changed at this point in time. In the case of the normal respiratory commensals, such as S. pneumoniae, NTHi and M. catarrhalis, the possibility of bacterial replacement with an organism that will be more harmful to the host has created debate.

New conjugate pneumococcal vaccines have become available and the results of several studies on populations in which the vaccines have been trialed have been examined. Trials in the American Indian population looked at the efficacy of the parenterally administered heptavalent formulation conjugated to a nontoxic diphtheria variant (Pevnu®, Wyeth). The study found that the overall pneumococcal load in the respiratory tract remained unchanged postvaccination despite a significant reduction in the vaccine-type pneumococcal carriage rate, thus indicating the vaccine was associated with replacement carriage in the nasopharynx [25]. Furthermore, clinical trials in Finland evaluating the effect of pneumococcal vaccines on OM demonstrated that the number of episodes of OM caused by nonvaccine serotypes of pneumococcus increased by 33% despite a demonstration of protection against serotypes included in the vaccine [26]. A similar replacement of pathogenic bacteria was not observed in the respiratory tract following mucosal vaccination with a whole-cell NTHi preparation in Papua New Guinea [77], yet most individuals make strain-specific immune responses postinfection with NTHi.

Control of absolute bacterial numbers and age of colonization are important in the predisposition to infection in individuals with high levels of bacterial colonization. Studies in developed and developing communities have established that the risk for development of OM increases when either the number of potential pathogenic bacteria, S. pneumoniae, NTHi, M. catarrhalis, colonizing the nasopharynx is increased or the age of the patient is less than 3 months when primary colonization occurs [28–31].

Antibiotics have had mixed success in the treatment of respiratory infections. Acute OM is the most common reason for prescribing antibiotics in young children [9]. However, lack of diagnostic specificity for bacterial causes; self-resolution of infection, in some cases in short time periods; and increased bacterial resistance, have resulted in mixed recommendations and outcomes in the use of antibiotic treatment for OM [12]. Effective immune responses at the mucosal site that control colonization and are upregulated to effectively prevent infection would therefore be a preferable therapeutic outcome. This could only be achieved by the development of new vaccines for most of these respiratory pathogens.

Factors likely to affect choice of therapy
Mucosal immunization
Since the late 18th Century, of the 25–30 vaccines available for human use, very few have been administered nonparenterally [32], despite one of the most successful disease eradication programs being achieved using an oral vaccine. The control of polio was achieved in many countries in the world through oral vaccination programs. However, once eradicated from a population, the continued use of the vaccine has proven questionable due to the live attenuated virus posing a risk of disease. Since the mucosal portals of the body are the entry for many infectious diseases, vaccines that increase the integrity of the mucosal barriers through specific immune mechanisms should have a significant impact on disease incidence. It is clear from both animal and human studies that when antigen is presented directly to immune-induction sites, such as the gut-, nasal- and bronchial-associated lymphoid tissues (GALT, NALT and BALT, respectively), protective responses are induced within the respiratory tract. Animal experiments have provided a great deal of information on the mechanisms of mucosal immune regulation such that it is now possible to
predict and certainly determine, whether a given antigen formulation will result in tolerance or polarize the mucosal response towards Th1 or Th2 [33]. Determination of pathogen-specific protective responses required in the respiratory tract will enable relatively well-targeted vaccine development programs.

The GALT and NALT are the major inductive sites of the mucosal immune system, although other minor networks have been described [34,35]. For the development of effective human mucosal vaccines against pathogenic respiratory bacteria, an oral or other mucosally delivered formulation would be most desirable for a number of reasons. First, oral administration is most likely to pose the least number of complications, although there are advances that are simplifying airway delivery. Second, no sophisticated administration procedures are required. Third, a number of mucosal vaccine formulations are stable without refrigeration, eliminating the ‘cold chain’ requirement of many vaccines. Fourth, mucosal administration is not invasive and, as such, would most likely have better subject compliance.

While these are very positive advantages for mucosal vaccination, one issue that remains unresolved is that at this point in time many mucosal vaccines do not induce adequate long-term immunity. Three essential criteria have been determined for a successful mucosal immunization strategy [36]:

- The effective delivery of antigen to the mucosal immune induction site
- The enhancement of mucosal immune responses by the use of mucosal immunomodulators (such as bacterial enterotoxins, cytokines, adjuvants and delivery systems)
- Choosing a regime and route of immunization that will induce protective responses and immunological memory at the desired mucosal site and preferably systemically as well

**Antigen delivery**

Delivering antigens by means other than injection remains one of the most significant challenges facing new generation vaccines and a high priority area in vaccine development [4]. Therefore, why is it that delivering vaccines via the mucosal route has posed such a challenge? A number of factors contribute to the problem. These include:

- The desire for nonreplicating agents due to perceived risks with live or attenuated microorganisms
- The need to overcome mucosal tolerance [37]
- The capacity to deliver the required antigen doses to the inductive sites of mucosal tissues [38]
- The choice of inductive site since this can influence the resulting acquired immune response [39,40]
- The choice of adjuvant and/or carrier system
- Whether these will preserve antigen conformation [41].

This is in addition to knowledge of the nature of both the protective antigens and immune responses that are required (not discussed in this review but is a key issue). It is the complexity of these issues that highlights the fact that there appears to be no single answer to effective mucosal vaccination approaches, although there are many innovative and promising developments within the field of mucosal vaccine delivery systems (Table 1).

A number of adjuvants have been investigated for potential use with mucosal delivery. The most commonly used in animal studies of mucosal immunization have been the protein enterotoxins, cholera toxin (CT) and E. coli heat-labile toxin (LT). The use of these enterotoxins as native or modified adjuvant subunits have recently been reviewed [42]. One of the potential limitations with these enterotoxins is that the native adjuvant activity is related to the enterotoxiny and so some of the current challenges are to develop modified CT and LT products that retain adjuvancy but not toxicity [43]. Both these adjuvants have shown promising results as mucosal adjuvants for oral and nasal delivery of a variety of antigens, including both mucosal and serum antibody responses. Therefore, forms of these adjuvants safe for human use would be quite advantageous.

Monophosphoryl lipid A (MPL) is an adjuvant that has been used in humans and has potential for mucosal delivery formulation. MPL in the pneumococcal–CRM197 vaccine was safe when administered parenterally to healthy toddlers. The results showed a dose-dependent effect on both Th1 and Th2 responses to the carrier protein but did not enhance serum IgG to any of the polysaccharide antigens [44]. Nasally formulated hepatitis B surface antigen (HbsAg), tetanus toxoid (TT) and FluShield® influenza vaccine in MPL in animal studies resulted in enhanced mucosal and systemic immunity [45]. The most significant difference between the mucosal and parenteral delivery in these studies was the production of specific IgA in mucosal secretions following intranasal vaccination. MPL-based adjuvants have also been successfully used to boost immune responses in mouse studies, resulting in substantial protection from aerosol challenge with M. tuberculosis [46]. This result is particularly exciting due to the poor efficacy of the BCG in countries with endemic M. tuberculosis and could be used to orally boost responses using a subunit vaccine.

Another agent that has recently shown promise as a mucosal adjuvant is the synthetic oligodeoxynucleotides containing Cpg stimulatory sequences. This adjuvant has been used with the H. influenzae type b polysaccharide (PRP) vaccine and when delivered mucosally, resulted in both systemic and mucosal antibody, particularly mucosal IgA [47] (as observed for the vaccines in the Baldridge study [48]). To date, application using Cpg DNA sequences are predominantly in animal studies. Very limited data are available on any human clinical trials, however, it is reported that the preliminary data from some early stage human trials are encouraging. Hence, this may be a very promising technology with both mucosal and parenteral adjuvant application [48]. Other adjuvants are regularly reported with model antigens but recently, a lipid core peptide (LCP) adjuvant has been used with group A streptococci antigens [49] that currently appears to have potential as a mucosal adjuvant suitable for human application.
Many studies have investigated bacterial systems for delivery of antigens. These have ranged from whole-killed-cell vaccines [50] to more complex engineered micro-organisms [51]. Some of the advantages of bacterial delivery systems are that they contain their own adjuvants, they naturally form and maintain their microsphere structure and once the recombinant technologies have been incorporated, they can be efficient to produce. Some of the limitations are potential toxic effects, poor or ineffective immune responses to the vaccine antigens, possible pathogenic issues with live micro-organisms and issues with the use of antibiotic resistance genes in their preparation. As such, while some of our existing vaccines are based on whole-cell systems and many of the bacterial systems indicated in Table 1 show definite promise, a broader acceptance for human use needs to be demonstrated. *Salmonella* species have been extensively used as a system for investigating live bacterial carrier systems and paved the way for investigations into a range of such carriers [52]. The range of human clinical trials using these systems has not been extensive and in those that have been done, the results would suggest that there is still a need for more development of these systems (reviewed in [53]).

Studies with nonliving bacterial systems in mice using *Vibrio cholerae* ghosts expressing a major outer membrane protein from *Chlamydia trachomatis* [53] and *E. coli* ghosts expressing an *H. influenza* antigen in recombinant S-layers [54], showed induction of immune responses to the respective antigens but demonstrated a need for further development of the system. The bacterial ghost technology is limited to Gram-negative bacteria and has been effective against the pig respiratory pathogen, *Actinobacillus pleuropneumoniae* [55]. Such nonliving bacterial systems are most likely quite safe as oral vaccines but whether they will meet the requirements of drug regulators remains an issue.

*Mycobacterium* sp. has been evaluated for mucosal delivery as both a vaccine and carrier of other antigens or immunomodulators. Oral and nasal delivery of formulated mycobacteria and BCG vaccines have been shown to be protective in mouse studies [56–59], demonstrating the feasibility of mucosal delivery as a safe and practical method for controlling tuberculosis. In addition, recombinant *M. bovis* BCG-based vaccines have been investigated for both immunopotentiating of the BCG vaccine and as delivery of heterologous antigens (reviewed in [60,61]). BCG is not the only currently available whole cell-based vaccine for bacterial respiratory infections to be investigated for mucosal delivery. The acellular pertussis vaccine delivered intranasally has been investigated in a mouse model for induction of both mucosal and systemic antibody responses in mice [62]. In this study, intranasal immunization induced titers of IgG in the serum and IgA in the lungs that were protective against bacterial challenge and which were reported to be more effective against infection than when mice were immunized subcutaneously.

Microparticles, such as ISCOMs and liposomes, have been investigated quite extensively [63]. ISCOMs are a licensed carrier for some veterinary vaccines. As a mucosal carrier, it has been used in both oral and intranasal immunization studies and appears to produce a mixed Th1- and Th2-type immune response [64]. It has also been used to investigate delivery of a *M. tuberculosis* antigen [65]. Microparticle technologies have been more extensively studied for viral respiratory pathogens but there have been few studies reported investigating the potential for delivery of bacterial antigens. Oral delivery of a liposome formulation containing outer membrane proteins from *NTHi* enhanced nasal clearance in a mouse model [66], whereas pneumococcal polysaccharide delivered in a liposome formulation induced a relatively poor antipolysaccharide response [67]. The development of new formulations of liposomes and other microparticles continues as a means of targeting antigen to the mucosa-associated lymphoid tissues [68] and they certainly have potential for safe use in humans.

Research efforts are also focusing on even smaller particles for antigen delivery. Nanoparticles have been developed specifically for nasal immunization. These are cationic cross-linked polysaccharides enclosed in lipidic bilayer and comprise of different formulations. Among the antigens tested, nanoparticles have been used to deliver DNA [69], group C meningoccal antigens [70] and antineoplastic proteoglycans from *M. vaccae* [71]. The results of these studies demonstrate potential for this technology to be applied for delivery of other bacterial antigens.

In concluding, a section on mucosal delivery would not be complete without mentioning edible vaccines. These have recently been reviewed by Mason and colleagues [72]. Potato, tomato, banana, legumes, cereals and alfalfa are edible species that have been transformed with varying degrees of success. Various issues associated with both the production of and immunization with plants as vaccine delivery vehicles remain to be resolved. One possibility is that they may be effective in boosting immune responses that were primed by a parenteral vaccine, as has been found for a clinical trial of the HBsAg expressed in potato [Y THANVALA, ROSWELL PARK CANCER INSTITUTE, NY, USA, PERC COMM].

Despite the excellent research efforts, antigen delivery systems remain one of the key issues in the development of preventative therapies for respiratory bacterial infections. A recent issue of the journal *Vaccine* (vol. 21, issue 9–10, 2003) was specifically dedicated to immunomodulating agents and provides a more extensive review of this field. In this section we have highlighted a range of developments in the mucosal delivery of vaccines and cited examples where there have been studies associated with the respiratory bacteria discussed in this review. What is apparent is that there remains a need for more extensive evaluation of a number of the more promising technologies to determine their suitability for inducing protective immune responses in the respiratory tract against a broader range of bacterial pathogens. There is some excellent basic research with many of the technologies discussed but for some there are obstacles
Mucosal immunization

Five-year view

In 5 years it is feasible that some of the current developments in mucosal delivery systems will be in clinical trials. The availability of genomes for most of these bacteria means that a new range of antigens will have been evaluated as vaccine candidates. A new generation of pneumococcal vaccines will have been developed to overcome the efficacy and serotype coverage problems associated with the current conjugate vaccine. It is desirable that there will be acceptable delivery systems available for routine use in humans and that we will have knowledge from human vaccine studies that enables the identification of the type of immune mechanisms required for pathogen-specific protection.

Key issues

- Establishment of these respiratory pathogens as a priority in the development of effective multivalent vaccines.
- Knowledge of the immune responses that control the bacterial colonizers of the respiratory mucosal surfaces is essential to optimizing therapeutic initiatives.
- Identification of the measures of pathogen-specific protective responses required at the mucosal surfaces must be resolved to ensure vaccine efficacy.
- Determination of the requirement for a vaccine against a 'commensal pathogen' to induce sterile immunity against a microbe.
- Investigation on whether a vaccine that creates sterile immunity to a commensal would open a niche for new pathogens to colonize the nasopharynx.
- Adoption of appropriate and cost-effective pathways by regulatory authorities to enable rapid changes to vaccine formulation in cases where safety has been demonstrated and there is an established technology track record.
- Dissemination of information that ensures public awareness of the importance of maintaining vaccine compliance for pertussis and diphtheria vaccines.
- The design of therapeutic and prophylactic approaches that prevent resurgence of disease causing pathogens as has occurred with tuberculosis.
- Although not discussed in this review, identification of key antigens for inclusion in vaccines remains an issue for a number of these bacteria.
- Continued development and more timely advancement to clinical evaluation of effective multivalent vaccines as a more effective means of ensuring vaccine coverage against a range of pathogens.
- The development of mucosal delivery systems that are inexpensive, effectively target antigen to the immune induction sites and are safe for human use.
References

Papers of special note have been highlighted as:

• of interest
•• of considerable interest


•• Extensive analysis and review of antimicrobial resistance and what this means for the patient and health care system.


•• Excellent review on factors contributing to increased risk of carriage of potential respiratory pathogens and clinical infection.


• Demonstration of vaccine efficacy and strain replacement.


•• Good review on mucosal immune vaccination strategies


Demonstration of mucosal vaccination as a boosting mechanism.


Novel adjuvant.


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