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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
CD4 | CD8 | LCK

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SCIENCE AND SOCIETY

Maintaining protection against invasive bacteria with protein–polysaccharide conjugate vaccines

Andrew J. Pollard, Kirsten P. Perrett and Peter C. Beverley

Abstract | Polysaccharide-encapsulated organisms are the leading cause of bacterial meningitis and pneumonia in children. The use of protein–polysaccharide conjugate vaccines in developed countries over the past two decades has markedly decreased the burden of disease and mortality from these organisms through direct protection of the immunized and through herd immunity. In the next decade, the widespread use of conjugate vaccines in the developing world should prevent millions of deaths. In this Science and Society article, we describe how vaccine-induced immunity wanes rapidly after vaccination in early childhood and argue that strategies that sustain protection in the population must be considered.

The polysaccharide-encapsulated bacteria *Streptococcus pneumoniae* (pneumococcus), *Haemophilus influenzae* type b (Hib) and *Neisseria meningitidis* (meningococcus) are the leading causes of serious bacterial infections in young children, accounting for most of the cases of bacterial pneumonia and meningitis worldwide. Between 800,000 and 1 million children under 5 years of age die from pneumococcal disease annually[1], and Hib and meningococcus are thought to account for approximately 400,000 and 50,000 deaths, respectively, each year[2,3]. The huge global burden of disease and death caused by these bacteria comes despite the availability of highly effective vaccines; in the United Kingdom, the Hib vaccine was introduced into the infant immunization schedule in 1992, the serogroup C meningococcal (MenC) vaccine in 1999 and the pneumococcal vaccine in 2006. However, only 26% of children worldwide received a course of Hib vaccine in 2006 (see The Hib initiative website for statistics) and less than 10% received other conjugate vaccines. An all-party parliamentary group report on pneumococcal disease was launched in the House of Lords in the UK Parliament on 15 October 2008 (see the All-Party Parliamentary Group on Pneumococcal Disease Prevention in the Developing World report website), highlighting the importance that the UK Government has placed on the global disease burden that is caused by *S. pneumoniae*. In addition, the World Health Organization (WHO) has recommended the widespread introduction of Hib and pneumococcal vaccines, as well as the use of serogroup A meningococcal (MenA) vaccines in the meningitis belt of Africa (a vast area across sub-Saharan Africa that suffers cycles of epidemic meningococcal disease)[4]. In the next decade, these initiatives could change the picture of global child health.

In this Science and Society article, we consider the nature of the immune response to polysaccharide and protein–polysaccharide conjugate vaccines and discuss the components of short- and long-term protection against encapsulated bacteria after immunization. It is only now apparent that the persistence of immunity after infant immunization with conjugate vaccines is poor, and with the rapid global implementation of such vaccines, these observations have important implications for sustaining the benefits of these vaccines for the world's children.

Polysaccharide and conjugate vaccines

The polysaccharide capsules of *S. pneumoniae*, *H. influenzae* and *N. meningitidis* are virulence determinants that are composed of repeating saccharide units, the chemical nature of which defines the capsular type of the organism (BOX 1). For example, there are 91 different polysaccharides (serotypes) associated with pneumococci and 13 polysaccharides associated with meningococci (although only five serogroups of meningococcal polysaccharide — A, B, C, Y and W135 — commonly cause disease). Four pneumococcal polysaccharides were first used for the development of a vaccine in 1945 (REF. 5), and a vaccine containing 23 pneumococcal polysaccharides was developed in 1983 and is now in widespread use for the elderly population in many developed countries, including the United Kingdom.

Polysaccharides are T-cell-independent antigens, as clearly shown in studies examining immune responses to polysaccharides in mice[6], and they generally stimulate short-lived B-cell responses by cross-linking the B-cell receptor, which drives the differentiation of B cells to plasma cells to produce antibodies (FIG. 1a). New memory B cells are not produced in response to most polysaccharide vaccines[7]; instead, the terminal

Box 1 | **Polysaccharide and protein–polysaccharide conjugate vaccines**

***Haemophilus influenzae* type b vaccines**

Polysaccharide vaccines for *Haemophilus influenzae* type b (Hib) were first used in 1985 but were rapidly replaced in 1989 by protein–polysaccharide conjugate vaccines containing the Hib polysaccharide polyribosyl ribitol phosphate chemically conjugated to a protein carrier, such as diphtheria toxoid, tetanus toxoid or meningococcal outer membrane protein. These vaccines continue to be widely used either alone or in combination with other vaccine antigens for the prevention of Hib-mediated disease, mainly among preschool children.

Pneumococcal vaccines

A vaccine containing 14 different polysaccharides from *Streptococcus pneumoniae* was licensed in the United States in 1977 and was replaced by a 23-valent vaccine in 1983 for the prevention of pneumococcal disease in the elderly. This vaccine was first introduced in the United Kingdom in 2003 for universal immunization of adults aged over 65 years. A protein–polysaccharide conjugate pneumococcal vaccine containing seven serotypes was first used in the United States in 2000 (and in the United Kingdom in 2006). In this vaccine, the carrier protein is crossreacting material 197 (CRM_{197}; which contains a glycine to glutamic acid point mutation at position 52 in the A subunit of diphtheria toxoid). Two new conjugate vaccines are in development, one containing 10 polysaccharides (conjugated to protein D from *H. influenzae)* and another with 13 serotypes (conjugated to CRM_{197}).

Meningococcal vaccines

A quadrivalent meningococcal vaccine containing serogroup A, C, Y and W135 polysaccharides of *Neisseria meningitidis* was first licensed in the United States in 1981, and a bivalent A plus C vaccine is also available in some countries. The polysaccharide of serogroup A is *N*-acetyl mannosamine-1-phosphate, that of serogroup C is α2-9 *N*-acetyl neuraminic acid (NANA), that of serogroup Y is a co-polymer of NANA with glucose and that of serogroup W135 is a co-polymer of NANA with galactose. Serogroup C (MenC) conjugate vaccines were first used in the United Kingdom in 1999 (conjugated to either tetanus toxoid or CRM_{197}), and a quadrivalent A, C, Y and W135–diphtheria toxoid conjugate vaccine has been available in North America since 2005. Several new combination conjugate vaccines are in development, including A, C, Y and W135–CRM_{197} and A, C, Y and W135–tetanus toxoid.

differentiation of memory B cells to plasma cells depletes the memory B-cell pool, resulting in hyporesponsiveness to future vaccine doses. It is for this reason that we have argued that there is little justification for using these vaccines in young children[8,9]. Furthermore, disease burden caused by polysaccharide-encapsulated bacteria is highest in the first year of life, but plain polysaccharides are not generally immunogenic in infants[10], which limits their use as vaccines to prevent disease in children.

These limitations to the B-cell response hold true for most of the polysaccharides encapsulating the bacteria that cause severe infections in humans, including Hib, MenC and most pneumococcal polysaccharides, but there are some exceptions. For example, unlike MenC polysaccharides, and for unknown reasons, MenA polysaccharides are immunogenic from early infancy. In addition, in some studies[11], but not in others[12], MenA polysaccharides did not induce antibody hyporesponsiveness. Another exception are some zwitterionic polysaccharides (that is, ones that have both a positive and negative charge), such as the *Bacteroides fragilis* capsule[13] and serotype 1 pneumococcal polysaccharide[14], which can be presented in an MHC class II-dependent manner. Based on mouse studies[15], it has been suggested that marginal-zone B cells are involved in polysaccharide-induced immune responses[16], and maturation of the splenic marginal zone and an ability to respond to polysaccharides in humans both occur at about 18 months to 2 years of age[17]. However, direct evidence of this has not been obtained from human studies.

Chemical conjugation of the polysaccharide to a protein carrier — such as tetanus toxoid, diphtheria toxoid or crossreactive material 197 (CRM_{197}; which is a mutated diphtheria toxoid) — directs processing of the protein carrier by polysaccharide-specific B cells and presentation of the resulting peptides to carrier-peptide-specific T cells in association with MHC class II molecules (FIG. 1b). So, a conjugate polysaccharide vaccine induces a T-cell-dependent response from early infancy and induces an anamnestic (memory) response to a booster dose of the vaccine[7]. The main B-cell subset that is involved in the immune response to conjugate vaccines in humans is unknown; however, the characteristics of the immune response that is induced by conjugate vaccines (such as the induction of immunological memory and avidity maturation) strongly indicate that follicular B cells are probably activated and form germinal centres. Unlike the response to plain polysaccharide vaccines described above, these responses to conjugate vaccines might provide long-term immunity through the production of new memory B cells.

The immunogenicity of different conjugate vaccines varies as a result of differences in the chemical nature of the polysaccharide (such as the length of the saccharide chain)[18], the amount of unconjugated polysaccharide in the vaccine and the nature of the carrier protein[19]. For example, the Hib–outer membrane protein (Hib–OMP) conjugate vaccine is markedly more immunogenic than Hib–CRM_{197} (REF. 20).

Definition of the nature of the primary response to the capsular polysaccharides of these bacteria is further complicated by prior exposure to the organism. Hib, pneumococcus and meningococcus are organisms that colonize the human nasopharynx. Indeed, 60% of healthy children will have been colonized with one or more capsular serotypes of pneumococcus by 1 year of age[21] and 4.2% of school-age children in the United Kingdom today are colonized with Hib despite immunization[22]. Moreover, high rates of meningococcal carriage have been documented among unimmunized populations of adolescents and young adults[23]. It remains unclear whether carriage of these bacteria induces a T-cell-dependent or T-cell-independent immune response. Therefore, immunization might induce a mixture of primary responses in those individuals who have never been colonized and secondary immune responses in individuals who have been affected by prior colonization.

The initial immune response

Most children in the world receive their primary immunizations in the Expanded Programme on Immunization (EPI) schedule at 6, 10 and 14 weeks of age (EPI was launched in 1974 by the WHO at a time when only 5% of the world's children received immunizations) or in the North American schedule at 2, 4 and 6 months of age. In Europe, immunization programmes are diverse, but most are based on either a North American, UK (2, 3 and 4 months) or Scandinavian (3 and 5 months) schedule. From an immunological perspective, vaccination in early infancy is not ideal, as the immune response at this age is generally of low magnitude and does not persist well (see below), which is in contrast to the more robust and sustained immune responses that can be induced in older children (see discussion below concerning MenC responses at

different ages)[24]. However, immunization programmes are largely directed at an early age because infectious diseases cause the highest morbidity and mortality rates in early life.

The immunogenicity of many vaccines in infancy is increased when immunization starts later (less interference from maternal antibody)[25,26], when more doses are given and when there is a greater length of time between doses[26–29]. So, schedules that start early and have only 1 month between doses (such as the EPI and UK schedules) generally generate lower immune responses to these vaccines at the end of the primary immunization course than schedules that start later, such as the North American schedule. It is clear that the size of the response must be weighed against protection from an early age, and this might be a particular problem for conjugate vaccines, the accepted protective threshold of which correlates with high levels of antibody. Despite these concerns, a primary UK schedule of two or three doses of the conjugate vaccines for Hib, pneumococcus and MenC generates antibody titres that are above the protective threshold, as measured at 1 month after the last immunization, in most infants[30,31]. Importantly, similar or greater responses are seen with just a single dose in children older than 1 year of age.

In the UK schedule, seven different vaccines are given (each in two or three doses) during the first year of life (see the WHO Vaccine Preventable Diseases Monitoring System website), and combination vaccines have been developed to decrease the needle burden for the infant and improve adherence to the immunization schedule[32]. Various examples of interference between antigens in combination vaccines have been described. For example, a decrease in the immunogenicity of the Hib conjugate vaccine occurs when it is incorporated into combination vaccines, particularly with *Bordetella pertussis* antigens, and this seems to have been one of the factors that contributed to a rise in Hib disease in the early part of the current decade in the United Kingdom[33,34] (FIG. 2). In another study, the use of a combination pneumococcal–MenC conjugate vaccine resulted in decreased immunogenicity of the MenC component of the vaccine and decreased responses to the concomitantly administered Hib and diphtheria vaccines[35]. These observations highlight the importance of careful evaluation of changes in immunization schedules and clearly show that immunization in early infancy does not guarantee protection, even in the short term. Decisions about immunization schedules made now could have dramatic effects on population protection in the coming decades.

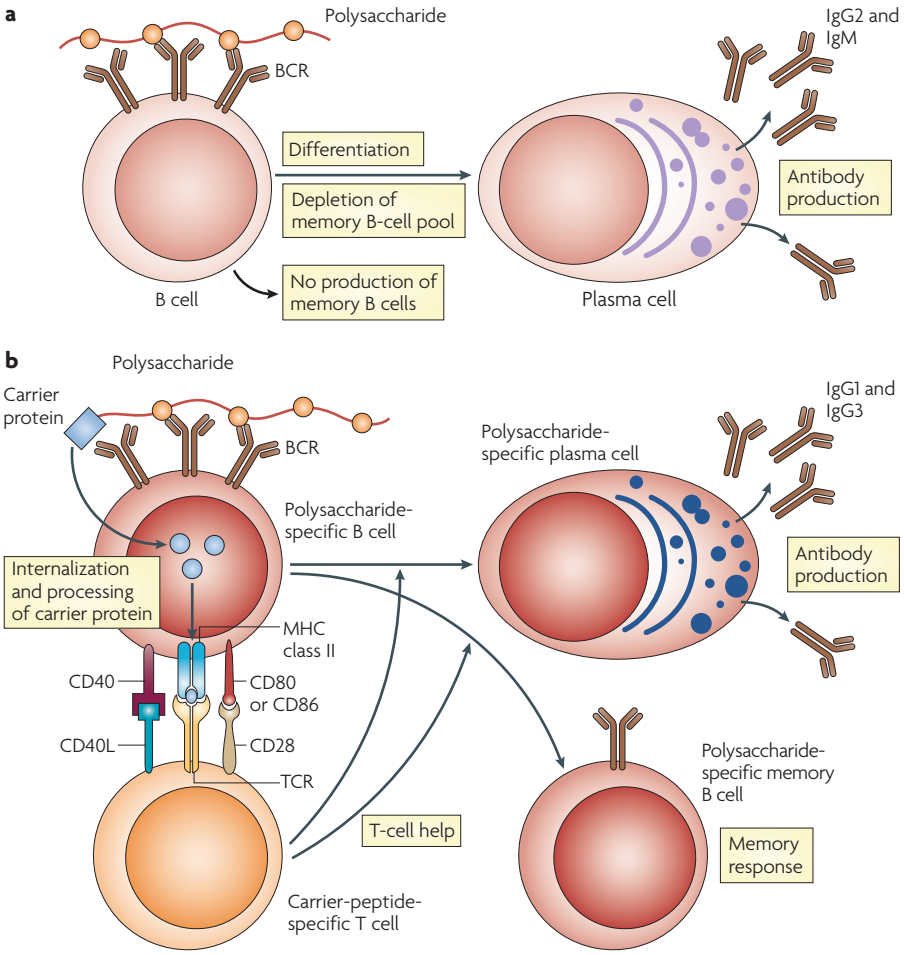

Figure 1 | **The immune response to polysaccharide and protein–polysaccharide conjugate vaccines. a** | Polysaccharides from the encapsulated bacteria that cause disease in early childhood stimulate B cells by cross-linking the B-cell receptor (BCR) and drive the production of immunoglobulins. This process results in a lack of production of new memory B cells and a depletion of the memory B-cell pool, such that subsequent immune responses are decreased. **b** | The carrier protein from protein–polysaccharide conjugate vaccines is processed by the polysaccharide-specific B cell, and peptides are presented to carrier-peptide-specific T cells, resulting in T-cell help for the production of both plasma cells and memory B cells. CD40L, CD40 ligand; TCR, T-cell receptor.

The introduction of the Hib vaccine into immunization schedules in wealthy countries 15–20 years ago resulted in a decrease in invasive Hib infections (FIG. 2) and highlighted the potential for control of diseases that are caused by other polysaccharide-encapsulated bacteria[36]. It was assumed that direct protection of the vaccinated individual by a capsule-specific antibody would be the main mechanism of protection, as indicated by studies of naturally acquired antibody and passive protection in agammaglubulinaemic children[37,38]. However, following use of the Hib conjugate vaccine (with various different protein carriers), the large reduction in disease among unvaccinated members of the population showed that the effectiveness of these vaccines is increased by the combination of direct protection of the individual through the production of serum antibodies and protection of the wider population through herd immunity.

Herd immunity occurs if the transmitters (those individuals or cohorts in the population who have a high rate of colonization and transmit the organism to susceptible contacts) are immunized, so that they no longer acquire the organism themselves and cannot drive its transmission in the population. After the introduction of a pneumococcal conjugate vaccine for young children in the United States, a decrease in the incidence of disease among unvaccinated adults was observed, showing that young children were responsible for driving infection with the vaccine serotypes in the wider population[39–41].

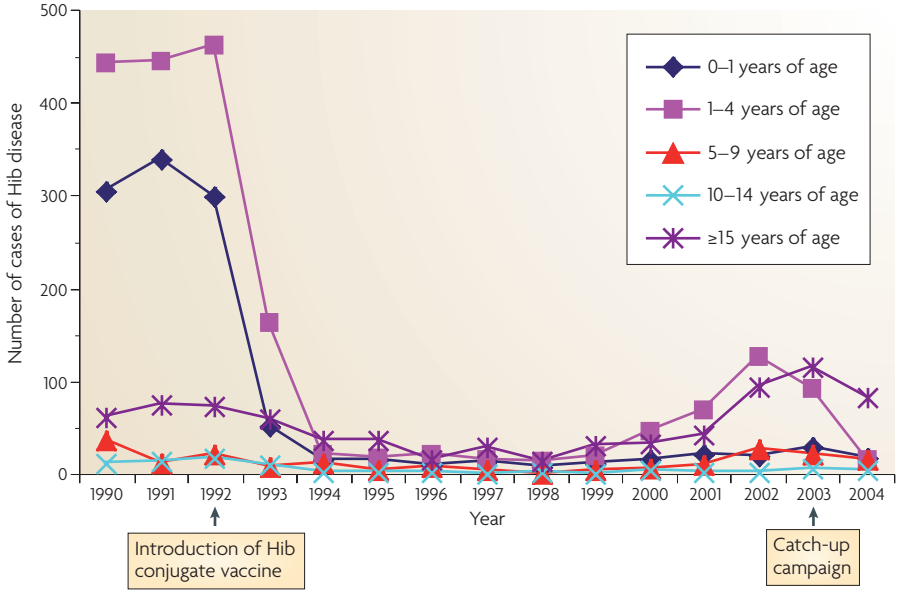

Figure 2 | **Epidemiology of *Haemophilus influenzae* type b disease in England and Wales from 1990 to 2004 by age group.** (adapted from publicly available data, see the Health Protection Agency website). The *Haemophilus influenzae* type b (Hib) protein–polysaccharide conjugate vaccine was introduced in the United Kingdom in 1992 and led to a rapid decrease in the number of cases of disease in all age groups. An upsurge in disease cases from 1999 probably resulted from the decreased immunogenicity of Hib vaccine when included in new combination vaccines. Alternatively, it could have resulted from interference from other concomitant vaccines, perhaps in combination with decreased natural boosting (as a result of decreased disease carriage). Disease has now been controlled again with a catch-up campaign that was implemented in 2003, the addition of a booster dose for 12-month-old children that was added in 2006 and an additional dose for preschool children that was introduced in 2007 to catch any child who did not receive a booster (not shown).

In the United Kingdom, the introduction of MenC conjugate vaccine in 1999 included a massive catch-up campaign in which all individuals under the age of 19 (later extended to 24) years were immunized with the MenC conjugate vaccine (three different vaccines were used conjugated to either tetanus toxoid or CRM_{197}). The incidence of MenC disease fell among both the immunized and unimmunized sections of the population[42] from almost 1,000 cases in 1999 to only 28 cases in 2006, whereas the rates of MenB, which is not covered by the vaccine, remained constant. The highest rates of meningococcal carriage are observed among adolescents and young adults, and vaccine efficacy against carriage of MenC in this age group was 75% (REF. 43). As there is almost no carriage in the first decade of life, even before the vaccine was introduced (possibly as a result of competition by other colonizing bacteria at this age), these data indicate that adolescents might drive the transmission of MenC in the wider population. Indeed, in the Netherlands, where both an immunization programme of children of 14 months of age and a catch-up campaign were introduced, a similar effect of herd immunity was observed[44]. So, the huge impact of the MenC vaccine campaign for the wider population in the short term seemed to depend on immunity among these vaccinated teenagers[43].

Long-term protection

It is probable that long-term protection after immunization against encapsulated bacteria depends on the maintenance of three mechanisms: the persistence of functional antibodies, the maintenance of immunological memory and herd immunity (BOX 2).

Antibody persistence. Antibody titres induced by infant immunization, even after three doses of MenC vaccine, do not persist well; antibody levels fall below the protective threshold in 50% of infants by 1 year of age, and only as few as 12% of vaccinated infants have persistent seroprotection by 4 years of age[45] (FIG. 3). Of note, protection from infection after infant immunization decreases along a similar trajectory to the antibody titres, with little evidence of ongoing protection beyond 1 year following infant immunization with the MenC vaccine[42]. Antibody persistence is better when the first dose is given after 12 months of age, but vaccination does not seem to induce sustained levels of protective antibody in a high proportion of children until much later in childhood[46] (FIG. 3). Protection is measured using a functional assay that tests the ability of antibody-containing serum to kill meningococci in the presence of exogenous complement. Although there is some compelling evidence to suggest that the levels of positive protection in the population in this assay correlate well with vaccine effectiveness[47], it is possible that the assay underestimates the degree of protection that is afforded by the vaccine in any one individual, as has been suggested by passive protection studies using human sera in animal models[48]. The rapid waning of protection in early childhood after infant immunization is also documented for Hib vaccines[49]. At least in young children, antibody levels might fall too rapidly after a primary immunization course with a protein–polysaccharide conjugate vaccine to provide long-term protection against this disease.

B-cell memory. The second mechanism of protection is immunological memory, which is generally defined as an anamnestic response to a booster dose of a vaccine. B-cell memory responses have been observed even among those who did not make a detectable primary response to the vaccine[49]. In addition, B-cell memory theoretically could provide long-term protection in those individuals for whom antibody levels have waned below the protective threshold. Unfortunately, in susceptible individuals the encapsulated bacteria are known to invade rapidly after acquisition, often within a few days. In this case, the memory B-cell response, which takes 4 or more days to become established after re-encounter with antigen, is too slow[50,51], except in those cases where there is a prolonged incubation period (FIG. 4). For example, in children who suffer from Hib disease despite prior vaccination (vaccine failures), the immune response to Hib infection is greater than the response in an unvaccinated individual who suffers from the disease. These children mount a memory immune response to infection but still suffer from Hib disease, which supports our view that the presence of immunological memory does not guarantee protection[52]. These observations strongly suggest that B-cell memory might not be as important as long-lasting antibodies for long-term protection against a rapidly invasive pathogen.

Box 2 | **Immunological memory and persistence of antibody**

Short-term immune responses

Short-term immune responses following immunization are usually described by the antibody levels in the serum at 28–42 days after the last dose of the vaccine. The short-term immune response that occurs 1 month after vaccination is presumably mediated by the production of antibody by a mixture of short- and long-lived plasma cells that reside in the bone marrow and other lymphoid tissues.

The short-term effectiveness of vaccines is a combination of the direct protection of the individual through the production of antibodies following immunization and through herd immunity, which decreases the chance of transmission in the population.

Long-term immune responses

Long-term immune responses are usually described by two factors:

The persistence of a protective level of serum antibody months or years after immunization. The persistence of antibody is presumably mediated by long-lived antibody-secreting cells in the bone marrow and/or turnover of memory B cells (to produce new plasma cells) as a result of direct or bystander stimulation. Maintaining serum antibody levels might be particularly important for preventing diseases that have a short incubation period (less than 4 days, such as meningococcal disease), for which the memory B-cell responses described below are too slow.

The magnitude of the antibody response to a booster dose of vaccine. The response to a booster dose of vaccine is more rapid and of greater magnitude than a primary response as a result of the availability of pre-existing memory B cells during booster immunization. This has therefore been the most commonly used means of demonstrating the presence of B-cell memory in vaccinology. The determinants of the number of memory B cells that are present at a given time-point presumably include: the magnitude of the initial B-cell response following immunization; the time since immunization; and the balance between proliferation and cell death in the memory B-cell pool. These memory responses are presumably important in controlling infections with a long incubation period (more than the 4 days it takes for a memory response to develop), such as rabies and hepatitis B.

Vaccine effectiveness. The longer-term effectiveness of a vaccine is determined by a combination of persistence of antibody, immunological memory (here defined as the ability to make a secondary response to re-exposure to the antigen) and the persistence of herd immunity, which reduces transmission of the organism in the population.

Herd immunity. The third mechanism by which population protection is preserved is through herd immunity. The maintenance of herd immunity over years or decades depends on the sustained ability of the immune response to prevent acquisition of the organism by the individuals or cohorts of the population who are the main transmitters, and this is presumably in turn mediated by mucosal antibodies (or serum antibodies that leak onto the mucosa). Also, the circulation of MenC, as shown in carriage studies[43], was decreased to low levels by the initial vaccine campaign, and modelling studies predict that this decrease in transmission will have a sustained effect on disease control, such that even in the absence of immunity it would take a long time for circulation to increase and MenC disease to return[53]. It is now more than 9 years since the MenC vaccine was introduced in the United Kingdom, and there is good evidence that antibody levels have waned among much of the child population (FIG. 3). Therefore, it might be expected that herd immunity would also decline. However, the rates of MenC disease have actually continued to fall each year, and in the past 12 months there have been no recorded deaths from this disease[54].

As can be seen from FIG. 3, individuals over 16 years of age have almost all sustained high levels of protection from MenC (measured using the serological correlate of protection in a bactericidal assay) nearly a decade after the immunization campaign[46], presumably as a one-off effect of vaccination of adolescents at the start of the campaign. However, it seems unlikely that adolescents are the sole transmitters of MenC disease, as they do not have the close contact with young children that is necessary for transmission of the disease to this age group. Notably, the oldest members of the 1999 MenC vaccination campaign cohort are now around 30–35 years of age; therefore, most new parents in the United Kingdom are likely to be immune to MenC, which would prevent transmission of this disease to young children. Based on these observations, we propose that the current population immunity against MenC in the United Kingdom depends largely on direct protection of young adults by their own antibodies and on the consequent herd immunity that results from the block of disease transmission between the current cohort of teenagers and from immune parents to susceptible young children.

As assessed at the end of 2008, most children aged 3–14 years are susceptible to MenC disease based on the specific bactericidal antibody titres in this cohort (FIG. 3). However, as described above, these children are presumably protected from MenC through herd immunity, as the incidence of disease is currently low. However, a decade from now, these children will be between 13 and 24 years of age, so the model of population protection and transmission of disease described above might predict the return of meningococcal disease when these children become adolescents and young adults (who are the transmitters). If these arguments are correct, sustained population immunity against MenC disease might be best achieved over future decades by the addition of an adolescent booster of the MenC vaccine.

In summary, the persistence of bactericidal antibody seems to be the determinant of individual protection against MenC, and herd immunity is the most important mechanism of population protection, which is achieved by antibody persistence in the transmitters. B-cell memory might also have a role among those individuals who have many days of colonization before invasion (so that there is time for a memory response to protect them), but this mechanism of protection might be less important. Perhaps the key to long-term population protection will be sustaining serum antibody levels in the transmitters above a protective threshold.

Maintaining serum antibody levels

We have discussed that maintenance of serum antibody levels above a protective threshold is the crucial determinant of individual immunity after immunization with protein–polysaccharide conjugate vaccines, as an individual can succumb to disease more rapidly than immunological memory can resurrect adequate levels of protective antibody. There are only limited data on the mechanisms that are responsible for antibody persistence in humans and to explain why immunization in infancy does not lead to sustained immune responses. However, it is probable that this relates, at least in part, to limited survival signals for plasma cells in the bone marrow during infancy[55].

Although many children develop poor immune responses during infancy, those with higher initial B-cell responses (measured as a greater number of memory B cells in peripheral blood following the primary course of immunization in infancy) have greater antibody persistence at 1 year of age[56]. This indicates that the magnitude of the initial germinal centre reaction

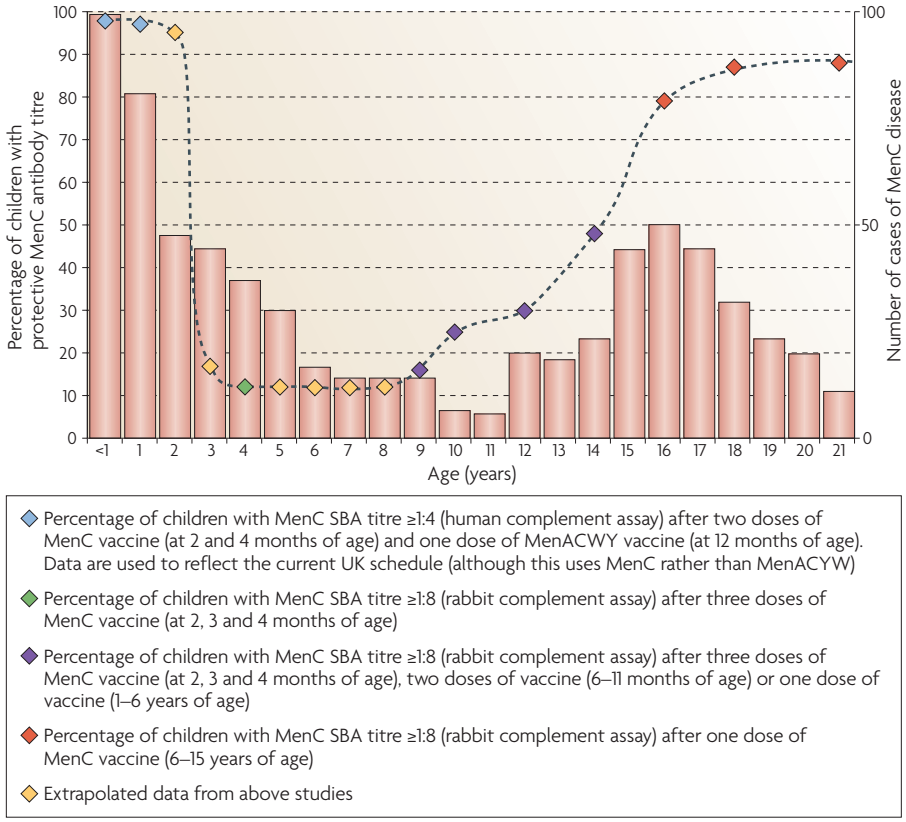

Figure 3 | **Antibody persistence following serogroup C meningococcal immunization.** The bars show the age distribution of serogroup C meningococcal (MenC) cases in England and Wales from April 1998 to March 1999 before the implementation of the MenC vaccine (adapted from data provided by the Health Protection Agency). The dashed line shows the percentage of vaccinated individuals in the population in 2008 who had a MenC-specific serum bactericidal antibody (SBA) titre above the protective threshold of 1:8. A cohort of children aged 2–14 years have low levels of protective antibody and could remain susceptible to disease as they reach their mid-teens, an age at which disease rates were high and outbreaks were common before the introduction of the MenC vaccine[46,62,63] (K.P.P. and A.J.P., unpublished observations). Data points along the dashed line were obtained from REFS 46,62,63 and K.P.P. and A.J.P., unpublished observations. The unpublished data are extrapolated from a study funded by the Oxford Partnership Comprehensive National Institute for Health Research (NIHR) Biomedical Research Centre Programme and the Thames Valley NIHR Comprehensive Local Clinical Research Network, with additional support from GlaxoSmithKline Vaccines and the European Society for Paediatric Infectious Disease.

during infant vaccination might determine longer-term protection from the disease. Strategies to enhance the initial B-cell response to immunization using more effective adjuvants or by adjusting immunization schedules could improve the persistence of antibody. It is noteworthy that the memory B-cell response to conjugate vaccines is still smaller in unprimed 12-month-old infants than in young adults[24] and that young adults seem to have more sustained antibody responses[46]. A key issue in sustaining antibody levels, therefore, is the age at which vaccination is administered.

In addition to appropriate signals for long-lived plasma cells and bystander turnover of memory B cells[57], other factors could also have a role in sustaining serum antibody levels (FIG. 4). As discussed above, differences in the nature of the vaccine might be important in determining the magnitude, quality and persistence of the immune response. For example, control of Hib disease was achieved among Alaskan natives after the vaccine was introduced in 1991. However, following a switch from a vaccine that contained OMP as the carrier protein to one that used CRM_{197}, the incidence of disease rose from 19.8 to 91.1 cases per 100,000 individuals per year in children of less than 5 years of age from 1996, compromising population control of the disease[58].

Natural boosting of immunity through colonization with bacteria or exposure to crossreacting antigens might be an important mechanism for maintaining antibody levels. For example, we have recently shown that the nasopharynx of school-age children (who only received three infant doses of Hib and no booster) in Oxfordshire, United Kingdom, is an important reservoir of Hib[22], and this could facilitate ongoing transmission of the organism to susceptible individuals. It is also possible that natural boosting from exposure to the organism can help to maintain immunity after immunization in early life. If natural boosting is important in the long term, increasing population immunity with additional booster doses of a vaccine during childhood could eventually decrease the circulation of Hib and result in lower antibody levels in older children and adults owing to decreased natural boosting. The polysaccharide capsules of many important pathogenic bacteria are also crossreactive with those of other colonizing organisms — for example, *Escherichia coli* K100 capsule crossreacts with the type b capsule of Hib[59] — and exposure to these crossreacting antigens could also assist in maintaining immunity.

The most obvious approach to overcoming poorly sustained immune responses is the boosting of immunity among those who are especially susceptible in the population, particularly preschool children. In 2006, the UK Department of Health recognized that poor persistence of antibody titres in infants was associated with waning of the effectiveness of the Hib and MenC vaccines beyond 1 year following infant immunization. Consequently, a booster dose of a combination Hib–MenC vaccine was added to the schedule at 1 year of age (see the WHO Vaccine Preventable Diseases Monitoring System website). Data on the persistence of antibody titres after a 12-month booster of these vaccines are limited, and further observation is required to determine whether a booster of the MenC vaccine at 12 months will maintain direct protection in the population through to the next period of susceptibility in the second decade of life. Data from a study of the Hib conjugate vaccine at various different ages provides some reassurance by indicating that boosters of this vaccine in the second year of life should maintain protection against Hib disease for at least the next 5 years[60].

An alternative intervention to protect society from the diseases these bacteria cause is the provision of booster doses of vaccine to prevent their transmission by aiming at the transmitters (rather than targeting of those who are susceptible). In the case of the MenC vaccine, the target groups are adolescents and young adults; one immunization strategy therefore could be to

concentrate on immunization in the second decade of life, during which rates of carriage are high and immune responses are more robust and sustained.

Implications

The poor persistence of antibody following immunization in infancy is a problem that has been highlighted by the use of protein–polysaccharide conjugate vaccines, as protection against the serious diseases that the polysaccharide-encapsulated bacteria cause seems to depend on high levels of antibody. These observations emphasize the role of careful disease and serological surveillance in the decades after the introduction of a new vaccine to detect unanticipated effects of the immunization programme on the dynamics of immunity, carriage and disease within the population.

Tetanus vaccine was first deployed in the 1930s, and it is now well established that at least five doses of this vaccine should be administered to provide long-term protection. The most obvious approach to provide more sustained protection with conjugate vaccines is to rediscover this principle and give more doses during childhood. For MenC vaccine, a schedule of two or three doses in the first 6 months of life, followed by boosters at 12 months, 3–5 years and in adolescence, would probably provide excellent protection in the first decade of life and, given the sustained response to MenC vaccine noted after adolescent boosting, for many years beyond. However, the herd immune effect that was noted after the MenC vaccination catch-up campaign in various countries indicates that an alternative approach is possible. In this case, fewer doses would be given in infancy so that population protection would rely on reduced transmission as a result of improved teenage and adult immunity. With herd immunity, this could be achieved by dropping the infant doses from the immunization schedule indicated above or even by offering a highly immunogenic two-dose schedule in adolescence alone, perhaps alongside the newly introduced cervical cancer vaccine against human papilloma virus.

The situation is not the same for the Hib or the pneumococcal conjugate vaccines, as the transmitters are mainly young children and not teenagers and young adults (as is the case for MenC). The current immunization strategy should, and does, focus on the induction of high levels of antibody in children under 5 years of age, which is best achieved by infant immunization with boosting in the second year of life. There is a considerable burden of pneumococcal disease in older adults, and boosting of pneumococcal responses throughout life might be required to provide wider disease control, particularly with the introduction of new vaccines in the next 12 months that cover a broader range of pneumococcal serotypes, including those that are especially prevalent in adult disease.

So, the solution to maintaining immunity might be as simple as immunizing the cohort that mediates the herd effect or the strategic use of booster doses of vaccine through periods of renewed susceptibility, as determined by waning levels of antibody. Alternatively, a better understanding of the mechanisms that contribute to poor immune responses in infants and of the mechanisms by which adjuvants and vaccine formulations potentiate immune responses, might allow for the development of second generation conjugate vaccines that can generate larger and more long lasting antibody responses in infants.

Over the next decade, there will be a huge deployment of conjugate vaccines against Hib and pneumococcus in low-income countries, which could save millions of lives in the second decade of this century. However, we predict that waning population immunity will continue to challenge our ability to sustain protection of the world's children in the years ahead. Therefore, consideration of booster doses of pneumococcal and Hib vaccines should be a priority.

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FURTHER INFORMATION

The Oxford Vaccine Group:
http://www.paediatrics.ox.ac.uk/ovg/
The Hib initiative:
http://www.hibaction.org/aboutdisease.php
WHO Vaccine Preventable Diseases Monitoring System:
http://www.who.int/vaccines/globalsummary/immunization/ScheduleSelect.cfm
All-Party Parliamentary Group on Pneumococcal Disease Prevention in the Developing World report:
http://www.appg-preventpneumo.org.uk/report.cfm
Health Protection Agency: http://www.hpa.org.uk

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