

Review

Pneumococcal vaccines: mechanism of action, impact on epidemiology and adaption of the species

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Abstract

Pneumococcal infections elicited by *Streptococcus pneumoniae* (pneumococcus) (pneumonia, otitis media, sinusitis, meningitis) are frequently occurring diseases that are associated with considerable morbidity and mortality even in developed countries. Pneumococci colonise the nasopharynx of up to 50% of children, and up to 5% of adults are pneumococcal carriers. Two pneumococcal vaccines are currently in clinical use. One of them contains 23 capsular polysaccharides of the as yet known 91 different pneumococcal serotypes. Because polysaccharide vaccines primarily induce a B-cell-dependent immune response, this type of vaccine prevents bacteraemia but does not efficiently protect the host against pneumococcal infection. In 2000, a vaccination programme was launched in the USA making use of a novel pneumococcal conjugate vaccine containing capsular polysaccharides derived from the seven most frequent pneumococcal serotypes causing pneumococcal disease in children <2 years of age. Conjugation of capsular polysaccharides with a highly immunogenic protein, i.e. a non-toxic diphtheria toxoid, induces a B- and T-cell response resulting in mucosal immunity and thus effectively protects against vaccine serotypes that induce invasive pneumococcal disease, thereby at the same time reducing vaccine serotype carrier rates. Pronounced herd immunity resulted in a decrease in invasive pneumococcal diseases in vaccinees and non-vaccinees as well as reduced antibiotic resistance rates. However, recent studies report that serotypes eradicated by the vaccine are being replaced by non-vaccine pneumococcal serotypes. This so-called ‘replacement’ might soon threaten the success of vaccine use.

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Keywords: *Streptococcus pneumoniae*; Vaccine; Conjugated; Antibiotics; Resistance; Epidemiology

1. Introduction

Although pneumococcal diseases are frequent and vaccine development was started early, the development of an efficacious vaccine was not successful for a long time. The main reason is the low immunogenicity of polysaccharides, which are the target of opsonising antibodies. Two types of vaccines are currently in clinical use: polysaccharide vaccines; and pneumococcal conjugate vaccines.

Polysaccharide vaccines have been available since the mid 1980s. These vaccines contain purified capsular polysaccharides from 23 pneumococcal serotypes (1, 2, 3, 4, 5, 6b, 7F, 8,

9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F). Polysaccharides primarily induce a B-cell-dependent immune response via release of immunoglobulin M (IgM) [1]. Such polysaccharide vaccines are not recommended for use in children <2 years of age, probably due to their immature immune system. Vaccination of adults with polysaccharide vaccines requires re-vaccination after 5–6 years [2]. However, non-responders to immunisation are especially frequent in older patients [3]. It has also been observed that antibodies against bacterial capsular polysaccharides are difficult to induce in response to non-conjugated polysaccharide vaccines for corresponding meningococcal and *Haemophilus* vaccines [4,5].

The heptavalent pneumococcal conjugated vaccine (PCV-7) contains capsular polysaccharides from those

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pneumococci (4, 6B, 9V, 14, 18C, 19F and 23F) that are most frequently involved in paediatric infections. Capsular polysaccharides of PCV-7 are conjugated to highly immunogenic cross-reactive material 197 (CRM₁₉₇), a non-toxic diphtheria toxoid protein. Employment of this pneumococcal vaccine is particularly successful in the vaccination of young children. Similar to *Haemophilus influenzae* type B conjugate vaccination, CRM₁₉₇-specific type 2 helper T (Th2) cells interact with B-cells that have bound and internalised the polysaccharide–CRM₁₉₇ complex via polysaccharide-specific IgM and subsequently present the processed CRM₁₉₇ protein along with MHC II to effector T-cells. This type of adaptive immune response is characterised by antibody isotype switching and the generation of memory B-cells.

PCV-7 was approved in the USA in 2000 and since then in several additional countries. Different national schedules are applied. According to the Strategic Advisory Group of Experts (SAGE) of the World Health Organization (WHO), clinical efficacy in children has been demonstrated in two schedules: a 6-week, 10-week, 14-week schedule; and a 2-month, 4-month, 6-month schedule, which was followed by a booster dose at 12–15 months of age.

The 23-valent polysaccharide vaccine is primarily designed for use in older children and adults who are at risk for pneumococcal disease. It is not licensed for use in children <2 years of age. In some countries it is recommended by the public health authorities for all adults at the age of 60 years or older.

2. Pneumococcal bacteriology and antibiotic resistance

Streptococcus pneumoniae is a Gram-positive encapsulated bacterium (Fig. 1). The bacterial polysaccharide capsule contributes to the overall virulence of the pathogen and protects the bacterium from phagocytosis. Uncapsulated pneumococci such as the laboratory reference strain R6 are considered to be non-pathogenic. Ninety-one different capsu-

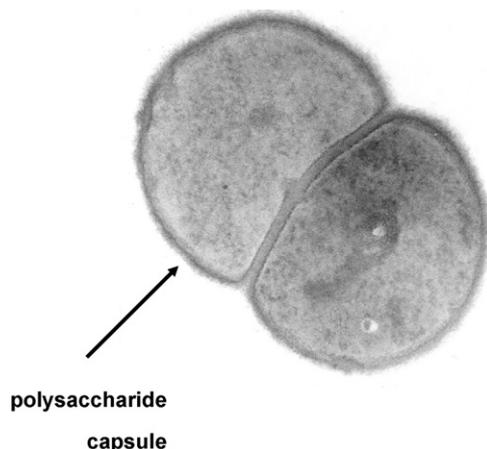


Fig. 1. Scanning electron microscope image of *Streptococcus pneumoniae*.

lar types (i.e. serotypes) have been described so far. Serotypes that are assessed by cross-reacting antibodies are summarised in serogroups.

Increased pneumococcal resistance worldwide is mainly due to the spread of multiresistant clones of the pathogen [6]. The current nomenclature of these clones is defined by the Pneumococcus Molecular Epidemiology Network and consists of the country of origin, the serotype (in superscript) and a consecutive number (e.g. Spain^{23F}-1 or England¹⁴-9) [7]. Current data demonstrate that pneumococcal resistance has also become a problem in countries with traditionally low resistance rates, such as Germany. A German study, which included children and adolescents <16 years of age suffering from invasive pneumococcal infections, showed that almost 29% of the pneumococci isolated were macrolide-resistant [8]. Thus, the proportion of macrolide-resistant species has increased three-fold in just 7 years (1997, 8.7%; 2004, 29%). Macrolide-resistant strains are of particular interest because macrolide resistance is associated with clinical treatment failure [9]. In contrast to penicillin resistance, which might be relevant in the treatment of pneumococcal pneumonia only at very high minimum inhibitory concentrations (MICs) (>4 mg/L) [10], a Canadian observational study identified 64 so-called ‘breaking through bacteremias’ during macrolide treatment. MIC determination of these isolates revealed that even slightly elevated MICs of ≥ 1 mg/L may lead to treatment failure [9].

3. Burden of pneumococcal disease

Pneumococci colonise the nasopharynx of ca. 50% of children and ca. 2.5% of adults [11]. Because humans are the only reservoir for these bacteria, it is theoretically possible to eradicate pneumococci by sufficient vaccination, similar to the pox virus.

Pneumococcal diseases primarily affect toddlers and adults [12]. The vaccination recommendations mentioned below reflect age-related incidence maxima. Pneumococci are the most frequent pathogens causing community-acquired pneumonia, otitis media, sinusitis and meningitis. Invasive pneumococcal disease has a mean mortality of 10%, which may rise to >30% in risk groups (e.g. nursing home residents >65 years of age) [13]. In Germany, the incidence of these invasive pneumococcal diseases is between 4.6/100 000/year for patients <65 years and 16.2/100 000/year for patients ≥ 65 years [14].

Since the mid 1980s, the US Centers for Disease Control and Prevention (CDC) has been performing an observational study (Active Bacterial Core Surveillance (ABCs)) to monitor invasive community-acquired pneumococcal disease comprehensively in nine different states with a current surveillance population of ca. 18.5 million people [15]. Invasive pneumococcal disease is defined by the presence of bacteria in body fluids that are usually sterile, including blood, cerebrospinal fluid, pleural fluid, ascites

or synovial fluid. After introducing PCV-7 in the USA, the high vaccination coverage rate required for reaching herd immunity (70–80%) was achieved very quickly. Data from the ABCs study allow an estimation of the effects of PCV-7 by comparing pre- and post-vaccine implementation data regarding the incidence of invasive pneumococcal infections, serotype distribution and antimicrobial resistance.

4. Immune responses induced by *Streptococcus pneumoniae* (Fig. 2)

Besides phagocytosis and intracellular killing by alveolar macrophages and neutrophil granulocytes (innate immunity), acquired humoral immunity is an important part of the host defence against pneumococci. As in other bacterial infections eliciting humoral responses, such a response requires processing and presentation of bacterial antigen in secondary lymphoid tissues. Recent data demonstrate that dendritic cells in particular are involved in this process. Immature dendritic cells process and present pneumococcal peptides along with MHC II complexes to naive CD4⁺ T-cells in the presence of co-stimulatory molecules such as CD80 and CD86. Such peptide antigen presentation results in T-cell proliferation and expansion of effector T-cells. IgM secreted by B-cells aids B-cells to capture and internalise soluble antigen via immunoglobulin receptors, which in turn promotes their capacity to mount increased IgM responses (Fig. 2A). Moreover, in the presence of co-stimulatory molecules, B-cells are also able to present processed antigen complexed with MHC II molecules to T-cells.

B-cells, which are usually stimulated by Th2 cells and cytokines (i.e. interleukin (IL)-4, IL-5 and IL-6), differentiate into IgG-secreting plasma cells (Fig. 2B). IgG plays an important role in opsonophagocytosis of and complement-induced cytotoxicity against pneumococci by professional phagocytes. Appropriately triggered B-cells also release IgA, which is deposited on mucosal surfaces to protect against pneumococcal colonisation.

In summary, current concepts consider polysaccharides as T-cell-independent antigens. However, in a recent in vitro study Stephen et al. [17] demonstrated that capsular polysaccharides of serotype 1 – even without conjugated protein – can be presented by dendritic cells to CD4⁺ cells. However, the meaning of these observations for man is not yet understood.

5. Advantages and disadvantages of the currently available pneumococcal vaccines

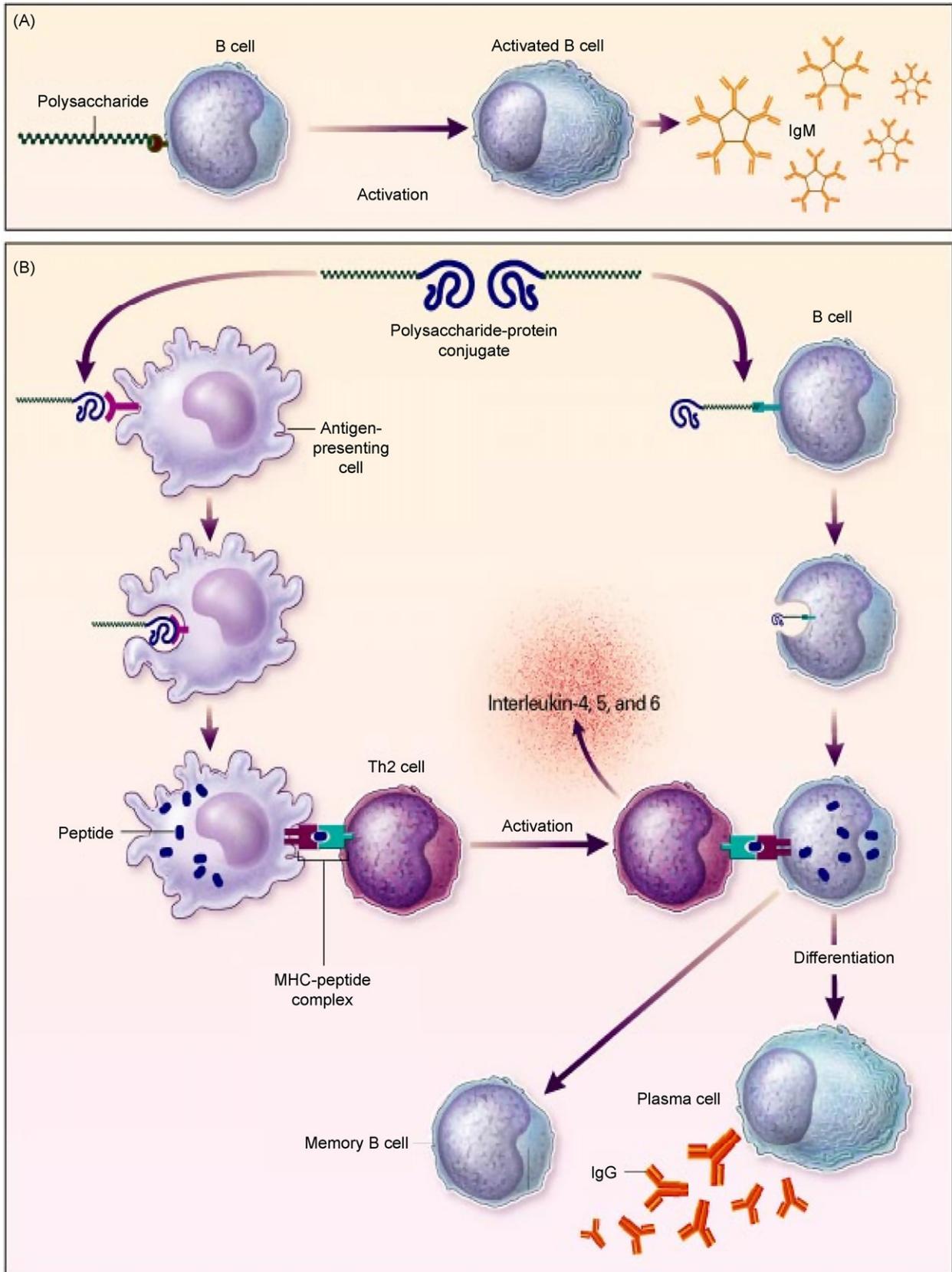
PCV-7 elicits mucosal immune responses in immunised hosts most probably due to induction of IgA antibodies. Mucosal immunity enables asymptomatic carriers to

eradicate colonising pneumococci of vaccine serotypes. Furthermore, PCV-7 is effective in preventing invasive disease progression of vaccine serotypes. A disadvantage of conjugate vaccines is their low coverage of pneumococcal serotypes, which, for example, would result in protection against just 50% of pneumococcal infections occurring in German adults [14]. In contrast to the aforementioned conjugate vaccines, polysaccharide vaccines do not induce mucosal immunity and thus do not affect carrier rates [18] or herd immunity. In addition, the occurrence of upper and lower respiratory tract infections cannot be prevented by a previous pneumococcal polysaccharide-based vaccination, although less severe disease courses have been reported. However, a major advantage of such a vaccine is the large number of included pneumococcal serotypes, leading in theory to a vaccine coverage of ca. 80% of adult pneumococcal infections [14].

6. Is vaccination with pneumococcal polysaccharide vaccines still useful?

Vaccination of adults with the 23-valent polysaccharide vaccine is clinically useful despite its relatively low and timely restricted efficacy. This becomes evident from a Spanish trial [19] where it was shown that out of 524 patients hospitalised with diagnosed pneumococcal pneumonia, the portion of patients (11%) who received the 23-valent pneumococcal vaccine within 5 years before hospitalisation indeed showed significantly less bacteraemia (15% vs. 35%) compared with non-vaccinees. Despite a less favourable prognosis due to higher mean age as well as a higher risk of pneumonia-induced death according to the Pneumonia Severity Index, vaccinees showed a quicker defervescence (1.7 days vs. 2.9 days) and could be released from the hospital faster (9.4 days vs. 11.3 days). Mortality of vaccinees was also much lower (1.6% vs. 6.1%) even if this difference did not reach statistical significance [20].

The benefit of pneumococcal polysaccharide vaccines for public health is also supported by one of the largest intervention studies ever performed [21]. Between 1995 and 1998, among all Stockholm residents >65 years of age ($n = 259\,627$), 100\,242 individuals were vaccinated against pneumococcal and influenza viral infections. Subsequently, hospitalisations occurring during December 1998 to May 1999 were recorded. Expectedly, reduced pneumonia- and influenza-associated diseases were observed in the immunised group. More specifically, overall mortality was lower by 57% (95% confidence interval 55–60%) in the vaccinee group compared with non-vaccinees (15.1 vs. 34.7 deaths per 1000 residents; $P < 0.0001$) [21]. Unfortunately, the applied study design does not allow discrimination between the effects mediated by pneumococcal vaccines compared with influenza vaccines because both vaccines were administered simultaneously.



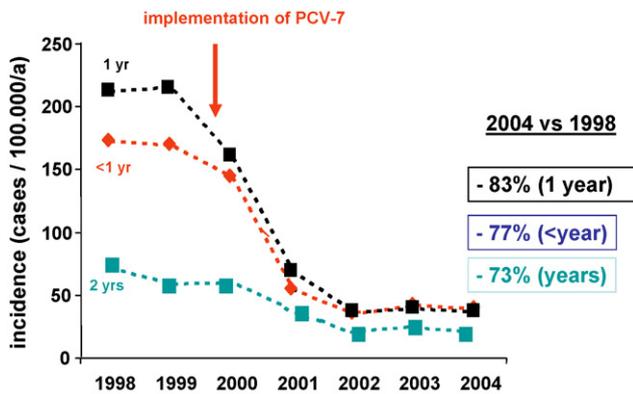


Fig. 3. Reduction of invasive pneumococcal diseases in the USA since introduction of the conjugate vaccine.

7. Efficacy of the pneumococcal conjugate vaccine

7.1. Direct effects

In the USA, the incidence of invasive pneumococcal diseases in 2004, i.e. after introduction of the vaccine, was reduced by 77% in children <1 year of age, by 83% in children at 1 year and by 73% in 2-year-old children (Fig. 3) [22]. These data demonstrate the rapid reduction of invasive pneumococcal diseases in the target population. Additional data also support a decrease in non-invasive pneumococcal infections such as acute otitis media (–20%) in the US population [23]. Moreover, in a large, randomised controlled trial, PCV-7 reduced the rate of vaccine type-associated acute otitis media by 57% [24], although the efficacy of the PCV-7 vaccine compared with the 23-valent polysaccharide vaccine to protect against acute otitis media was not demonstrated.

7.2. Herd immunity

Children are the main reservoir of *S. pneumoniae*, with colonisation rates of up to 50% [11]. Because the heptavalent conjugate vaccine leads to eradication of the corresponding serotypes, persons in contact with children should consequently benefit from the vaccination of children. The incidence of invasive pneumococcal diseases in the USA was reduced in 2004 compared with 1998 by 41% in the group aged 20–39 years (mostly representing the children's parents), by 20% in the group aged 40–64 years and by 31% in the group aged ≥ 65 years, largely representing the grandparents. The latter benefit strongly from children's vaccination

[22], as this generation exhibits the highest mortality with respect to invasive pneumococcal diseases [13].

Current data from the German Competence Network for Ambulant Acquired Pneumonia (CAPNETZ) also demonstrate that the risk of pneumococcal pneumonia increased proportionally with the number of children in the household [25]. Therefore, according to current concepts, vaccination with the heptavalent conjugate vaccine indeed can interrupt the typical infection chain from grandchildren to grandparents.

Herd immunity also exhibits benefits for subgroups with an increased risk of invasive pneumococcal diseases. Data from the USA show that the incidence of invasive pneumococcal diseases in non-vaccinated patients with human immunodeficiency virus (HIV) infection was reduced by 37% after the implementation of PCV-7 [26].

7.3. Reduction of resistance rate

As mentioned above, the worldwide increasing pneumococcal resistance to antibiotics, particularly to macrolides, is an issue of major concern. Recently, Stevens et al. [27] published results of a longitudinal observational study on invasive pneumococcal diseases in Georgia. In this study, the incidence of invasive pneumococcal disease remained unchanged before the introduction of vaccination (1994 and 1999, 30.2/100 000/year). Just 2 years after the introduction of vaccination the incidence of invasive pneumococcal disease was lowered by two-thirds (2002, 13.1/100 000/year). Remarkably, at the same time the proportion of macrolide-resistant species increased from 4.5% to 9.3% during the observation period of 1994 and 1999, and again decreased in 2002 to 2.9%.

Currently, fluoroquinolone-resistant pneumococcal strains do not play a role in most countries. The prevalence in Germany was 0.4% in 2003 [28], and in the USA fluoroquinolone resistance ranges between 1% and 3% [15]. Similar to macrolide resistance, fluoroquinolone resistance is clearly associated with treatment failure in discordant therapy [29,30]. In the USA, a longitudinal study investigated the proportion of fluoroquinolone-resistant invasive pneumococcal isolates between 1998 and 2002 [15]. Initially it showed an increased resistance for all serotypes until 2001, followed by a decrease in 2002. However, when non-vaccine serotypes were differentiated from vaccine serotypes, an overall reduction of fluoroquinolone

Fig. 2. Antibody responses to polysaccharide antigens and polysaccharide–protein conjugates. (A) A polysaccharide antigen binds to an immunoglobulin M (IgM) receptor on the surface of a B-cell in lymphoid tissues. Once B-cells are activated they produce and then secrete IgM antibody molecules. The individual Fab segments of the IgM molecule have only a moderate affinity, but because there are 10 such segments an IgM molecule has a high avidity. (B) In contrast, some polysaccharide–protein conjugates will be taken up by dendritic cells, which present peptides from the protein portion of the conjugate to type 2 helper T (Th2) cells. Other conjugate molecules bind to B-cells, which have IgM receptors specific for the carbohydrate moiety, and will undergo endocytosis and be processed by the B-cell; the resulting peptides will be expressed with class II MHC molecules on the surface of the B-cell. This complex is recognised by the activated Th2 cell, which then secretes interleukin (IL)-4, IL-5 and IL-6, which cause the B-cell to differentiate and express IgG molecules with polysaccharide specificity. These cells mature in the lymphoid follicles; only cells that express very high-affinity IgG molecules become plasma cells and secrete high-affinity IgG that binds strongly to the encapsulated bacteria and mediates opsonic activity and complement-mediated bactericidal activities (modified after [16]).

resistance was only based on reduction of vaccine serotypes. Moreover, a further increase in fluoroquinolone resistance was observed in serotypes not covered by the vaccine. This finding indicates a continued selection pressure because of high fluoroquinolone use.

Finally, a longitudinal analysis of the ABCs study data also demonstrates a reduction of invasive penicillin-resistant pneumococcal species after the introduction of vaccination [31].

8. Replacement: threat to vaccination success

Eradication of vaccine serotypes in asymptomatic carriers has created an ecological niche for non-vaccine serotypes ('replacement'). Data from the CDC (Table 1) show an almost complete reduction of invasive pneumococcal disease in children <2 years of age. This reduction is caused by the almost complete eradication of vaccine serotypes and, to a lesser extent, of vaccine-associated serotypes where a cross-immunity is induced by vaccination [22]. In contrast, diseases caused by non-vaccine serotypes at the same time increased by 45%.

Alaska natives exhibit a high risk of invasive pneumococcal disease, which can in part be attributed to crowding and poor socioeconomic status. Owing to a US government launched vaccination programme, current vaccination rates are high in this community and there is only minor migration, thus providing appropriate conditions for cohort studies. A recent study by Singleton et al. [32] documented a re-increase in invasive pneumococcal disease after introduction of the vaccine in this community in 2001. Between 2001–2003 and 2004–2006 there was an 82% increase in invasive disease in Alaskan native children ($P=0.02$). Since 2004, the rate of invasive pneumococcal disease caused by non-vaccine serotypes increased by 140% compared with the pre-PCV-7 implementation period (i.e. from 95/100,000 in 1995–2000 to 229/100,000 in 2004–2006; $P=0.001$). During the same period, a 96% decrease in vaccine serotype-mediated diseases was observed. Importantly, the non-vaccine serotype 19A was found to account for 28.3% of invasive pneumococcal diseases in this cohort [32], and an emergence of this pneumococcal serotype has also been reported in other populations [22,31].

Genetic analyses of pneumococcal isolates collected after the introduction of the vaccine demonstrate that the increases

in most non-vaccine serotypes is not due to a de novo emergence of new pneumococcal clones but rather to expansion of pre-existing clones of non-vaccine serotypes [33]. In contrast to other non-vaccine serotypes, the disturbing increase in resistant isolates of serotype 19A is only partially due to expansion of a pre-existing clone (sequence type (ST) 199) that was rare before the introduction of vaccination. Results of multilocus sequence analyses show that some 19A isolates collected in the post-PCV-7 implementation era are of different STs that were originally related to other serotypes (e.g. serotype 4) [34]. These isolates are called 'vaccine escape recombinants' and are probably the result of recombination events. Brueggemann et al. [35] have identified at least three recombinational events that lead to serotype 19A strains with different STs. They assume that there was a main event around 2003 when a fragment containing the capsular locus and two adjacent penicillin-binding proteins was transferred from a strain of ST199 and serotype 19A to a strain of ST695 and serotype 4. The resulting strain of ST695 and serotype 19A strain has been increasing rapidly since 2003 [35]. Thus, capsular switching is another mechanism that contributes to the rise of non-vaccine serotypes.

Apparently, no cross-immunity exists between serotype 19F, which is included in PCV-7, and serotype 19A. Furthermore, purified polysaccharides derived from pneumococcal strain 19F exhibit the least potent immunogenicity profile of all serotype-derived polysaccharides included in the vaccine. Recent data show that there is no difference between polysaccharide and conjugate vaccine-induced antibody titres to 19F [36]. The reason for this apparently weak immunogenicity of serogroup 19 is unknown.

In the long run, the increase in non-vaccine serotype-induced disease may pose a threat to the vaccination success that has been achieved. There is not only a re-increase in disease incidence but also a re-increase in antibiotic resistance. Currently available data indicate a clear shift of the serotypes in penicillin-resistant pneumococci. In 1999, most penicillin-resistant pneumococci belonged to serotypes 14, 9F, 23F, 19F and 6B. However, in 2005 the majority of penicillin-resistant pneumococci belonged to non-vaccine serotype 35B, and especially isolates of serotype 19A [31].

Different concepts are currently being worked out to improve the pneumococcal conjugate vaccine and to counteract replacement. Basically, this would be possible by the identification of a serotype-independent antigen. The suitability of pneumococcal membrane proteins as vaccine

Table 1

The reduction in incidence rates of invasive pneumococcal disease depends on the serotype [22]

Serotype	Cases/100 000 persons/year		Change (%) (1998–1999 vs. 2004)	95% CI
	1998–1999	2004		
Vaccine serotypes	160	3	–98	–99 to –97
Vaccine-associated serotypes (without 6A and 19A)	13	3	–79	–68 to –63
Non-vaccine serotypes	13	19	+45	+71 to +261
Serotype 19A	5	13	+148	+10 to +91

CI, confidence interval.

antigens is being examined. However, although they induce a sufficient vaccination response in animal experiments, not all of these antibodies against pneumococcal surface proteins are helpful in the fight against infection in vivo. A possible explanation for this discrepancy between antibody titres and protection is that some of the cell wall proteins lie under the capsule polysaccharides, which can prevent binding of the corresponding antibody [37]. However, most recent data show that immunisation of mice with intact purified pneumococcal proteins resulted in an antibody-independent, but strongly CD8⁺ T-cell-dependent, protection against pneumococcal colonisation [38].

Another possibility to prevent replacement by adaptation of the selection pressure is regular monitoring and new composition of vaccine serotypes. Currently, the introduction of a 13-valent conjugated pneumococcal vaccine is being worked on. It is already in Phase III of clinical development (<http://www.clinicaltrials.gov>). Approval of this vaccine is being sought for older adults who have until now been receiving the non-conjugated vaccine. For technical reasons, production of a conjugate vaccine that contains all or at least many serotypes is currently not possible.

9. Conjugate vaccine also for adults?

A current German study by de Roux et al. [36] shows that the presently available conjugate vaccine is also effective and safe in older people. This study demonstrated that PCV-7, in comparison with the polysaccharide vaccine, does not only induce a more effective antibody response but also fewer local reactions even after re-vaccination within 1 year. Re-vaccination, which is recommended for the polysaccharide vaccine after 5 years, frequently causes pronounced local reactions in patients. Furthermore, it also seems to induce a selective immune tolerance to capsular polysaccharides [39]. In contrast, re-vaccination with the conjugate vaccine induced antibody titres similar to those obtained subsequent to the initial vaccination [36].

10. Open questions

To date, it is unknown whether non-vaccine serotypes exhibit the same fitness and virulence as vaccine serotypes. The much higher frequency of vaccine serotypes in the pre-PCV-7 implementation era among colonising and pathogenic pneumococcal isolates suggests that these serotypes have advantages compared with non-vaccine serotypes. These advantages could hypothetically consist of faster replication, decreased immunogenicity, increased virulence, enhanced airborne spread, resistance to environmental factors or even direct inhibition of a competing pneumococcal strain in the natural habitat, i.e. the human nasopharynx. Unfortunately, there are no studies addressing these questions. At least the aforementioned ST695 serotype 19A has been isolated

mainly from patients with meningitis and bacteraemia, suggesting that there is no decrease in virulence [35].

If non-vaccine serotypes are less virulent and less fit compared with vaccine serotypes, the clinical spectrum, the clinical course and/or the spectrum of patients with pneumococcal diseases might change. This issue should be addressed in future epidemiological studies. However, the emergence of the 19A clonal complex demonstrates that non-vaccine serotypes are also able to spread easily even without the selective pressure induced by PCV-7.

11. Conclusions

The pneumococcal conjugate vaccine induces humoral/mucosal immunity and leads to eradication of the serotypes covered by the vaccine. Expansion of non-vaccine serotypes into the ecological niches (replacement) threatens the long-term efficacy of pneumococcal vaccination programmes. Hopefully, the 13-valent conjugate vaccines currently under development will help to overcome these concerns.

Acknowledgement

We thank Bernard Beall, PhD, Chief of the Streptococcus Laboratory, Centers for Disease Control & Prevention, Atlanta, USA for helpful discussions and critical reading.

Funding: Bayer, Pfizer, Wyeth, MSD, sanofi-aventis and Astra Zeneca.

Competing interests: M.W.P. has received lecture fees from Wyeth and MSD. T.W. has received lecture fees from Wyeth, MSD, GSK and sanofi-aventis, and is a member of the Advisory Boards of the aforementioned companies. H.L. participates in clinical studies and is an advisor to Wyeth.

Ethical approval: Not required.

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