Streptococcus pneumoniae colonisation: the key to pneumococcal disease

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Streptococcus pneumoniae is an important pathogen causing invasive diseases such as sepsis, meningitis, and pneumonia. The burden of disease is highest in the youngest and oldest sections of the population in both more and less developed countries. The treatment of pneumococcal infections is complicated by the worldwide emergence in pneumococci of resistance to penicillin and other antibiotics. Pneumococcal disease is preceded by asymptomatic colonisation, which is especially high in children. The current seven-valent conjugate vaccine is highly effective against invasive disease caused by the vaccine-type strains. However, vaccine coverage is limited, and replacement by non-vaccine serotypes resulting in disease is a serious threat for the near future. Therefore, the search for new vaccine candidates that elicit protection against a broader range of pneumococcal strains is important. Several surface-associated protein vaccines are currently under investigation. Another important issue is whether the aim should be to prevent pneumococcal disease by eradication of nasopharyngeal colonisation, or to prevent bacterial invasion leaving colonisation relatively unaffected and hence preventing the occurrence of replacement colonisation and disease. To illustrate the importance of pneumococcal colonisation in relation to pneumococcal disease and prevention of disease, we discuss the mechanism and epidemiology of colonisation, the complexity of relations within and between species, and the consequences of the different preventive strategies for pneumococcal colonisation.

Lancet Infect Dis 2004; 4: 144–54

Streptococcus pneumoniae is a common cause of invasive disease and respiratory-tract infections in more and less developed countries. Risk groups for diseases caused by pneumococci, such as meningitis, sepsis, and pneumonia, include young children, elderly people, and patients with immunodeficiencies. Each year, 1 million children younger than 5 years old die from pneumonia and invasive diseases. In the USA, the annual number of fatal pneumococcal infections is 40 000.1 Community-acquired pneumococcal meningitis has a very high case-fatality rate (20% and 50% in more and less developed countries, respectively). Depending on age, 30–60% of survivors develop long-term sequelae including hearing loss, neurological deficits, and neuropsychological impairment.

Protection against pneumococcal infections is mediated by opsonin-dependent phagocytosis. Antibody-initiated complement-dependent opsonisation, which activates the classic complement pathway, is thought to be the major immune mechanism protecting the host against pneumococcal infections. The mechanism of clearance depends on the interaction of type-specific antibodies (IgA, IgM, IgG), complement, and neutrophils or phagocytic cells from lung, liver, and spleen. Functional or anatomical asplenia and cirrhosis of the liver both predispose to severe pneumococcal infection. Congenital deficiencies in immunoglobulin or complement are also associated with predisposition to pneumococcal infection. S pneumoniae is part of the commensal flora of the upper respiratory tract. Together with Moraxella catarrhalis, Haemophilus influenzae, Neisseria meningitidis, Staphylococcus aureus, and various haemolytic streptococci, they colonise the nasopharyngeal niche. Though colonisation with pneumococci is mostly symptomless, it can progress to respiratory or even systemic disease (figure 1). An important feature is that pneumococcal disease will not occur without preceding nasopharyngeal colonisation with the homologous strain.1,2 In addition, pneumococcal carriage is believed to be an important source of horizontal spread of this pathogen within the community. Crowding, as occurs in hospitals, day-care centres, and prisons, increases horizontal spread of pneumococcal strains.2,3,4 Because the highest frequency of pneumococcal colonisation and the highest crowding index are found in young children, this risk group is thought to be the most important vector for horizontal dissemination of pneumococcal strains within the community.1,2 Therefore, part of the strategy to prevent pneumococcal disease focuses on prevention of nasopharyngeal colonisation, especially in children.

Owing to the key role of nasopharyngeal colonisation in pneumococcal disease and pneumococcal spread, we focus in this review on the different features of nasopharyngeal colonisation in children. To elucidate the route of pneumococcal disease, we discuss current knowledge on the mechanism of colonisation, the epidemiology and determinants of pneumococcal carriage, and the status of prevention of colonisation by means of vaccination.
Dynamics of nasopharyngeal colonisation
The upper respiratory tract is the ecological niche for many bacterial species. In children, the nasopharyngeal flora become established during the first months of life. A broad variety of microorganisms including S pneumoniae, H influenzae, and M catarrhalis can colonise the nasopharyngeal niche. Every individual is likely to be colonised with these pathogens at least once during life. In general, there is simply asymptomatic carriage; but in some cases, colonisation is followed by disease. Colonisation is commonly followed by horizontal dissemination of the pathogens to individuals in the direct environment, leading to spread within the community. The reported rates of bacterial acquisition and carriage depend on age, geographical area, genetic background, and socioeconomic conditions. The local host immune response has an important regulatory role in the trafficking of pathogens in the upper respiratory tract. A poor mucosal immune response might lead to persistent and recurrent colonisation and consequently infection, whereas a brisk local immune response to the pathogen will eliminate colonisation and prevent recolonisation. In general, mucosal immunity matures earlier than systemic immunity, and is present from the age of 6 months. IgG and secretory IgA antibodies directed against capsular polysaccharides and surface-associated proteins have been observed in saliva of children in response to colonisation with S pneumoniae.

Nasopharyngeal colonisation is a dynamic process in terms of the turnover of colonising species and serotypes. Moreover, interspecies competition is thought to occur and to interfere with the composition of the nasopharyngeal flora. First, the balance between the resident flora and transient invaders is important. The resident flora, including α-haemolytic streptococci, inhibit colonisation by S pneumoniae, H influenzae, S aureus, and M catarrhalis. The importance of this inhibitory role was shown by Ghaffar and colleagues, who found a competitive balance between α-haemolytic streptococci and S pneumoniae and H influenzae, which could be altered by antibiotics. A negative association between viridans streptococci and S pneumoniae, H influenzae, and M catarrhalis has also been reported, with the last three becoming predominant during upper-respiratory-tract infections.

Furthermore, the different pathogenic species show a competitive relationship. In-vitro studies by Pericone and colleagues showed a positive relation between N meningitidis and S pneumoniae. Growth of S pneumoniae increased in the presence of meningococci, a process probably mediated by meningococcal catalase. However, meningococcal growth was decreased in the presence of pneumococci or pneumococcal culture supernatant. The researchers attributed the latter effect to the presence of pneumococcal peroxide. This inhibitory effect of S pneumoniae was also observed in co-cultures with H influenzae and M catarrhalis. Moreover, S pneumoniae can interfere with the growth of S aureus; this effect has also been attributed to pneumococcal hydrogen peroxide. We showed in a cross-sectional carriage study of 3200 children that the competition between S aureus and S pneumoniae contributes substantially to the age-related dynamics of
nasopharyngeal colonisation in children. Our findings have been confirmed by Regev-Yochay and co-workers. We also found that parallel to the age-related decline in pneumococcal colonisation, caused by the maturation of the immune system, there was a simultaneous increase in *S aureus* carriage rate, from 10% in the first years of life to a maximum of 50% at the age of 10 years. In addition to these ecological interactions, the composition of the nasopharyngeal niche is influenced by environmental factors such as crowding and smoking. There is limited evidence on the competition between the different pneumococcal serotypes. For example, Lipsitch and colleagues used a mouse model of intranasal carriage of pneumococci to test whether there is competition between pneumococcal strains.

### Table 1. Pneumococcal colonisation and serotype-distribution studies

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<th>Ref</th>
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<th>Country</th>
<th>Number of children</th>
<th>Age</th>
<th>Risk group</th>
<th>Type of culture</th>
<th>Carriage (%)</th>
<th>Coverage with 7-valent conjugate vaccine (%)</th>
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RTI=respiratory-tract infection; URTI=upper-respiratory-tract infection; AOM=acute otitis media; SCD=sickle-cell disease; DCC=day-care centre; OPD=outpatient department; COME=chronic otitis media with effusion. *Increasing with age. †Coverage for 9-valent conjugate vaccine. ‡Including cross-reactive serotypes. §Average for all isolates of the study.
Streptococcus pneumoniae colonisation

They found that mice carrying a serotype 6B strain as resident strain showed reduced colonisation with a serotype 23F pneumococcus when challenged intranasally with the latter strain. This inhibitory effect could be overcome by increasing the dose of the challenge strain. Interference in this complex pattern of interaction and inhibition by means of vaccination could have serious and unpredictable consequences for the composition of the entire nasopharyngeal population.

Mechanism of colonisation

The pneumococcal outer surface is covered by a polysaccharide capsule. Capsular polysaccharides are highly heterogeneous, and almost 100 different capsular serotypes have been described so far. The polysaccharide capsule is the most important virulence factor of pneumococci because it protects the bacteria from phagocytosis. Reduced expression results in greater access of antibodies and complement to the pneumococcal surface, and hence increased clearance by the immune system. Capsular polysaccharides are highly immunogenic. Antibodies against them protect against infection with the homologous serotype by induction of opsonophagocytosis. The antigenicity of the capsule is type-specific; however, cross-reaction can occur because of shared polysaccharides.

The layer underneath the capsule, the cell wall, consists of polysaccharides and teichoic acid and serves as an anchor for cell-wall-associated surface proteins. The cell wall is the cause of the intense inflammatory reaction that accompanies pneumococcal infection, since it stimulates the influx of inflammatory cells and activates the complement cascade and cytokine production. The cell wall is believed to be protected from the host response by the surrounding polysaccharide capsule.

Colonisation by S pneumoniae requires adherence to the epithelial lining of the respiratory tract. Asymptomatic colonisation involves pneumococcal binding to cell-surface carbohydrates (N-acetyl-glycosamine) on non-inflamed resting epithelium. Adherence to these sugars is mediated by cell-wall-associated surface proteins, such as pneumococcal surface adhesin A (PsaA; figure 2). In addition, the surface proteins contribute to the hydrophobic and electrostatic surface characteristics of pneumococci and might facilitate adherence to host cells partly through non-specific, physicochemical interactions. In general, colonisation is not followed by symptomatic disease. Conversion of asymptomatic colonisation to invasive disease requires the local generation of inflammatory factors such as interleukin 1 and tumour necrosis factor, as seen in the presence of viral infections. This inflammatory cascade changes the type and number of receptors on target epithelial and endothelial cells. Pneumococcal cell-wall choline shows increased affinity for one of these upregulated receptors, the platelet-activating-factor receptor (PAFr). The pneumococcus has increased affinity via its cell-wall phosphocholine (ChoP) for PAFr. Moreover, a second choline-binding protein, CbpA, shows increased affinity for immobilised sialic acid and lacto-N-neotetraose, and binds directly to the polymeric Ig receptor (pIgR), which increases migration through the mucosal barrier (transcytosis). Pneumococcal IgA1 protease cleaves opsonising IgA, which results in a change (neutralisation) of surface charge and increases the physical proximity of ChoP to the PAFr.

**Figure 2. Interaction between S pneumoniae and epithelial cells. Neuraminidase (NanA) decreases the viscosity of the mucus and exposes the N-acetyl-neuraminic acid (GlcNAc) receptors on the epithelial cells, which can interact with pneumococcal surface-associated proteins such as PsaA. In response to cytokine stimulation, host epithelial cells upregulate the platelet-activating-factor receptors (PAFr). The pneumococcus has increased affinity via its cell-wall phosphocholine (ChoP) for PAFr. Moreover, a second choline-binding protein, CbpA, shows increased affinity for immobilised sialic acid and lacto-N-neotetraose, and binds directly to the polymeric Ig receptor (pIgR), which increases migration through the mucosal barrier (transcytosis). Pneumococcal IgA1 protease cleaves opsonising IgA, which results in a change (neutralisation) of surface charge and increases the physical proximity of ChoP to the PAFr.**
mucin, decreasing the viscosity of the mucus. Neuraminidase also cleaves glycolipids, glycoproteins, and oligosaccharides, and thus is thought to bring about exposure of N-acetyl-glycosamine receptors on the host epithelial cells. The neuraminidase activity of viruses such as influenza and parainfluenza viruses might thereby contribute to the increased adherence of pneumococci observed during viral infections. Variability in the composition, expression, or exposure of surface-associated proteins could explain differences in colonisation and invasion capacities between strains. The complexity of this process is underlined by studies in which reversible phenotypic variation within pneumococcal strains and its role in host interaction were identified. Transparent phase variants show greater adherence than opaque variants. This phenotypic variation is associated with lower expression of capsule polysaccharides and higher expression of certain cell-surface proteins and carbohydrate-containing cell-wall structures. With increasing knowledge about the mechanisms of colonisation, surface-associated proteins have become of major interest as potential vaccine candidates. Although surface-associated proteins such as pneumolysin and pneumococcal surface protein A (PspA) elicit protection against systemic diseases, PsA and CbpA are promising candidates for prevention of colonisation. In theory, better protection against colonisation and infection with *S pneumoniae* might be expected when a combination of proteins with distinct roles in bacterial virulence is used.

**Pneumococcal colonisation in children**

Nasopharyngeal colonisation of *S pneumoniae* in children mainly depends on age. We investigated the age-dependent carriage rate in a large cohort of healthy children and adolescents aged 1–19 years. The peak incidence of pneumococcal colonisation was 55% at the age of 3 years. There was then a steady decline until a stable prevalence of 8% was observed after the age of 10 years. Although most other colonisation studies have not extended the age-group studied into adulthood, those that did have also shown a decline. By contrast, the nasopharyngeal niche becomes colonised during the first year of life. Therefore, pneumococcal carriage shows an increase before the age of 2 years (table 1). For example, in a Finnish study the frequency of nasopharyngeal carriage in children aged 2–24 months increased from 13% for under 6 months to 43% in children older than 19 months. The proportion increased during respiratory infections to 22–45%, which supports the theory of greater adherence during (viral) infections.

In the healthy population, risk factors also seem to determine the frequency of pneumococcal carriage. Independent determinants for nasopharyngeal colonisation are ethnicity, crowding, environmental features, and socioeconomic factors. Socioeconomic and environmental risk factors include family size (specifically the number of older siblings), income, smoking (passive and active), and recent antibiotic use. Crowding is a major factor in colonisation and in spread of pneumococcal strains. In young children, especially, day-care visits are associated with significantly increased colonisation rates (table 1). In a study in the Netherlands, the relative risk of nasopharyngeal colonisation by pneumococci in children who attended day-care centres compared with children who were cared for at home was 1.6. In addition, that study showed increased genetic clustering among pneumococcal isolates, which accords with previous reports. This finding supports the hypothesis of increased horizontal spread of specific pneumococcal strains among attenders at day-care centres. In agreement with these findings, Raymond and colleagues reported a colonisation rate of up to 82% in infants living in an orphanage. Close relatedness between the pneumococcal isolates was found in that study, suggesting frequent horizontal spread.

Ethnic groups at increased risk of pneumococcal colonisation as well as invasive disease are African American, native American (Apache and Navajo), and Alaskan native populations. The risk of invasive pneumococcal diseases in children aged 24–35 months is 64.7 cases per 100 000, whereas black people in the USA have a rate of 116.4 per 100 000, and native Americans 73–227 cases per 100 000. The risk of invasive disease in the native American population is increased to such an extent that the US Advisory Committee for Immunization Practices (ACIP) has recommended pneumococcal vaccination for this population in all age-groups. For children attending day-care centres the risk of pneumococcal infection is so high that immunisation with a seven-valent pneumococcal conjugate vaccine (Prevnar, Wyeth, USA) covering the most prevalent serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F is advised. Pneumococcal colonisation, especially with antibiotic-resistant bacteria, is also increased as a result of recent antibiotic treatment. The selection of antibiotic-resistant pneumococci at the nasopharynx is commonly assumed to be the cause of the spread of resistant pneumococcal strains within the community. Consequently, several multidrug-resistant clones have already spread throughout the world.

Not all risk groups for pneumococcal diseases show increased rates of colonisation compared with the general population. For example children with HIV infection and sickle-cell disease have similar colonisation rates to healthy children (table 1). This similarity is a result of the underlying immune disorder: instead of a defect or augmented challenge of the primary defence mechanism against pneumococcal invasion, the immune disorder is related to an impaired response to or clearance mechanism for pneumococci after invasion has occurred. In children with HIV/AIDS, the numbers of CD4-positive T cells, necessary for an appropriate antipolysaccharide response, are decreased. In children with sickle-cell disease, splenic function, involved in direct phagocytosis and initiation of the antipolysaccharide response, is impaired. However, the primary mucosal barrier, including the mucosal immune response, is still intact in these patients.

Though variable colonisation rates have been observed in different areas of the world (table 1), colonisation rates tend to be higher during respiratory tract infections and otitis media and in risk groups such as attenders at day-care centres. In addition, colonisation rates tend to be higher
Serotype distribution among pneumococcal isolates

The serotype distribution among nasopharyngeal carriage isolates varies slightly by country, age-group, and type of cohort. Europe and the US show similar serotype distributions with minor differences in several serotypes. For example, in the Netherlands, serotypes 19F (19%), 6B (16%), 6A (13%), 9V (7%), and 23F (7%) are most frequently found among children under 3 years of age. In Greece, similarly, the most predominant serotypes among children younger than 2 years are 6B, 19F, 23F, 14, and G;14,15 and in Finland, serotypes 6B (16%), 23F (14%), 19F (14%), and 6A (9%) are most prevalent. In the USA, serotypes 6B, 14, 19F, and 23F are also common. In Asia, similar serotypes and serogroups have been found among nasopharyngeal isolates in healthy children. For example, in India, the most common serogroups are 6, 14, 19, and 15;66 in Vietnam the commonest serogroups are 19, 23, 14, and 18;67 in Indonesia is slightly different, with the most common being 6 (25%) and 23 (21%) followed by 15 (8%), 33 (8%), 19 (6%), 12 (5%), and 3 (4%). In Kenya, serotype 13 was with 15, 14, 6B, and 19F most commonly present. In South Africa, a similar distribution was found with the exception of serotype 13, which was not found at all.68

No major differences have been found in serotype distribution between children with risk factors such as attendance at day-care centres or upper-respiratory-tract infections and healthy children.21,42,74 By contrast, an important variable is the age-group investigated. In general, the frequency of vaccine serotypes declines with age. In our study in the Netherlands, nasopharyngeal carriage of vaccine-type strains generally declined from 30% at age 1 year to 3% at 8 years, after which a stable prevalence was observed until age 19 years. By contrast, non-conjugate vaccine serotypes, especially serotypes 3, 8, 10, 11, and 15 showed an increase to the age of 7–10 years, after which there was a delayed decline compared with the vaccine serotypes.

In general, the serotype distribution among nasopharyngeal isolates from different parts of the world is similar. This similarity is also reflected by the potential conjugate vaccine coverage (table 1). As shown by Lloyd-Evans and colleagues, invasive disease originates from nasopharyngeal colonisation with the homologous serotype. Therefore, the serotype distribution of colonisation isolates should be an indicator of invasive disease, antibiotic resistance profiles, and potential vaccine coverage. However, certain serotypes and genotypes seem to cause higher rates of invasive diseases when corrected for prevalence of nasopharyngeal colonisation. Brueggemann and colleagues found serotype-specific and clone-specific differences in invasive-disease potential with an increased capacity to cause disease for specific serotype 14 and 18C clones. The most commonly carried serotypes, 6B, 19F, and 23F, are least invasive, whereas certain non-vaccine serotypes (8, 38, 33F) are infrequent colonisers but appear to be more invasive. This is also true for serotypes 5, 7F, and 1.10 This knowledge is extremely important in view of the replacement of colonising strains observed after conjugate vaccination. Therefore, surveillance of pneumococcal invasive disease and colonisation isolates remains a necessity in those countries where large-scale pneumococcal vaccination is initiated.

Current vaccine strategies

The ACIP has recommended vaccination against pneumococcal infections for several risk groups. Although the 23-valent vaccine, with a theoretical coverage of 85–90% of circulating strains, is immunogenic in adults and children older than 5 years, young children (<2 years) have a severely impaired antibody response to polysaccharide vaccination.10,101–103 Therefore, the recommendations of the ACIP in 1997 excluded the major risk group of children under 2 years of age. The remaining groups were immunocompetent children older than 2 years at increased risk of illness and death associated with pneumococcal disease because of chronic cardiac and pulmonary diseases, individuals older than 2 years with functional or anatomical asplenia, and immunocompromised patients older than 2 years. Fortunately, the new generation of conjugate vaccines is highly immunogenic in children under 2 years.
Dagan and co-workers,\textsuperscript{113} for example, showed a decreased disease in the vaccinated community—ie, herd immunity. elicit protection against pneumococcal colonisation and individual protection, diminished colonisation is thought to proven data are often missing.\textsuperscript{105,106,108,109} Moreover, several much lower and more difficult to measure because culture-mucosal diseases such as pneumonia and otitis media is caused by vaccine serotype strains. The efficacy against conjugate vaccine is highly effective against invasive diseases\textsuperscript{107} The polysaccharide booster, because this step improves setting, conjugate vaccination is followed by a at increased risk of pneumococcal diseases. In the latter children under 2 years of age and in children aged 2–5 years valent conjugate vaccine Prevnar (Wyeth, USA) for all childhood recommendations for pneumococcal vaccination beings from birth. The ACIP has therefore changed the serotypes conjugated to a carrier protein inducing a T-cell-memory.\textsuperscript{104} In several large studies, a seven-valent conjugate vaccine had almost 100% efficacy against invasive diseases caused by the included serotypes.\textsuperscript{105,106} The new vaccines contain polysaccharides of seven to 11 pneumococcal serotypes conjugated to a carrier protein inducing a T-cell-dependent immune response that is present in human beings from birth. The ACIP has therefore changed the childhood recommendations for pneumococcal vaccination in 2000. The current advice is vaccination with the seven-valent conjugate vaccine Prevnar (Wyeth, USA) for all children under 2 years of age and in children aged 2–5 years at increased risk of pneumococcal diseases. In the latter setting, conjugate vaccination is followed by a polysaccharide booster, because this step improves pneumococcal antibody titres in this age-group.\textsuperscript{107} The conjugate vaccine is highly effective against invasive diseases caused by vaccine serotype strains. The efficacy against mucosal diseases such as pneumonia and otitis media is much lower and more difficult to measure because culture-proven data are often missing.\textsuperscript{105,106,108,109} Moreover, several investigators have shown a significant reduction in nasopharyngeal carriage of vaccine-type pneumococci as a result of conjugate vaccination.\textsuperscript{99,100,101} In addition to individual protection, diminished colonisation is thought to elicit protection against pneumococcal colonisation and disease in the vaccinated community—ie, herd immunity. Dagan and co-workers,\textsuperscript{111} for example, showed a decreased colonisation rate in siblings of children attending day-care centres who were vaccinated with a nine-valent conjugate vaccine. Moreover, penicillin and multidrug resistance is common among pneumococcal strains, especially among the conjugate vaccine serotypes. Therefore, there have been suggestions that conjugate vaccination will also reduce resistance among pneumococcal strains in vaccinated individuals as well as the open community as a result of herd immunity.\textsuperscript{99} Recently, Dagan and colleagues\textsuperscript{114} have shown a significant reduction in penicillin and multidrug resistance among carriage strains as a result of vaccination with a nine-valent conjugate vaccine.

The vaccines with seven to 11 serotypes inevitably do not cover all serotypes. Protection also depends on the geographical area, with potential coverage of the seven-valent conjugate vaccine for invasive strains of over 85% for the USA, 60–70% for Europe, and around 55% for Asia,\textsuperscript{89} although a large proportion of these differences might be explained by variation in blood-culture practices.\textsuperscript{115} In addition to the limited coverage of these conjugate vaccines, another long-term risk should be considered. Because of the limited coverage of circulating pneumococcal strains by the conjugate vaccine, the remaining non-vaccine serotype strains will actually benefit from this selective immunological pressure. Replacement may occur, causing a shift in serotype strains circulating in the population and, consequently, in disease. Since the start of large-scale vaccination trials, replacement has been observed in individuals colonised with pneumococci as well as in patients with acute otitis media.\textsuperscript{79,85,118} So far, the effect of this event on invasive diseases remains unclear. However, though not yet significant, the first alarming findings have been reported on partial replacement of invasive strains with non-vaccine serotypes in vivo.\textsuperscript{119} In addition, Brueggemann and co-workers\textsuperscript{89} have shown a high invasive capacity for certain non-vaccine serotype strains, which may also imply that replacement of carriage will lead to replacement of disease. Thus, close monitoring of serotype distribution among invasive as well as colonisation strains remains of major importance. Nine studies have investigated the effect of conjugate vaccination on nasopharyngeal colonisation (table 2). Two studies found no significant effect on the overall pneumococcal colonisation nor on vaccine type carriage.\textsuperscript{94,110} In the remaining studies, a positive effect of vaccination was found on colonisation of vaccine-serotype pneumococci. However, replacement of these strains with

### Table 2. Conjugate vaccination studies investigating the effect of vaccination on colonisation rate, serotype distribution, and replacement

<table>
<thead>
<tr>
<th>Ref</th>
<th>Year</th>
<th>Country</th>
<th>Age (months) children</th>
<th>Number of Follow-up (months)</th>
<th>Risk group</th>
<th>Vaccine schedule</th>
<th>Carriage (%) of vaccine types</th>
<th>Carriage (%) of replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>2000–01</td>
<td>UK</td>
<td>2</td>
<td>607</td>
<td>24–60</td>
<td>7-valent</td>
<td>Vaccine group</td>
<td>Control group</td>
</tr>
<tr>
<td>117</td>
<td>1998–99</td>
<td>Netherlands</td>
<td>12–72</td>
<td>383</td>
<td>26</td>
<td>7-valent</td>
<td>25/43</td>
<td>21/41</td>
</tr>
<tr>
<td>118</td>
<td>NS</td>
<td>USA</td>
<td>2</td>
<td>260</td>
<td>10</td>
<td>9-valent</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>119</td>
<td>1998–99</td>
<td>USA</td>
<td>7–12</td>
<td>577</td>
<td>11</td>
<td>9-valent</td>
<td>63</td>
<td>65</td>
</tr>
<tr>
<td>95</td>
<td>1997</td>
<td>South Africa</td>
<td>2</td>
<td>500</td>
<td>9</td>
<td>9-valent</td>
<td>54</td>
<td>61</td>
</tr>
<tr>
<td>96</td>
<td>1996–97</td>
<td>Israel</td>
<td>12–35</td>
<td>262</td>
<td>24</td>
<td>9-valent</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td>111</td>
<td>1997</td>
<td>Israel</td>
<td>2</td>
<td>75</td>
<td>11</td>
<td>4-valent</td>
<td>44</td>
<td>52</td>
</tr>
<tr>
<td>94</td>
<td>1995</td>
<td>USA</td>
<td>2</td>
<td>81</td>
<td>13</td>
<td>7-valent</td>
<td>47</td>
<td>53</td>
</tr>
<tr>
<td>112</td>
<td>1994</td>
<td>Israel</td>
<td>12–18</td>
<td>263</td>
<td>12</td>
<td>7-valent</td>
<td>43</td>
<td>57</td>
</tr>
</tbody>
</table>

CV—conjugate vaccine; NS—not stated; PV—polysaccharide vaccine. *Depending on the season. 116 Efficacy data do not include the effect of the polysaccharide booster. 117 valent pneumococcal vaccine conjugated to outer membrane protein of N meningitidis.
non-vaccine serotypes reduced the effect on overall pneumococcal colonisation in most cases.

New vaccine strategies

New vaccine strategies focus on the use of pneumococcal surface-associated proteins. This approach has several advantages. First, the production of protein vaccines is expected to be cheap and therefore within reach of developing countries. Second, a protein-based vaccine is expected to elicit protection in all age-groups, including children younger than 2 years. Finally, if highly conserved proteins or protein epitopes are used as vaccine components, broad and serotype-independent protection can be expected. However, the degree and type of protection will be influenced by the function of the proteins included in the vaccine. We illustrate this effect by discussing the most promising protein vaccine candidates.

PspA, one of the family of structurally related choline-binding surface proteins, can interfere with complement fixation by blocking recruitment of the alternative pathway through reduction of the amount of C3b deposited on the pneumococci, thereby reducing the effectiveness of the complement-receptor-mediated pathways of clearance.120–123 This process is particularly important when bacterial invasion has occurred and suggests a significant role for PspA in the maintenance of invasive pneumococcal disease. Studies on active immunisation with PspA in animals show a protective effect against invasive infections and to a lesser extent against mucosal disease and nasopharyngeal carriage.124–127 The first phase I vaccination trial with a single recombinant PspA variant in human beings showed that broadly cross-reactive antibodies to heterologous PspA molecules were elicited,128 which were found to protect mice challenged intraperitoneally with pneumococci.129

Another candidate is PsaA, a member of the family of metal-binding lipoproteins, part of an ABC transporter complex thought to be involved in the transport of manganese into pneumococci.130,131 This protein is mainly involved in asymptomatic colonisation.132 The first immunisation studies with PsaA have shown significant protection against colonisation but limited to modest protection against invasive infections.132–134 Seo and colleagues135 showed that oral vaccination of mice with PsaA encapsulated in microalginate microspheres elicited significant protection against colonisation, pneumonia, and septicaemia from an oral challenge. These findings suggest that vaccination with PsaA elicits primary protection against colonisation with secondary protection against invasive disease. However, clinical studies on the correlation between antibodies to PsaA and the risk of pneumococcal acute otitis media have had contradictory results. Rapolä and co-workers136,137 showed an association between higher titres of anti-PsaA and lower risk of pneumococcal acute otitis media, but only in children older than 9 months, whereas in younger children the risk was increased with higher anti-PsaA concentration. These findings suggest a basic difference among age-groups with respect to protection by antibodies to PsaA, and perhaps to the origin of the antibody response. A higher anti-PsaA titre might be associated with increased pneumococcal contacts in the past—ie, through colonisation as well as through infection. Consequently, it might explain the relation with the underlying increased susceptibility to pneumococcal acute otitis media rather than a lower risk of infections.

Pneumolysin is a protein that also contains a choline-binding domain and is thought to interfere with host immunity and inflammatory responses by various functions, including complement fixation and inhibition of phagocyte function. It also inhibits ciliary activity in the bronchus and is thus important in pathogenesis of pulmonary infection.138 Knock-out mutagenesis of genes encoding pneumolysin has suggested a role in virulence, in colonisation as well as in infection.139–141 Several research groups have described the protective properties of pneumolysin against challenge with pneumococci in mice, albeit only against invasive disease.142,143 The combination of PspA and pneumolysin yields complementary protection to invasive disease in animals.144,145 The combination of PsaA and PspA prevents colonisation and otitis media in animals.146,147 Hence, depending on the target, differing combinations of vaccine components can be used. The optimum combination of proteins to be chosen for vaccination purposes remains to be investigated.

Alternative routes of vaccination have also been explored. Several studies144,147,148 have suggested that administration of a vaccine via the oral or nasal route is as effective as systemic application. In addition, Lynch and colleagues146 found that intranasal administration of a conjugate vaccine plus interleukin 12 not only elicited protection against invasive disease but also, in contrast to intramuscular administration, induced protection against nasal carriage. The latter effect occurs through the induction of substantial mucosal IgA responses. Mucosal routes of administration are highly preferable because they are less invasive and because so many other vaccines are already administered intramuscularly to children, as part of community vaccination programmes. Moreover, in contrast to pneumococcal conjugate vaccines and polysaccharide vaccines, protection is also expected in children with HIV/AIDS, even during progression of disease, because of the intact mucosal immune response in these patients.149

Discussion

Nasopharyngeal colonisation provides an important key to the burden of pneumococcal disease and its prevention. Colonisation not only is obligatory for invasive disease, but also provides the basis for horizontal spread of pneumococci.

Although the major goal of all vaccine strategies is to reduce the burden of pneumococcal disease, they involve also prevention of pneumococcal colonisation. Opinion about reduction in colonisation ranges from “secondary aim” to “fortunate side-effect”. However, the importance of this essential link in pathogenesis has seldom received full attention.

The natural route of infection with S pneumoniae starts with colonisation, which may progress to invasive disease if natural immunological barriers are crossed. Therefore,
a rational aim is to prevent colonisation, thus eliciting protection against invasive disease. Moreover, prevention of nasopharyngeal colonisation of S pneumoniae might also decrease horizontal spread of pneumococcal strains, thus improving herd immunity.2,3,115,16 This possibility supports the use of polyvalent polysaccharide-based vaccines such as the 23-valent polyvalent conjugate vaccine, or future protein-based vaccines consisting of surface-exposed proteins involved in colonisation and adherence such as PsaA, CbpA, and neuraminidase. An alternative to vaccination could be the use of anti-attachment agents such as receptor analogues or agents like xylitol, N-acetylcyctein, or the recently identified S-carnosynthesis.156 None of these agents results in complete eradication of pneumococcal colonisation, but the same is true for vaccination: by prevention of colonisation without complete eradication of pneumococcal carriage, the immunological pressure will skew selection of non-covered serotypes or genotypes. Moreover, if the nasopharyngeal niche is cleared, replacement with other species might occur. Veennhoven and colleagues observed that pneumococcal conjugate vaccination resulted in fewer middle-ear fluid cultures with vaccine-serotype pneumococci, but in an increase of three times in cultures positive for S aureus.167
Moreover, we have found competition within the individual between S pneumoniae and other microbes such as H influenzae, M catarrhalis, and N meningitidis has been shown in vitro. A possible solution for this problem might be to aim strictly for prevention of invasive disease and leave nasopharyngeal colonisation unhampered, although mucosal disease can then still occur. Such disease cannot occur with the currently available vaccines, but might with future protein-based vaccines including disease-related proteins such as PspA, pneumolysin, the phosphate transporter family, and autolysins.38,144 A second option might be to consider the different amounts of protective antibodies necessary for systemic and mucosal protection. Pelton and colleagues99 have suggested that higher titres of immune-protective antibodies are needed for mucosal protection against S pneumoniae colonisation and infection than for systemic infection. Although highly speculative, one possibility is to adjust the conjugate vaccines to such an extent that the antibody titres induced are adequate to prevent invasive pneumococcal disease but insufficient to eradicate pneumococcal colonisation. However, such an approach would require individual monitoring and will not be achievable in the setting of large-scale vaccination.
In conclusion, although pneumococcal colonisation is mostly asymptomatic, it is the first step in the pathogenic route of pneumococci towards invasive disease. Moreover, it plays a crucial part in the prevention of pneumococcal infections and horizontal spread of virulent strains. There is a natural balance between pneumococci and co-colonising bacterial species, which could influence the outcome of vaccination strategies. These facts underline the key role for pneumococcal colonisation in pathogenesis and prevention of pneumococcal infections, which justifies extensive consideration in decision-making about mass vaccination and future vaccine strategies.

Conflicts of interest
None declared.

References
Streptococcus pneumoniae colonisation

Review

Preventing pneumococcal disease among infants and young children.


