Development of vaccines against common colds

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Respiratory tract viruses are particularly significant causes of illness and death in children and in the elderly. Vaccines offer the possibility of decreasing the severity and complications of viral respiratory disease, but development has been delayed by numerous factors. First, there are more than 200 serologically distinct RNA and DNA virus species and strains which cause an essentially similar spectrum of disease. Some re-infect at high efficiency despite little antigenic variation, while others exhibit extensive coat protein variability.

Vaccine candidates show variable efficacy in partially immune adults, the immunocompromised and the elderly, and may be ineffective or pathogenic in neonates or in the presence of maternal antibodies. However, effective childhood vaccines are essential to prevent severe disease due to respiratory syncytial virus (RSV) and parainfluenza and to reduce virus transmission to adults. A number of promising vaccines are in clinical trial, and it is likely that vaccines against RSV and parainfluenza will be licensed within the next 5–10 years. Mucosal delivery and the use of novel adjuvants offers the prospect of better vaccines against influenza. The ultimate goal is to develop multivalent mucosal vaccines offering protection against a spectrum of respiratory infections.

Despite advances in infectious diseases and the introduction of new vaccines, viruses transmitted via the respiratory tract remain the major cause of community morbidity (Fig. 1) and hospitalisation in the industrialised world and mortality in non-industrialised countries. Of the 200 or more serologically distinct viruses that cause sporadic or epidemic respiratory infections, rhinoviruses, coronaviruses, respiratory syncytial virus (RSV), parainfluenza viruses (PIV), influenza viruses A and B, cytomegalovirus, and adenoviruses are the most frequent (Table 1). The relative importance of different agents depends on age, and respiratory viruses are a particularly significant causes of illness and death in children. RSV bronchiitis is the single most frequent single cause of infantile hospitalisation in industrialised countries, causing up to 70% of infant hospitalisations during each winter season. Over 80% of children hospitalised with RSV bronchiolitis are less than 6 months old.
In addition, viral bronchiolitis may have a long-lasting influence on the subsequent development of recurrent wheeze in childhood and asthma diagnosis. It is clear that respiratory viruses are also important pathogens in other age groups and that treatment and prevention should also be focused on those with compromised cardiac, immune, and respiratory systems of any age, and the elderly. In this respect, RSV and influenza are the most important causes of morbidity, hospitalisation and mortality among elderly persons.

Fig. 1 Proportion of GP consultations due to various conditions. Data from 78 UK general practices, 1999 (http://www.rcgp-bru.demon.co.uk).

Table 1 Agents causing common colds in older children and adults

<table>
<thead>
<tr>
<th></th>
<th>Number of serotypes</th>
<th>% of colds</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>100+</td>
<td>60</td>
<td>All year (peaks)</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>2</td>
<td>15</td>
<td>All year (peaks)</td>
</tr>
<tr>
<td>Influenza viruses</td>
<td>3</td>
<td>&lt; 10</td>
<td>Epidemics</td>
</tr>
<tr>
<td>Parainfluenza viruses</td>
<td>4</td>
<td>&lt; 10</td>
<td>All year (peaks)</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>2</td>
<td>&lt; 10</td>
<td>Epidemics</td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>47</td>
<td>&lt; 10</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Enteroviruses</td>
<td>40+</td>
<td>&lt; 10</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td><strong>Atypical bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>1</td>
<td>&lt; 10</td>
<td>5-yearly cycle</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>1</td>
<td>&lt; 10</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Figures are approximate and highly dependent on the age of the subjects, the period, and location of study. No large study has been performed using modern molecular diagnostic methods.
Special problems in the development of respiratory vaccines

In order to develop new respiratory virus vaccines, it is necessary to understand clinical and immunological facts about infectious agents and to gain information about protective and non-protective immune responses. Although it is clear that specific neutralising serum antibody correlates with protection in many cases, it is not clear whether this antibody is itself responsible for protection. With localised mucosal infection being sufficient to cause disease, viruses confined to the respiratory tract do not need to pass into areas permeated by serum antibody. Local mucosal antibody or T-cell responses may be necessary and sufficient for protection, and serum antibody may merely be a marker of immunological priming. Second, it is important to appreciate that many respiratory viruses are themselves relatively non-lytic. The disease is, in large part, a result of immune and inflammatory responses to the infection. Therefore, vaccine-induced immunity may actually enhance symptoms.

The general approach to vaccine development for respiratory pathogens is similar to that for other pathogens, but live, locally delivered vaccines may have significant advantages. They may be effective even in the presence of maternal antibody, which is particularly important for RSV, parainfluenza and measles vaccines. However, some live vaccines have been insufficiently attenuated or unstable, reverting to pathogenicity in young children. The use of formalin-inactivated measles and RSV vaccines have led to disease potentiation following natural infection, almost certainly due to inappropriate T-cell priming. The availability of animal models greatly accelerates vaccine development. It is now possible to study human respiratory infections in mice (e.g. RSV and parainfluenza). On the other hand, the lack of convenient models for studying other infections (e.g. rhinovirus) has been a major limitation for vaccine progress.

Respiratory vaccines may need to be tailored to different groups. For instance, an optimally attenuated live virus vaccine designed for young children may be over-attenuated for adults. Similarly, the requirements for a vaccine that prevents re-infections in the elderly or for use in the immunocompromised may differ. Finally, vaccines that require an intact cold chain may be inappropriate for non-industrialised countries.

Effective immunisation of neonates has posed particular problems. Maternal antibodies afford partial protection and may inhibit some immune responses. Several studies show that very young children develop poor antibodies in response to primary viral infection and the convalescent titre is lower than in older children. The fact that the immune system of neonates is not fully developed may impede responses to some antigens, particularly T-cell dependent responses.
The existence of diverse viral serotypes makes vaccine preparation difficult. In particular, frequent mutations of viral proteins of RNA viruses (e.g. influenza) require preparation of new vaccine each year. Additionally, non-human viruses may acquire ability to infect humans, as occurred in Hong Kong in 1997. There are several general problems which have impeded rhinoviral vaccine development: (i) the large number of relatively stable viral serotypes would necessitate a highly polyvalent vaccine; (ii) the effects of rhinoviral vaccination would be to reduce the frequency of common colds, but would not be expected to impact greatly on respiratory morbidity and mortality in otherwise healthy people; (iii) there is no good animal model to facilitate development; and (iv) there is an expectation that anti-rhinoviral drugs may have a place in controlling rhinoviral disease in the near future.

Vaccines for specific viruses

Respiratory syncytial virus

Although RSV causes common colds in adults and is increasingly recognised as an important pathogen in the elderly, RSV bronchiolitis is most frequent at 2–6 months of life. An ideal vaccine would, therefore, be effective soon after birth, would confer life-long immunity, and avoid induction of potentially pathogenic immune responses. However, natural immunity against RSV is short-lived and re-infections with serologically similar strains are frequent throughout life. Anti-RSV vaccine would, therefore, have to induce ‘better-than-life’ immunity but, realistically, good protection for the duration of the winter season might be all that could be achieved. Even if no effective vaccine can be developed for use in infants, a vaccine for susceptible adults would be well worth having. The best prospect for improving on natural immunity is to discover mechanisms that serve to limit or inhibit immune responses during natural infection and to design a vaccine that does not induce such effects.

Despite 40 years of research and development, no RSV vaccine has yet been licensed for human use. The field has undoubtedly been slowed by the experience with formalin-inactivated and alum-precipitated whole RSV vaccines tried in children. These induced good ELISA binding but poorly neutralising antibodies, non-protective but disease enhancing immune responses. During subsequent RSV outbreaks, the frequency of RSV infection was equal to, or greater than, that in control groups immunised with parainfluenza vaccine. Moreover, about 80% of RSV vaccinees needed hospitalisation compared to about 5% of controls. At least two deaths occurred amongst RSV vaccinees as a result of disease augmentation.
It is clear that insights into the mechanisms causing disease enhancement are necessary to the development of safe RSV vaccines. It will never be possible to repeat such trials with human volunteers, so good animal models are essential for these adverse vaccine effects to be understood. From experiments in mice, it is clear that balanced T-cell responses (both Th1 and Th2 priming, both CD4 and CD8) tend to be less pathogenic than polarised responses\(^7\).

In man, natural re-infection with RSV generally causes mild disease and high level of local mucosal IgA appears the best predictive correlate of protection. Live attenuated viruses are, therefore, strong vaccine candidates. However, most attenuated strains seem to either loose immunogenicity or revert to pathogenicity in young children. There is a long history of live attenuated vaccine development; as early as 1966, a cold-passaged RSV strain (\(cp52\)) was selected for testing. This vaccine was safe and immunogenic in adults and older children, but not sufficiently attenuated in seronegative infants\(^9\). New RSV strains generated by 5-fluorouracil mutagenesis were also tested in children and infants, but again were either insufficiently attenuated or genetically unstable. The most promising RSV vaccine candidate so far was \(ctps\)RSV 248/404 derived by cold-passage and acquisition of two mutations that lead to temperature sensitivity. This vaccine was relatively safe in infants and protected against challenge with second dose of vaccine\(^10\). A purified F subunit vaccine (PEP-2) is being investigated as booster immunization following \(ctps\)RSV 248/404 and is aimed for elderly and high-risk children\(^11\).

Subunit and peptide vaccines are being developed for use in non-naïve individuals at high risk of developing pulmonary complications upon RSV infection. A recently developed subunit candidate, BBG2Na, is a fusion protein between the 130–230 residues of the G protein and the albumin-binding domain (BB) of streptococcal G protein. This has shown considerable promise in mice\(^12\). The results of a phase 1 clinical trial have now been published\(^12\).

The availability of reverse genetics may accelerate RSV vaccine development. Using this approach, it is possible to engineer in multiple known attenuating mutations and thus develop stable, fully attenuated live RSV vaccines. In addition, ability to insert cytokine genes into live RNA viruses provides an interesting and informative experimental approach to understanding the potential mechanisms for inducing protective immunity. Recombinant technology has also been used to make viruses expressing glycoproteins from the different RSV groups. For example, live attenuated subgroup B RSV has been made expressing the F and G proteins from subgroup A\(^13\), and bovine RSV has been used to express human-RSV-F and RSV-G proteins. These have been tested in animal models and further trials are underway\(^14\).

Live recombinant vectors containing RSV antigens can induce good virus neutralizing antibody and cytotoxic T-cell responses. Vaccines of this
type include canarypox (ALVAC®), attenuated vaccinia (NYVAC®) and modified vaccinia virus Ankara (MVA). MVA is stable and safe, and can induce good antibody and T-cell responses (including CD8+ CTL) in animals, comparable to that induced by replication competent strains15. Recombinant viral vaccines containing RSV antigens were promising in rodents, but poorly immunogenic in primates, and did not reach clinical trials.

Recent studies have shown that neutralising antibody and CTL-mediated immune responses can be induced in rodent models by DNA vaccination. Vectors that express RSV-F or RSV-G antigens are novel vaccine candidates, but one study revealed that both were capable of sensitising for pulmonary eosinophilia16. Even so, DNA immunization is being explored and may become a viable vaccination strategy of the future.

**Influenza vaccines**

Influenza viruses are enveloped RNA viruses containing a segmented negative sense RNA genome. There are three types of influenza which cause disease in humans (A, B and C) grouped according to their internal proteins. Types B and C are largely restricted to humans, type C generally causing minor illness. The natural reservoir for type A viruses is aquatic birds, but they also infect pigs and horses. Only a limited number of type A influenza viruses infect man.

The major surface glycoproteins are the haemagglutinin (HA) and neuraminidase (NA). In common with many RNA viruses (which replicate via an RNA-dependent RNA polymerase) the frequency of spontaneous mutation is high. Host antibody exerts a selective pressure favouring new viral mutants, which are able to escape immune responses. The segmented nature of the influenza A genome allows the possibility of re-assortment of segments following a dual infection of a single animal host, and such an event may lead to the emergence of a virus which is novel for humans because it has surface antigens to which humans have little or no immunity. The gradual evolutionary process is termed antigenic drift, while a major change resulting in a new strain to which there is little hard immunity in the population is termed antigenic shift.

Although serotype-specific vaccines against influenza A were developed in the 1930s and are widely available, influenza A still causes 13,000–20,000 excess deaths a year in the UK17. About 11 million doses of influenza vaccine are administered to high risk and elderly patients in the UK each year, only about 5% of vaccinees being immunologically naïve children at risk. The death rate due to influenza infection in high-risk patients may be as high as 870 per 100,00018. Influenza vaccines currently used worldwide are virtually all split products or purified subunit vaccines. The strains that are included in immunization are
selected annually and recommended by the World Health Organization in co-operation with national public health institutions. While live attenuated vaccines were used in the former Soviet Union states, they are not widely used in Europe and the US. Formaldehyde-inactivated whole virus influenza vaccine has been licensed for use in humans and induces good immunity in naive individuals, but causes an unacceptably high rate of local side-effects. New, high-efficacy polyvalent influenza A vaccines are, therefore, required.

Various influenza vaccine approaches have been tried in humans over the past 20 years. Live attenuated influenza vaccines offer improved and long-lasting protection against influenza, may reduce viral spread, and can be administered as intranasal sprays or drops. In addition, they elicit good T-cell responses and local secretory IgA. However, temperature-sensitive influenza mutants are often genetically unstable, precluding further development. Cold-adapted viral vaccines are immunogenic and well tolerated in young adults, children, and the elderly. Field trials demonstrated over 90% efficacy of trivalent cold-adapted influenza vaccine in children aged 18–71 months. From various studies in the elderly, it seems that cold-adapted vaccines are less efficacious, but combined intranasal cold-adapted and intramuscular inactivated vaccine provided significantly higher protection than inactivated vaccine alone. There is a growing interest in improving influenza vaccine efficacy by the use of novel adjuvants. Innovative adjuvants tested in animals or in phase I clinical studies include Quil A or QS-21, squalene derivative MF59, liposomes, immunostimulating complexes (ISCOMS), and cytokines. Experimental local mucosal adjuvants include the modified heat-labile toxin from Escherichia coli.

Recently, advances in reverse genetics have allowed genomic manipulation so that mutations necessary for virus attenuation are preserved and a mutant is genetically stable. Future development is anticipated focused on the identification of genes responsible for virus growth and the study of stable live vaccine strains for nasal administration. Recombinant vaccines expressing influenza sequences are under investigation. Among a variety of vectors tested experimentally are pox viruses, Salmonella or even isolated flagellae. The interest in using Gram-negative rods as expression vectors for foreign peptides lies in their ability to colonise mucosal surfaces and induce mucosal immunity, serum antibodies, and specific CTL responses. Experiments indicated that oral or intranasal delivery was also able to evoke secretory IgA in the lungs and protection was sustained over several months. The issue of mucosal delivery of influenza vaccine is again discussed later in this chapter. Although the US Advisory Committee on Immunization Practices (ACIP) did not recommend vaccination of infants and children under 2 years in
Parainfluenza vaccines

Human parainfluenza virus types 1, 2 and 3 (PIV1, PIV2 and PIV3) cause serious lower respiratory tract infections in infants and young children world-wide; of these viruses, the most often isolated is PIV3. Among children hospitalized for respiratory tract infections, about 18% are due to PIV. Although generally considered a disease of childhood, the parainfluenza viruses have been recognized causes of respiratory illness in the immunocompromised and in the elderly, who usually have only upper airway symptoms of disease.

To date, there is no licensed parainfluenza vaccine. The first PIV vaccine candidates were generated in the 1960s by inactivation with formalin. These vaccines were targeted to young children and infants; they were immunogenic, but could not prevent PIV disease and, therefore, were soon abandoned. Research on new vaccines was held up for some 20 years until a new isolate of PIV3 (cp45) was obtained by cold-passage. This vaccine candidate was safe, immunogenic, and genetically stable in seronegative infants. However, only 11% of seropositive children developed a 4-fold increase in haemagglutinin titres and only 22% acquired nasal IgA antibodies.

A live bovine PIV vaccine with significant antigenic relatedness to human PIV3 was developed to prevent human parainfluenza. Initially tested in 6–36-month-old children, the vaccine was safe, infectious, immunogenic, and genetically stable. Follow-up studies were recently completed in children younger than 6 months and demonstrated that it also was immunogenic in the majority of these young infants.

Recombinant bovine PIV3 (in which fusion and haemagglutinin genes were replaced with their human PIV3 counterparts) and human PIV3 attenuated due to the presence of the bovine PIV3 nucleocapsid protein are being evaluated in animal models as vectors for the delivery of other viral antigens such as RSV-G and RSV-F proteins, or measles haemagglutinin. This valuable strategy may in the future lead to the development of multivalent vaccine against respiratory diseases very likely targeted to paediatric patients.

Adenovirus vaccines

The human adenoviruses consist of almost 50 serotypes divided into six serogroups (A–F) characterised by differential tissue tropism. These viruses...
are highly transmissible and cause an estimated 5–15% of all respiratory disease in children, mostly those under 5 years of age. Symptoms include pharyngitis, tonsillitis, bronchitis, and pneumonia. In adults, adenoviruses trigger large epidemics of acute respiratory disease in closed populations in residential homes, long-term care facilities and the military. In young paediatric patients, serotypes 1, 2, 3 and 5 are predominantly responsible for adenovirus-related respiratory disease and, therefore, there is a need for the vaccine composed of these serotypes to be delivered to young children. Adenoviral infections are increasing within the population of immunocompromised patients and have severe consequences with a fatality rate often up to 60% depending on the nature of the immunodeficiency. Additionally, corticosteroid treatment of those patients may re-activate latent adenovirus infection.

Oral, live-attenuated vaccines against serotypes 4 and 7 of adenovirus were routinely used since the 1970s in army recruits, and this strategy successfully eliminated frequent epidemics at trainee camps. However, the production of this vaccine was discontinued in 1995 and subsequently adenoviral infections have re-emerged, causing a significant burden of disease in military camps. Work is underway to re-introduce this safe and effective vaccine.

**Current developments and trends**

**Mucosal immunization**

Mucosal immunization represents an attractive method of vaccine delivery. It is simple, fast and non-invasive, does not involve use of needles, and can be done by untrained personnel. The use of mucosal administration is particularly appropriate for diseases that start at mucosal sites, inducing local immunity able to control the initial stages of infection. Intranasal or oral vaccinations have been explored for at least 30 years and, as a result, some currently available vaccines are delivered via these routes or are being tested for such delivery. Respiratory vaccines have been also delivered via the oral route. The safe and effective vaccine for adenovirus infections is a good example of an oral vaccine for respiratory viral infection.

The Swiss vaccine ‘Nasalflu Berna’ is a trivalent influenza virus vaccine delivered as a nasal spray. It comprises influenza virosomes formulated from inactivated influenza surface glycoproteins (H1N1, H3N2 and B), lecithin, and the heat-labile toxin from *E. coli* bacteria. The 150 nm particles are delivered by a spray device in phosphate buffered saline twice over a one week period and induce a ≥ 4-fold rise in IgG antibody titres in most
recipients. In clinical trials, it has an 85% efficacy in adults and nearly 90% efficacy in children.

No significant adverse events were noted in any of the initial studies. As a result, the vaccine was licensed for use in Switzerland in 2000, was widely accepted, and used in over 100,000 people during the first season. However, 43 cases of Bell’s palsy were reported in recipients of the vaccine (one of whom was a prominent journalist) and sales were suspended pending investigation. There is no evidence to support a link between nasal vaccination and Bell’s palsy, but further investigation of this potential side-effect are on-going. This incident demonstrates how susceptible vaccine manufacturers are to adverse publicity, even before causal links between vaccination and possible adverse events are proven.

Maternal immunization

Vaccination of pregnant or pre-pregnant women is a promising approach to prevention of neonatal infection, while also reducing the circulation of common cold agents in the community. However, there is understandable apprehension concerning vaccination during pregnancy and concern that administration may be linked (even anecdotally) with premature birth, developmental defects, etc. According to the recommendations of the US Advisory Committee on Immunisation Practices, women should be immunised with influenza vaccine during their second or third trimester of pregnancy if these fall during the influenza season39. The aim of vaccination of pregnant women against RSV would be to boost maternal antibody levels, as passively-obtained specific antibodies are protective and an inverse relationship between maternal antibody titres and severity of RSV disease in the new-born has been shown40,41. The possibilities arise to use more vaccines for maternal immunisation as more potential candidate vaccines become available, possibly including PIV3 purified subunit vaccines.

Conclusions

In addition to causing simple common colds, respiratory viruses also lead to bronchitis, pneumonia, and acute sinusitis; they may be sometimes complicated by myocarditis, pericarditis, encephalitis or even acute respiratory distress syndrome (ARDS). Moreover, some respiratory viral infections are associated with the development of asthma or chronic obstructive pulmonary disease (COPD) exacerbation. There are currently few safe and successful vaccines which can prevent respiratory illness and disease burden within populations at risk; those available are
essentially limited to influenza and adenoviral vaccines. New vaccines are being developed and tested in animal models, and some are already in advanced clinical trials (e.g. PIV3 and RSV vaccines). It is hoped that some will soon be successful.

**Acknowledgements**

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