Combined schedules of pneumococcal conjugate and polysaccharide vaccines: is hyporesponsiveness an issue?

Katherine L O'Brien, Michael Hochman, David Goldblatt

Streptococcus pneumoniae is a major cause of morbidity and mortality in children less than 5 years of age. Prevention of pneumococcal disease and death in children in the developing world through vaccination with recently developed, highly efficacious pneumococcal conjugate vaccines (PCVs) is now possible. Schedules combining PCV with 23-valent pneumococcal polysaccharide vaccine (PPV23) have been studied and proposed as a means to expand disease protection against serotypes not included in the PCVs. Studies of group A and C meningococcal polysaccharide vaccine and repeated doses of PPV23 in adults and children have shown that a state of immune tolerance, or hyporesponsiveness, can develop to repeated polysaccharide vaccine antigen exposures. In this Review, we describe the evidence for and against this hyporesponsiveness and explore the possible mechanisms for such an occurrence.

Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA (K L O'Brien MD); **Department of Medicine, The Cambridge Hospital, Cambridge, MA, USA** (M Hochman MD); **and Immunobiology Unit, UCL Institute of Child Health, University College London, London, UK** (Prof D Goldblatt MBChB)

Correspondence to:
Dr Katherine L O'Brien, Johns Hopkins Bloomberg School of Public Health, 621 N Washington St, Baltimore, MD 21205, USA.
Tel +1 410 955 6931;
fax +1 208 988 0008;
klobrien@jhsph.edu

Introduction

Vaccination of infants and young children against pneumococcal disease has become a global health priority (figure). WHO estimates that every year up to 1 million children under 5 years of age die from pneumococcal disease, making it the most important cause of vaccine-preventable deaths in this age-group. An increase in the awareness of the pneumococcal disease burden, through improvements in laboratory and clinical surveillance methods, has coincided with the availability of highly efficacious pneumococcal polysaccharide-protein conjugate vaccines (PCV). Pneumococcal polysaccharides are T-cell independent antigens that are poorly immunogenic for important pneumococcal serotypes in infancy. The conjugation of pneumococcal capsular polysaccharide to carrier proteins results in a T-cell dependent immune response, characterised by increased antibody concentrations in infants, induction of memory cells, and a booster response upon subsequent antigenic exposures.[1–4] This same concept led to the development of conjugate vaccines for *Haemophilus influenzae* type b (Hib); widespread use of these vaccines led to a more than 90% decline in invasive Hib disease in children in the USA[5] and in other countries where the vaccines have been implemented.

In February, 2000, the first PCV, PnCRM7 (Prevnar, Wyeth Vaccines, Pearl River, NY, USA), was licensed in the USA and recommended for routine use in children younger than 2 years of age.[6,7] The vaccine contains polysaccharides or oligosaccharides to seven pneumococcal serotypes (4, 6B, 9V, 14, 18C, 19F, 23F), each conjugated to non-toxic diphtheria toxin material, CRM$_{197}$. Four large clinical trials of the seven-valent or closely related nine-valent PCV in the USA, South Africa, and The Gambia have reported vaccine efficacy between 77% and 97% for serious invasive pneumococcal disease caused by vaccine serotypes.[8–11] PCV efficacy against radiologically confirmed pneumonia has ranged from 19% to 37%.[9–12] The Gambian trial was able to assess the effect of PCV on mortality; there was a 16% reduction in all-cause mortality in children 3–29 months of age who received PCV compared with those who had not received the vaccine.[11] Since these trials were done with vaccines against only seven or nine pneumococcal serotypes, there are opportunities for an even greater vaccine effect with the eventual licensing and use of higher valency PCVs.

With the opportunity for pneumococcal disease prevention in the developing world on the near horizon, there is increasing attention on optimising vaccine schedules, introduction strategies (ie, catch-up or no catch-up), and products or combinations of products for maximum public-health impact. Different vaccine regimens, such as reducing the number of PCV doses or combining sequential doses of PCV and pneumococcal polysaccharide vaccine (PPV), are being considered by some decision makers. Combined PCV/PPV regimens

Figure: Ethiopian infant receiving routine Expanded Program on Immunization vaccination

have the theoretical possibility of broadening serotype coverage and they might also reduce the cost of vaccination. Pneumococcal vaccine strategy decisions should consider the proportionate and absolute serotype coverage, optimum immunological protection in vaccinated individuals, and the immunological pressure in the community.

The 23-valent pneumococcal polysaccharide vaccine (PPV23), licensed for use in adults and children more than 2 years of age in the USA since 1983 and in several other countries since then, was developed for the prevention of adult invasive pneumococcal disease and pneumonia. One dose contains 25 µg of each of the 23 serotypes; the dose was selected for the preceding PPV14 product following dose-ranging studies of 10, 25, and 50 µg per serotype.[13] PPV23 use has been limited in infants and young children because the T-cell independent immune response induced by PPV23 is poorly developed in young children. However, an evaluation of PPV efficacy in infants and young children in Papua New Guinea, a

country with one of the highest documented rates of childhood pneumococcal disease, was done in the early and mid-1980s.[14] In this study, PPV14 or PPV23 was given to children 6–59 months of age; children who were 6–17 months of age at vaccination received a second dose 12 months after their first dose. A 50% reduction in acute lower respiratory tract mortality was reported in children vaccinated before 2 years of age. Mortality from all-causes was 19% less in the vaccinated group.[14]

Other studies have failed to show efficacy of PPV against invasive disease or against otitis media in children.[15,16] On the basis of these and other findings, PPV23 has not been recommended for routine use in children younger than 2 years of age.

With the registration of PnCRM7, some health authorities have considered or recommended a combined schedule of PCV/PPV for some populations (eg, Australian aboriginal populations, some Native American populations, children with HIV or sickle-cell disease). A systematic review of the evidence on which such programmatic decisions could be

	Country	Age at vaccination		Number of patients		PCV product	Summary of findings
		Primary schedule	Booster dose	PPV23 boost	PCV boost		
Nurkka et al (2004a)[17]	Finland	2, 4, 6 months	12 months	51	51	11-valent Pnc-PD	PPV23 boost induced a greater response than PCV boost for all 11 serotypes
Kilpi et al (2003)[18]	Finland	2, 4, 6 months	15 months	187	648	Seven-valent PncOMPC	PPV23 boost induced a greater response than PCV boost for all seven serotypes
Ahman et al (1998)[4]	Finland	2, 4, 6 months	14 months	37	38	Four-valent PncD	PPV23 boost induced a greater response than PCV boost for all four serotypes
Nurkka et al (2001)[19]	Finland	2, 4, 6 months	15 months	29	30	Seven-valent PncCRM	PPV23 and PCV induced generally similar responses
Zangwill et al (2003)[20]	USA	2, 4, 6 months	12 months	60	60	Seven-valent PncOMPC	PPV23 boost induced a greater response than PCV boost for all seven serotypes
Anttila et al (1998)[21]	Finland	2, 4, 6 months	14 months	60	66	Four-valent PncD	PPV23 and PCV induced responses of similar magnitude; however, PCV booster—but not PPV23 booster—induced a significant rise in antibody avidity (p<0·01)
Nurkka et al (2005)[22]	Finland	2, 4, 6 months	12 months	7	49	Seven-valent PncOMPC	PPV23 booster induced a greater mucosal response than PCV booster for all four serotypes
Sigurdardottir et al (2002)[23]	Iceland	3, 4, 6 months	13 months	79	73	Eight-valent PncD or eight-valent PncT (3, 4, 6B, 9V, 14, 18C, 19F, 23F)	PPV23 and PCV induced generally similar responses
Nurkka et al (2004b)[24]	Finland	2, 4, 6 months	12–15 months	51	51	11-valent Pnc-PD	PPV23 boost induced higher opsonophagocytic titres than PCV boost for the measured serotypes
Goldblatt et al (2006)[25]	UK	2, 3, 4 months or 2,4 months	12 months	74	66	Nine-valent PncCRM	PPV23 induced a response of greater magnitude for seven of nine serotypes; however, the antibodies induced by PCV had higher avidity for all three serotypes tested
Goldblatt et al (2000)[26]	Ghana	6, 10, 14 weeks	12 months	8	6	Nine-valent PncCRM	PPV23 induced a response of greater magnitude for nine out of nine serotypes; however, the antibodies induced by PCV had higher avidity for all three serotypes tested
Huebner et al (2004)[27]	South Africa	6, 10, 14 weeks	18 months	35	31	Nine-valent PncCRM	PPV23 boost induced a greater response than PCV boost for nine out of nine serotypes
Blum et al (2000)[28]	Israel	12–18 months	24–30 months	40	35	Seven-valent PncOMPC	PPV23 induced a greater response for seven out of seven serotypes. PCV and PPV23 induced a similar opsonic antibody response to 6B
Chiu et al (1995)[29]	USA	17–20 months	19–22 months	..	..	Five-valent PncCRM (6B, 14, 18C, 19, 23F)	PPV23 and PCV induced generally similar responses

..=abstract does not report the number of study subjects. Pnc-PD=pneumococcal polysaccharide-*Haemophilus influenzae* protein D vaccine. PncOMPC=pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine. PncD=pneumococcal polysaccharide-diphtheria toxoid conjugate vaccine. PncCRM=pneumococcal polysaccharide CRM$_{197}$ protein conjugate vaccine. PncT=pneumococcal polysaccharide-tetanus protein conjugate vaccine.

Table 1: **Immunogenicity of PPV23 or PCV as a booster dose following PCV priming**

based has not been published. An important consideration is the immunological effects of combined PCV/PPV schedules. In this Review, we aim to assess these immunological issues.

Immunogenicity of PPV23 or PCV boost following PCV

PPV23 vaccine has been used in combination with various PCVs in preregistration studies to evaluate the priming nature of PCV. Studies with PCV/PPV23-combined regimens have also been done in patient populations for whom PPV23 is routinely recommended (eg, elderly people, individuals with sickle-cell disease or HIV infection). All such studies have been done using the PPV23 dose that is licensed for administration to adults (ie, 0·5 mL of vaccine containing 25 μg of each antigen).

Studies comparing the immunogenicity of PPV23 and PCV following a priming series of PCV are summarised in table 1. Several studies in children, using a variety of conjugate formulations and schedules in different countries, have shown that PPV23 vaccine following PCV priming results in a higher concentration of vaccine serotype antibodies than when PCV is used as the final dose. Few of these studies included children receiving PPV23 without previous PCV as a control group.

The dose of serotype-specific antigen in PPV23 is six to ten-fold that contained in PCV (ie, 25 μg versus 2 μg or 4 μg). The greater antigen dose may contribute to the higher titres observed; whether this is caused by enhanced stimulation of memory B cells or stimulation of a greater number of B cells overall than the smaller dose is unclear.

In addition to PPV23 eliciting higher antibody concentrations, the quality of the antibody seems to differ. Studies have evaluated avidity[21,26,28] or opsonic functional activity[24,25] of antibodies generated by PPV23 compared with PCV booster doses. In Finnish children, vaccination with PCV resulted in antibodies of greater avidity than those following PPV23,[21] whereas in Ghanaian children no difference was observed.[26] In children in the UK primed with two or three PCV doses and boosted with PPV23 or PCV at 12 months of age, the latter elicited antibodies of substantially higher avidity for all three serotypes studied (6B, 14, and 19F).[25] The functional activity of pneumococcal antibodies elicited following boosting with PPV23 or PCV has also had inconsistent results between studies. PPV23 resulted in antibodies of greater opsonic activity in Finnish children;[24] however, in Israeli children there was no difference.[28] Overall, a PPV23 boost results in an increased concentration of pneumococcal antibody compared with that generated following a PCV boost; the quality of these antibodies relative to those from a PCV boost are inconsistent. The reasons for the differences observed are not known. In view of the diversity of the populations studied, antigenic stimulation

from natural exposure to pneumococci through nasopharyngeal colonisation might explain some of these differences.

The age at immunisation is an important consideration if combined PCV/PPV23 infant immunisation schedules are intended to broaden the serotype coverage. PPV23 is believed to be poorly immunogenic for many serotypes at 12–15 months of age when most vaccine regimens would target a booster dose.[21] The programmatic complexity of managing two pneumococcal vaccines, one given only as a boosting dose, is substantial. Finally, there has been much speculation about the possibility that use of PPV23 as a booster dose might induce subsequent hypo-responsiveness to pneumococcal antigens encountered either through natural exposure or through subsequent vaccine exposure.

Basis of concern for hyporesponsiveness with PPV23

As discussed above, PPV23 as a booster dose following a priming series of PCV induces higher concentrations of antibodies than a PCV booster. Beyond the concerns of antibody quality and programmatic issues is the concern that use of PPV23 in infants might induce immunological hyporesponsiveness to subsequent pneumococcal antigenic exposure.

Studies have shown that repeated doses of bacterial polysaccharides might induce a state of immune tolerance, or hyporesponsiveness, to these vaccine antigens in children and adults; most of these data are from studies on group A and group C meningococcal polysaccharide vaccines (MPVs). Limited data are also available on repeated doses of PPV23 in adults and children.[30–40]

The first widely recognised observation of hypo-responsiveness following repeated doses of bacterial antigen vaccines was in infants immunised with group C MPVs.[34] When infants previously immunised with group C MPV at 3 months of age were reimmunised at 7 months and 12 months of age, not only was no memory response observed, as expected with a T-cell independent antigen, but second doses elicited serum antibody concentrations lower than those elicited by primary immunisation. Importantly, hyporesponsiveness was observed in children receiving either 25 μg or 100 μg of meningococcal antigen as the second dose, but not in those who received 10 μg, suggesting that the mechanism of action may relate to existing memory B cells being overwhelmed by large doses of antigen.

Additional evidence of group C meningococcal hyporesponsiveness comes from a study of infants in The Gambia who had received three doses of group C MPV before 2 years of age. These infants had significantly lower antibody responses to a second or third dose of group C MPV containing 50 μg of antigen than age-matched children who had received only one dose of vaccine (p<0·02).[35]

Polysaccharide vaccine given as a booster following conjugate vaccine priming can also interfere with immune responses to subsequent vaccine doses. Infants primed with meningococcal conjugate A/C vaccine, and boosted with group A/C MPV at 2 years of age showed a reduced antibody response (measured by the serum bactericidal antibody assay) to a second dose of MPV given at 5 years of age compared with age-matched children who had received conjugate meningococcal vaccine or no meningococcal vaccine at 2 years of age (p<0·001).[30]

Meningococcal serogroup C conjugate (MCC) vaccines have been studied for their ability to overcome the hyporesponsiveness induced by group C MPV. University students who received a dose of group A/C MPV were immunised 6 months later with either group A/C MPV or MCC vaccine.[40] In individuals receiving two doses of serogroup A/C MPV, serum bactericidal antibody titres were lower after the second dose compared with the first dose. However, MCC vaccine following serogroup A/C MPV induced serum bactericidal antibody titres similar to those seen 6 months earlier following serogroup A/C MPV. Although these results could be interpreted as the MCC vaccine "overcoming" the hyporesponsiveness induced by polysaccharides, a control group in this study that received MCC vaccine for the first time had serum bactericidal antibody titres significantly greater than individuals receiving MCC vaccine following serogroup A/C MPV (p=0·013). Therefore, MCC vaccine only partly overcomes the influence of the previous polysaccharide vaccine. This same pattern of responsiveness to MCC vaccine has been shown in infants and toddlers who have previously received either serogroup A/C MPV[38] or a quadrivalent MPV.[37]

Studies of hyporesponsiveness to meningococcal group A polysaccharides in infants have yielded mixed results. Group A meningococcal serum antibody concentration was found to increase after repeated doses of meningococcal polysaccharide.[32,41,42] In these studies only the concentration—not serum bactericidal antibody—of serum IgG was measured; nevertheless, these results suggest that because of its chemical structure, group A polysaccharide may not be handled by the immune system as a classic T-cell independent antigen. When the serum bactericidal antibody titres were measured, the response to group A meningococcal polysaccharide was lower in children who had received three doses of polysaccharide vaccine compared with control children who had received only one dose.[43] Similar hyporesponsiveness to group A and group C polysaccharides has also been described in adults.[31,36]

Evaluation of hyporesponsiveness to bacterial polysaccharide vaccines has not been limited to meningococcal vaccine. The administration of Hib polyribosylribitol phosphate vaccine showed that responses to a second vaccine dose did not differ from those responses in age-matched children receiving their first dose.[44] However, the children who did not respond to a second dose of vaccine were those with high polyribosylribitol phosphate antibody concentrations before the first dose. There was no specific evidence of hyporesponsiveness following polyribosylribitol phosphate vaccine in this study.

There have been few studies on immunological responses to multiple doses of PPV, and of these, many predate the development of sensitive and specific assays for antipneumococcal antibodies. Nevertheless, sufficient data exist to suggest that the phenomenon of hyporesponsiveness could be a concern for pneumococcal antigens or might even be a property of bacterial polysaccharides in general.

Multiple doses of PPV

Most studies on the antibody response to multiple doses of PPV have been done in adults.[45–52] All studies, except one, show that serotype-specific pneumococcal antibody concentrations following a second dose of PPV are lower than those measured following the first dose.[52] It is difficult to clearly attribute the hyporesponsiveness to an immunological consequence of the first vaccine dose because of confounding effects of age when studies are done in elderly people. Second doses are, by their nature, given when the study participants are older, and it is known that the immune response to PPV23 wanes with increasing age in elderly people. However, the interval between doses was 1 to 6 years, and it is unlikely that a pronounced immunological decline in adults could have occurred within such a short timeframe.

Only one study of multiple PPV doses showed no hyporesponsiveness.[52] The investigators evaluated the response to a first and second dose (given 6–9 years later) of PPV23 in 26 adults with chronic diseases and found that the rise in antibody levels against all pneumococcal serotypes after the second dose was similar to the rise detected after the first PPV23 dose. The interval between doses in this study was longer than the intervals between doses in the studies that documented hyporesponsiveness, suggesting that hyporesponsiveness to multiple doses of PPV23 could be a time-limited phenomenon.

Few studies have investigated multiple doses of PPV in children,[48,53–56] and most were done in the era when antibodies were measured with a radioimmunoassay, which lacks specificity for serotype-specific antibody. Despite these limitations, all but one of the studies showed that the antibody response to a second dose of PPV was lower than the response measured following the first dose, for at least some serotypes.

Konradsen and colleagues[53] studied responses to the PPV23 vaccine in children with sickle-cell disease who had received PPV14 5 years before. Responses to serotypes 1, 2, 3, 6A, 9N, and 25 were reduced following the second dose compared with the first dose.

In Finnish children immunised with PPV14 at 7–9 months of age and a second dose at 13 months of age, there was a reduced immune response to the second

dose compared with the first dose for those serotypes that were poorly or moderately immunogenic at the first dose.[54]

In a study of otherwise healthy children given PPV at 6 months and 12 months of age, a comparison group of children was vaccinated with a single dose of PPV at 12 months of age.[55] The serum antibody response to serogroups 7, 18, and 23 was greater in children first vaccinated at 12 months of age than in children receiving a second dose at 12 months of age. For type 3, the immune response to the second dose was at least as great as the response to the first dose at 6 months of age; however, in the children that had shown the highest response at 6 months of age, there was almost no response to the second dose given at 12 months of age. For children vaccinated with a second dose at 12 months of age, serotype 3 antibody concentration was lower after the second dose compared with before administration of the second dose. These inhibitory or depressed responses were not sustained at 24 months of age.

A study of children immunised at 6 months and 12 months of age with either PPV8 or PPV14, and then PPV14 at 2–5 years of age also evaluated children immunised for the first time with PPV14 at 2–5 years of age. Hyporesponsiveness was seen for serotype 23F but not serotypes 6A, 14, or 19F.[56]

By contrast with these four paediatric studies showing that a second dose of PPV results in a lower antibody concentration than that induced by the first dose, there is a single study that shows no evidence of hypo-responsiveness to multiple doses of PPV. The serotype-specific antibodies in infants immunised with a PPV12 vaccine at 3–5 months of age and then again at 9–11 months of age were greater following the second dose than after the first dose for virtually all serotypes.[48] In this same study, a second dose of vaccine given to adults resulted in a reduced mean fold antibody increase compared with that measured following the first dose for a variety of serotypes.[48]

These studies of multiple PPV doses in children and adults assessed a limited number of serotypes. Because of the structural diversity of pneumococcal antigens, their immunological properties are likely to vary; type 1 polysaccharide for instance is zwitterionic and thought to be handled as a classic T-cell dependent antigen.[57] Data from this limited number of serotypes might not apply to all those found in PPV23. Furthermore, these data on multiple PPV doses do not address the possible immunological risks of the proposed PCV/PPV sequential regimens. However, they do explore whether large pneumococcal antigen loads may interact with the immune system to depress its ability to respond to natural or vaccine-related pneumococcal antigenic challenges.

There are anecdotal observations of the immune system following systemic infections with polysaccharide-coated bacteria. In a chapter exploring pneumococcal polysaccharide as a paralysing agent on the immune system of mice, the authors drew an analogy between these observations in mice and those in human beings.[58,59] The authors of the chapter noted that many people with invasive Hib infections did not develop Hib antibodies during the recovery phase of the infection.[60] They also reported on the failure of an infant with type 14 pneumococcal meningitis to develop type 14 antibodies following vaccination despite his ability to mount an immune response to serotype 4;[60] a phenomenon that has also been observed by others.[61] A 9-month-old child

	Country	Age of dosing		Number of patients		PCV product	Summary of findings
		PPV23 and PCV	PCV alone	PPV23 and PCV	PCV alone		
de Roux et al (2005)[63]	Germany	70 years or more	70 years or more	78	110	Seven-valent PncCRM	A single dose of PCV induced a greater immune response for all seven vaccine serotypes than did a dose of PCV following PPV23 administration
Bernatowska et al (2004)[64]*	Finland	PPV23 was given before the study, PCV was given at 6 months to 19 years	6 months to 19 years	13	7	Seven-valent PncCRM	A comparison of the response in the two groups is not reported; however, children who previously received PPV23 responded "satisfactorily" to PCV
Blum et al (2000)[28]	Israel	PPV23 at 12–18 months, PCV at 24–30 months	24–30 months	26	67	Seven-valent PncOMPC	The response to PCV at 24–30 months of age was greater for six out of seven serotypes in the group that had previously received PPV23
Miiro et al (2005)[65]†	Uganda	PPV23 given before study, PCV at mean age of 37 years	24–56 years (mean 37 years)	54	55	Seven-valent PncCRM	The response to PCV in patients who had previously received PPV23 was similar to the reponse in patients who had not received PPV23

*Asplenic patients. †HIV-infected patients. PncCRM=pneumococcal polysaccharide CRM$_{197}$ protein conjugate vaccine. PncOMPC=pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine.

Table 2: Immunogenicity of PCV after PPV23 administration compared with PCV alone

developed serotype 19F meningitis and failed to respond to vaccination with serotype 19F pneumococcal antigen for the next 2 years; however, he did respond to other polysaccharide antigens (ie, Hib) and protein antigens (ie, tetanus toxoid).[61]

Efficacy trials of multiple dose PPV23 vaccine regimens have not been done, thus the clinical relevance of hyporesponsiveness is not clear. However, a placebo-controlled, blinded efficacy trial of PPV23 vaccine in HIV-infected adults in Uganda was terminated when there was evidence for an increase in the rate of pneumococcal disease among individuals who had received the PPV23 vaccine compared with those who had received placebo.[62] The researchers suggested that a mechanism for this observation might be the destruction of polysaccharide-responsive B-cell clones.

Hyporesponsiveness of PCV and PPV combined schedules

Various studies have evaluated the immunogenicity of schedules combining pneumococcal conjugate and polysaccharide vaccines (table 1 and table 2). Although the issue of primary interest is the immunological effect of PPV given following PCV priming, there are no published studies with a study design adequate to assess this issue. Four studies have, however, assessed the effect of PPV23 on subsequent administration of PCV, which addresses the effect of PPV23 on subsequent pneumococcal antigen exposures (table 2).

One paediatric study in Israel evaluated possible immune tolerance induced by PPV alone or PPV given as a priming dose before PCV.[28] Children were randomised to receive PCV7 or PPV23 at 12, 15, or 18 months of age followed by either PCV7 or PPV23 12 months later at 24–30 months of age. The pneumococcal antibody concentrations elicited by the second dose were compared with those elicited by PPV23 or PCV7 given as a first dose to age-matched children. The initial PPV23 dose impaired the response to subsequent PPV23 but not PCV7 given at 24–30 months of age.

By contrast, a dose of PPV23 impaired the response to a subsequent dose of PCV7 in elderly adults (70 years of age or more). Previous immunisation with PPV23 was found to suppress the antibody response to PCV7 for all seven serotypes compared with individuals who had not received PPV23 before their PCV7 dose.[63] There was no evidence that a first dose of PCV7 interfered with the response to a second dose of PCV7.

A study of asplenic children found that a previous vaccination with PPV23 did not affect the response to PCV7; however, the study's ability to detect differences was limited because only 13 children with previous PPV23 vaccination were compared with seven children without PPV23 vaccination.[64]

Two additional studies done in HIV-infected people are inconsistent in supporting or refuting the hyporesponsiveness hypothesis. The HIV-infection status might confound the immune response observations. HIV-infected Ugandan adults were randomised to receive either a single dose of PPV23 or placebo, followed by two doses of PCV. The immunogenicity of the PCV regimen did not differ between placebo and PPV23-vaccinated groups. However, those receiving PCV were not necessarily representative of the whole cohort since many did not survive to receive PCV.[65] A Greek study of HIV-infected children assessed the antibody response to two doses of PCV given 12 months apart. Most of the children had previously been immunised with PPV23.[66] A control group consisting of healthy, HIV-uninfected children also received a two-dose PCV regimen. The pneumococcal antibody concentrations in the HIV-infected children were lower than those found in the control children. It is not clear whether the reduced immunogenicity is attributable to the HIV-infection status of these children, or whether the preceding PPV23 vaccine might have contributed to reduced immunogenicity.[66]

In addition to the magnitude of the antibody responses, the antibody quality in people previously immunised with PPV23 is important. Antibody characterisation can direct hypotheses about the effect of such vaccines on various immune cells. For example, the avidity of antibodies elicited by booster doses of PPV23 is reduced for some serotypes compared with that elicited by PCV booster doses in children who have previously been primed with PCV.[21,26] This observation suggests that in immunocompetent children, large doses of pneumococcal polysaccharide antigen, like those found in PPV23, recruit naive and memory B cells providing a mixture of high and low avidity antibodies; however, the small dose of polysaccharide in PCV only stimulates memory cells and therefore antibodies with higher avidity. The subclass distribution of pneumococcal antibodies induced by pneumococcal polysaccharide challenge (in the form of a fractional dose of PPV23 given 7 years after priming) was assessed in children who had been primed with PCV in infancy and then boosted with either PPV23 or PCV at 13 months of age.[67] In children who had received only PCV before fractional PPV23, the IgG subclass response was characteristic of a memory immune response (ie, IgG1:IgG2 ratio greater than one) for two of the three PCV vaccine serotypes tested. In pneumococcal vaccine naive children and in children primed with PCV in infancy and boosted with PPV23, the response to a fractional dose of PPV23 was characteristic of an unprimed, naive host response (ie, IgG1:IgG2 ratio less than one).[67]

Further suggestive evidence of possible reduced immune responses following PPV23 comes from an efficacy trial of a combined PCV/PPV23 schedule to prevent otitis media in otitis-prone children.[68] The editorial that accompanied the study reported and commented on reduced efficacy following the

PPV23 boost compared with that seen before receiving the boost.[69] Since the trial did not include a group of children who received PCV alone without a PPV23 boost, it is difficult to assess whether the PPV23 itself resulted in the increased risk, or whether other factors such as changes in nasopharyngeal colonisation or age-related changes might have affected the risk of otitis media. Although the interpretation of the reduced efficacy of pneumococcal vaccine following receipt of PPV23 compared with the efficacy before its receipt is not clear, the possibility that PPV23 could suppress the immune response to pneumococcal antigens is one possible explanation.

By contrast, a Finnish study of infants who had received three primary doses of PCV at 2, 4, and 6 months of age assessed a booster dose of either PPV23 or PCV at 12 months of age. The efficacy of the PPV23 booster against otitis media was 65% (95% CI 34–81%) whereas the efficacy of the PCV booster was 60% (43–72%).[18]

Postulated mechanisms of hyporesponsiveness from PPV23 and supporting in-vitro evidence

The data summarised here are mostly observational data of reduced or impaired antibody responses following exposure of children or adults to multiple doses of PPV23. There are several mechanisms by which such an exposure could lead to a adverse immune response, but the mechanism by which large doses of pneumococcal polysaccharides, as found in PPV23, might induce subsequent hyporesponsiveness is unclear.

When added in large amounts in vitro, polysaccharides may be able to downregulate B cells.[70] Therefore, if the same phenomenon occurs in vivo, hyporesponsiveness might be related to the reduction in the reactive B-cell pool following a polysaccharide challenge, thereby resulting in a smaller pool of available reactive B cells when a subsequent dose or antigenic exposure is encountered. Some investigators have shown that hyporesponsiveness to meningococcal group C could last for 5 years following vaccination with the pure polysaccharide vaccine.[71] It is unclear why new B cells, derived from bone marrow, do not replenish the reactive B-cell pool. One speculation is that because polysaccharide antigens are so long-lived, persisting for years, they bind to new naive but reactive B cells that emerge from the bone marrow.

Studies in mice have led to similar speculation following challenge with large doses of polysaccharide.[58] In white mice, large doses of polysaccharides (500 μg), but not small doses (0·5–1·0 μg), rendered mice unresponsive to subsequent challenges with bacteria containing the polysaccharide and also impaired the immune response to additional immunising doses of polysaccharide.[59] The investigators postulated that polysaccharide antigen persisted in the animal and was excreted intact from immune cells, thereby recirculating

for long periods of time and neutralising polysaccharide-specific antibody, which resulted in the inability to detect such antibodies.

In mice immunised with ^{14}C-labelled type 3 pneumococcal polysaccharide, there was an exponential decay of the polysaccharide for about 6 days.[72] Subsequently, the rate of decay declined; however, free immunogenic type 3 polysaccharide was still detected in the mice even after 100 days. The study suggested that serum polysaccharide—rather than tissue depots—largely determines polysaccharide-specific antibody neutralisation. The authors noted that progressive re-entry of minute amounts of polysaccharide from tissue depots into the circulation results in polysaccharide continuously neutralising newly formed antibody over long periods of time. It is also possible that newly formed antibody might be immunosuppressive. Polysaccharide-specific IgG, IgM, or immune complexes might bind to inhibitory Fc receptors, thus preventing antibody production.[73]

Although studies of polysaccharide antigen immunology have historically focused on B cells, the contribution of other immune system cells to the regulation of B-cell responses cannot be ruled out. Studies have described the role of dendritic cells in the murine immune response to pneumococcus[74,75] and have focused on the in-vitro interaction between human-derived dendritic cells and purified pneumococcal polysaccharides.[76] In the latter study, dendritic cells that were fed polysaccharides displayed an altered cytokine profile when subsequently stimulated with lipopolysaccharide. The stimulation of immature human-derived dendritic cells with lipopolysaccharide classically results in the secretion of cytokines dominated by the immunostimulatory interleukin 12, yet after the ingestion of polysaccharide, lipopolysaccharide-stimulated dendritic cells were shown to produce mainly interleukin 10. Interleukin 10 has well-characterised immunosuppressive and immunomodulatory effects that might alter B-cell responses to polysaccharides.

T-cell suppressor activity has also been implicated in the reduced response to polysaccharides in murine studies. Heilmann[77] observed that to optimise the isolation of polysaccharide-specific B cells from mice immunised with pneumococcal polysaccharide, T-cell depletion of the mononuclear fraction was required, suggesting that suppressor T cells existed. Some research groups have shown that the adoptive transfer of putative suppressor T cells can modulate subsequent response to bacterial-derived polysaccharide antigens.[78] Other groups have shown that spleen cells from mice injected with low-dose meningococcal group A polysaccharide could, when adoptively transferred, inhibit meningococcal A responses in the recipient mouse.[79] So far, no data derived from human studies have identified polysaccharide-specific pneumococcal T cells.

Search strategy and selection criteria

We searched PubMed for relevant studies published from 1995, to March, 2006. Additional studies were retrieved by review of references from relevant articles and the authors' files. Search terms were "pneumococcal conjugate vaccine", "pneumococcal polysaccharide vaccine", and "invasive pneumococcal disease". We reviewed abstracts from the International Symposium on Pneumococci and Pneumococcal Diseases (1998–2006) and from the Interscience Conference on Antimicrobial Agents and Chemotherapeutics (1995–2005). Studies meeting any of the following criteria were included in this review: (1) studies comparing the booster response to PPV23 and PCV in children primed with PCV, (2) studies in which multiple doses of PPV23 were given, and (3) studies comparing the response to PCV in patients who had previously received at least one dose of PPV23 with patients who had not. Only reports in English language were reviewed.

Conclusion

Studies evaluating multiple doses of vaccines containing polysaccharide from various bacteria have shown an altered immune response to subsequent polysaccharide challenge. It is not clear from the efficacy data of human vaccine regimens whether these observations have important clinical implications and if so, to what degree any hyporesponsiveness might affect clinical disease prevention.

Studies appropriately designed to directly address whether hyporesponsiveness occurs following full-dose PPV23 in infants and children are needed; designs using a fractional dose of PPV23 would be one such approach. Moreover, studies to more clearly understand the immune responses to repeated natural and artificially administered pneumococcal antigens are needed to provide the appropriate interpretation and biological basis of clinical study observations. Until such areas of investigation provide a more comprehensive understanding of the effect of PPV23 use in infants, routine vaccination strategies that include large polysaccharide doses should consider the complex immune response that ensues.

Conflicts of interest

DG has accepted honoraria for participation in advisory boards from vaccine manufacturers including Wyeth, GSK, Novartis, and Sanofi-Aventis. DG's laboratory does contract research measuring pneumococcal antibodies for all the vaccine manufacturers with pneumococcal programmes (Wyeth, GSK, Merck, and Sanofi-Aventis). KLOB has had research funding from Wyeth, Merck, and Sanofi-Aventis, and has received advisory board honoraria from Wyeth and Sanofi-Aventis. MH declares that he has no conflicts of interest.

References

1 Rennels MB, Edwards KM, Keyserling H, et al. Safety and immunogenicity of heptavalent pneumococcal vaccine conjugated to CRM197 in United States infants. *Pediatrics* 1998; **101:** 604–11.

2 Kayhty H, Ahman H, Ronnberg PR, Tillikainen R, Eskola J. Pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine is immunogenic in infants and children. *J Infect Dis* 1995; **172:** 1273–78.

3 Dagan R, Melamed R, Zamir O, Leroy O. Safety and immunogenicity of tetravalent pneumococcal vaccines containing 6B, 14, 19F and 23F polysaccharides conjugated to either tetanus or diphtheria toxoid in young infants and their boosterability by native polysaccharide antigens. *Pediatr Infect Dis J* 1997; **16:** 211–16.

4 Ahman H, Kayhty H, Lehtonen H, Leroy O, Froeschle J, Eskola J. *Streptococcus pneumoniae* capsular polysaccharide-diphtheria toxoid conjugate vaccine is immunogenic in early infancy and able to induce immunologic memory. *Pediatr Infect Dis J* 1998; **17:** 211–16.

5 Bisgard KM, Kao A, Leake J, Strebel PM, Perkins BA, Wharton M. *Haemophilus influenzae* invasive disease in the United States, 1994–1995: near disappearance of a child vaccine preventable disease. *Emerg Infect Dis* 1998; **4:** 229–37.

6 American Academy of Pediatrics, Committee on Infectious Diseases. Policy statement: recommendations for the prevention of pneumococcal infections, including the use of pneumococcal conjugate vaccine (Prevnar), pneumococcal polysaccharide vaccine, and antibiotic prophylaxis. *Pediatrics* 2000; **106:** 362–66.

7 Advisory Committee on Immunization Practices. Preventing pneumococcal disease among infants and young children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2000; **49:** 1–35.

8 Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. *Pediatr Infect Dis J* 2000; **19:** 187–95.

9 Klugman KP, Madhi SA, Huebner RE, et al. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. *N Engl J Med* 2003; **349:** 1341–48.

10 O'Brien KL, Moulton LH, Reid R, et al. Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomised trial. *Lancet* 2003; **362:** 355–61.

11 Cutts FT, Zaman SM, Enwere G, et al. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. *Lancet* 2005; **365:** 1139–46.

12 Black SB, Shinefield HR, Ling S, et al. Effectiveness of heptavalent pneumococcal conjugate vaccine in children younger than five years of age for prevention of pneumonia. *Pediatr Infect Dis J* 2006; **25:** 306–11.

13 Cano FR, Querry MV, Tarrant CJ, et al. Antibody responses after immunization with polyvalent pneumococcal vaccine containing 10, 25, or 50 micrograms of 14 polysaccharide types per dose. *Proc Soc Exp Biol Med* 1983; **173:** 579–82.

14 Riley ID, Lehmann DM, Alpers MP, Marshall TF, Gratten H, Smith D. Pneumococcal vaccine prevents death from acute lower-respiratory-tract infections in Papua New Guinean children. *Lancet* 1986; **2:** 877–81.

15 Douglas RM, Miles HB. Vaccination against *Streptococcus pneumoniae* in childhood: lack of demonstrable benefit in young Australian children. *J Infect Dis* 1984; **149:** 861–69.

16 Teele DW, Klein JO, Bratton L, et al. Use of pneumococcal vaccine for prevention of recurrent acute otitis media in infants in Boston. The Greater Boston Collaborative Otitis Media Study Group. *Rev Infect Dis* 1981; **3** (suppl): S113–18.

17 Nurkka A, Joensuu J, Henckaerts I, et al. Immunogenicity and safety of the eleven valent pneumococcal polysaccharide-protein D conjugate vaccine in infants. *Pediatr Infect Dis J* 2004; **23:** 1008–14.

18 Kilpi T, Ahman H, Jokinen J, et al. Protective efficacy of a second pneumococcal conjugate vaccine against pneumococcal acute otitis media in infants and children: randomized, controlled trial of a 7-valent pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine in 1666 children. *Clin Infect Dis* 2003; **37:** 1155–64.

19 Nurkka A, Ahman H, Korkeila M, Jantti V, Kayhty H, Eskola J. Serum and salivary anti-capsular antibodies in infants and children immunized with the heptavalent pneumococcal conjugate vaccine. *Pediatr Infect Dis J* 2001; **20:** 25–33.

20 Zangwill KM, Greenberg DP, Chiu CY, et al. Safety and immunogenicity of a heptavalent pneumococcal conjugate vaccine in infants. *Vaccine* 2003; **21:** 1894–900.

21 Anttila M, Eskola J, Ahman H, Kayhty H. Avidity of IgG for *Streptococcus pneumoniae* type 6B and 23F polysaccharides in infants primed with pneumococcal conjugates and boosted with polysaccharide or conjugate vaccines. *J Infect Dis* 1998; **177:** 1614–21.

22 Nurkka A, Lahdenkari M, Palmu AA, et al. Salivary antibodies induced by the seven-valent PncOMPC conjugate vaccine in the Finnish Otitis Media Vaccine Trial. *BMC Infect Dis* 2005; **5:** 41.

23 Sigurdardottir ST, Ingolfsdottir G, Davidsdottir K, et al. Immune response to octavalent diphtheria and tetanus-conjugated pneumococcal vaccines is serotype and carrier-specific: the choice for a mixed carrier vaccine. *Pediatr Infect Dis J* 2002; **21:** 548–54.

24 Nurkka A, Poolman J, Henckaerts I, et al. Opsonophagocytic activity of antibodies induced by 11-valent pneumococcal conjugate vaccine. 4th International Symposium on Pneumococci and Pneumococcal Disease; Helsinki, Finland; May 9–13, 2004. Abstract PSV-41.

25 Goldblatt D, Southern J, Ashton L, et al. Immunogenicity and boosting following a reduced number of doses of a pneumococcal conjugate vaccine in infants and toddlers. *Pediatr Infect Dis J* 2006; **25:** 312–19.

26 Goldblatt D, Akoto AY, Ashton L, et al. Immunogenicity and the generation of immune memory following 9-valent pneumococcal conjugate vaccination in Ghanaian infants with sickle cell disease. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy; Toronto, Ontario, Canada; Sept 24–27, 2000. Abstract 688.

27 Huebner RE, Mbelle N, Forrest B, et al. Long-term antibody levels and booster responses in South African children immunized with nonavalent pneumococcal conjugate vaccine. *Vaccine* 2004; **22:** 2696–700.

28 Blum MD, Dagan R, Mendelman PM, et al. A comparison of multiple regimens of pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine and pneumococcal polysaccharide vaccine in toddlers. *Vaccine* 2000; **18:** 2359–67.

29 Chiu SS, Greenberg DP, Partridge S, et al. Safety and immunogenicity of a pentavalent pneumococcal conjugate vaccine in healthy toddlers. 35th Interscience Conference on Antimicrobial Agents and Chemotherapeutics; San Francisco, CA, USA; Sept 17–20, 1995. Abstract G71.

30 MacLennan J, Obara S, Deeks J, et al. Immunologic memory 5 years after meningococcal A/C conjugate vaccination in infancy. *J Infect Dis* 2001; **183:** 97–104.

31 Borrow R, Joseph H, Andrews N, et al. Reduced antibody response to revaccination and meningococcal serogroup A polysaccharide vaccine in adults. *Vaccine* 2001; **19:** 1129–32.

32 Kayhty H, Karanko V, Peltola H, Sarna S, Makela HP. Serum antibodies to capsular polysaccharide vaccine of group A *Neisseria meningitidis* followed for three years in infants and children. *J Infect Dis* 1980; **142:** 861–68.

33 MacLennan J, Obaro S, Deeks J, et al. Immune response to revaccination with meningococcal A and C polysaccharides in Gambian children following repeated immunisation during early childhood. *Vaccine* 1999; **17:** 3086–93.

34 Gold R, Lepow ML, Goldschneider I, Draper TL, Gotschlich EC. Clinical evaluation of group A and group C meningococcal polysaccharide vaccines in infants. *J Clin Invest* 1975; **175:** 1536–47.

35 Leach A, Twumasi PA, Kumah S. Induction of immunologic memory in Gambian children by vaccination in infancy with a group A plus group C meningococcal polysaccharide-protein conjugate vaccine. *J Infect Dis* 1997; **175:** 200–04.

36 Granoff DM, Gupta RK, Belshe RB, Anderson EL. Induction of immunologic refractoriness in adults by meningococcal C polysaccharide vaccination. *J Infect Dis* 1998; **178:** 870–74.

37 MacDonald NE, Halperin SA, Law BJ, Danzig LE, Granoff DM. Can meningococcal C conjugate vaccine overcome immune hyporesponsiveness induced by previous administration of plain polysaccharide vaccine? *JAMA* 2000; **283:** 1826–27.

38 Borrow R, Goldblatt D, Andrews N, Richmond P, Southern J, Miller E. Influence of prior meningococcal C polysaccharide vaccination on the response and generation of memory after meningococcal C conjugate vaccination in young children. *J Infect Dis* 2001; **184:** 377–80.

39 MacDonald NE, Halperin SA, Law BJ, Forrest B, Danzig LE, Granoff DM. Induction of immunologic memory by conjugated *vs* plain meningococcal C polysaccharide vaccine in toddlers: a randomized controlled trial. *JAMA* 1998; **280:** 1685–89.

40 Richmond P, Kaczmarski E, Borrow R, et al. Meningococcal C polysaccharide vaccine induces immunologic hyporesponsiveness in adults that is overcome by meningococcal C conjugate vaccine. *J Infect Dis* 2000; **181:** 761–64.

41 Gold R, Lepow ML, Goldschneider I, Draper TF, Gotschlich EC. Kinetics of antibody production to group A and group C meningococcal polysaccharide vaccines administered during the first six years of life: prospects for routine immunization of infants and children. *J Infect Dis* 1979; **140:** 690–97.

42 Gold R, Lepow ML, Goldschneider I, Gotschlich EC. Immune response to human infants to polysaccharide vaccines of groups A and C *Neisseria meningitidis*. *J Infect Dis* 1977; **136:** S31–35.

43 MacLennan J, Obaro S, Deeks J, et al. Immune response to revaccination with meningococcal A and C polysaccharides in Gambian children following repeated immunisation during early childhood. *Vaccine* 1999; **17:** 3086–93.

44 Kayhty H, Karanko V, Peltola H, et al. Serum antibodies after vaccination with *Haemophilus influenzae* type b capsular polysaccharide and responses to reimmunization: no evidence of immunologic tolerance or memory. *Pediatrics* 1984; **74:** 857–65.

45 Torling J, Hedlund J, Konradsen HB, et al. Revaccination with the 23-valent pneumococcal polysaccharide vaccine in middle-aged and elderly persons previously treated for pneumonia. *Vaccine* 2003; **22:** 96–103.

46 Jackson LA, Benson P, Sneller VP, et al. Safety of revaccination with pneumococcal polysaccharide vaccine. *JAMA* 1999; **281:** 243–48.

47 Mufson MA, Hughey DF, Tuner CE, et al. Revaccination with pneumococcal vaccine of elderly persons 6 years after primary vaccination. *Vaccine* 1991; **9:** 403–07.

48 Borgono JM, McLean AA, Vella PP, et al. Vaccination and revaccination with polyvalent pneumococcal polysaccharide vaccines in adults and infants. *Proc Soc Exp Biol Med* 1978; **157:** 148–54.

49 Linnemann CC, First MR, Schiffman G. Revaccination of renal transplant and hemodialysis recipients with pneumococcal vaccine. *Arch Intern Med* 1986; **146:** 1554–56.

50 Heidelberger M, Dilapi MM, Siegel M, et al. Persistence of antibodies in human subjects injected with pneumococcal polysaccharides. *J Immunol* 1950; **65:** 535–41.

51 Musher DM, Groover JE, Rowland JM, et al. Antibody to capsular polysaccharides of *Streptococcus pneumoniae*: prevalence, persistence, and response to revaccination. *Clin Infect Dis* 1993; **17:** 66–73.

52 Davidson M, Bulkow LR, Grabman J, et al. Immunogenicity of pneumococcal revaccination in patients with chronic disease. *Arch Intern Med* 1994; **154:** 2209–14.

53 Konradsen HB, Pedersen FK, Henrichsen J. Pneumococcal revaccination of splenectomized children. *Pediatr Infect Dis J* 1990; **9:** 258–63.

54 Koskela M, Leinonen M, Haiva VM, et al. First and second dose antibody responses to pneumococcal polysaccharide vaccine in infants. *Pediatr Infect Dis* 1986; **5:** 45–50.

55 Sell SH, Wright PF, Vaughn WK, et al. Clinical studies of pneumococcal vaccines in infants. I. Reactogenicity and immunogenicity of two polyvalent polysaccharide vaccines. *Rev Infect Dis* 1981; **3** (suppl)**:** S97–107.

56 Lawrence EM, Edwards KM, Schiffman G, et al. Pneumococcal vaccine in normal children. Primary and secondary vaccination. *Am J Dis Child* 1983; **137:** 846–50.

57 Cobb BA, Wang Q, Tzianabos AO, Kasper DL. Polysaccharide processing and presentation by the MHCII pathway. *Cell* 2004; **117:** 677–87.

58 Felton LD, Ottinger B. Pneumococcus polysaccharide as a paralyzing agent on the mechanism of immunity in white mice. In: Nahmias AJ, O'Reilly RS, eds. Comprehensive immunology: immunology of human infection: part 1. Bacteria, mycoplasmae, chlamydiae, and fungi. New York: Plenum Medical Book Co, 1981: 28.

59 Felton LD, Ottinger B. Pneumococcus polysaccharide as a paralyzing agent on the mechanism of immunity in white mice. *J Bacteriol* 1942; **43:** 94–95.

60 O'Reilly RJ, Anderson P, Ingram DL, et al. Circulating polyribophosphate in *Hemophilus influenzae*, type b meningitis. Correlation with clinical course and antibody response. *J Clin Invest* 1975; **56:** 1012–22.

61 Pichichero ME. Immunological paralysis to pneumococcal polysaccharide in man. *Lancet* 1985; **2:** 468–71.

62 French N, Nakiyingi J, Carpenter LM, et al. 23-valent pneumococcal polysaccharide vaccine in HIV-1-infected Ugandan adults: double-blind, randomised and placebo controlled trial. *Lancet* 2000; **355:** 2106–11.

63 de Roux A, Schmoele-Thoma B, Ahlers N, et al. Different immune response after sequential use of pneumococcal polysaccharide and pneumococcal conjugate vaccine. 8th Annual Conference on Vaccine Research; Baltimore, MD, USA; May 9–11, 2005. Abstract S4.

64 Bernatowska E, Kayhty H, Mikoluc B, Piotrowska-Jastrzebska J. Immune response to pneumococcal conjugate vaccine in asplenic children and teenagers. 4th International Symposium on Pneumococci and Pneumococcal Disease; Helsinki, Finland; May 9–13, 2004. Abstract PSV-12.

65 Miiro G, Kayhty H, Watera C, et al. Conjugate pneumococcal vaccine in HIV-infected Ugandans and the effect of past receipt of polysaccharide vaccine. *J Infect Dis* 2005; **192:** 1801–05.

66 Spoulou VI, Tsoumas DL, Papaevangelou VG, Mostrou GI, Theodoridou MC. Immunogenicity and immunological memory induced by a 7-valent pneumococcal CRM197 conjugate vaccine in symptomatic HIV-1 infected children. *Vaccine* 2005; **23:** 5289–93.

67 Sigurdardottir ST, Davidsdottir K, Jonsdottir I. IgG subclass responses to pneumocccal polysaccharide (PPS) booster 6 years after pneumococcal conjugate (Pnc) vaccination in infancy. Effect of PPS booster at 13 months. 4th International Symposium on Pneumococci and Pneumococcal Diseases; Helsinki, Finland; May 9–13, 2004. Abstract PSV-51.

68 Veenhoven R, Bogaert D, Uiterwaal C, et al. Effect of conjugate pneumococcal vaccine followed by polysaccharide pneumococcal vaccine on recurrent otitis media: a randomized study. *Lancet* 2003; **361:** 2189–95.

69 Peltola H, Schmitt J, Booy R. Pneumococcal conjugate vaccine for otitis media—yes or no? *Lancet* 2003; **361:** 2170–71.

70 Yokochi T, Kato Y, Sugiyama T, et al. Lipopolysaccharide induces apoptotic cell death of B memory cells and regulates B cell memory in antigen-nonspecific manner. *FEMS Immunol Med Microbiol* 1996; **15:** 1–8.

71 Granoff DM, Gupta RK, Belshe RB, Anderson EL. Induction of immunologic refractoriness in adults by meningococcal C polysaccharide vaccination. *J Infect Dis* 1998; **178:** 870–74.

72 Howard JG, Christie CH, Jacob MJ, et al. Studies on immunological paralysis III. Recirculation and antibody neutralizing activity of 14C-labeled type III pneumococcal polysaccharide in paralyzed mice. *Clin Exp Immunol* 1970; **7:** 583–96.

73 Heyman B. Regulation of antibody responses via antibodies, complement and Fc receptors. *Annu Rev Immunol* 2000; **18:** 709–37.

74 Colino J, Shen Y, Snapper CM. Dendritic cells pulsed with intact *Streptococccus pneumoniae* elicit both protein and polysaccharide-specific immunoglobulin isotype responses in vivo through distinct mechanisms. *J Exp Med* 2002; **195:** 1–13.

75 Balazs M, Martin F, Zhou T, Kearney J. Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses. *Immunity* 2002; **17:** 341–52.

76 Meltzer U, Goldblatt D. Pneumococcal polysaccharides interact with human dendritic cells. *Infect Immun* 2006; **74:** 1890–95.

77 Heilmann C. Vaccination-induced activation of human blood T cells suppressing pneumococcal polysaccharide-specific B cells. *Acta Pathol Microbiol Immunol Scand [C]* 1987; **95:** 65–69.

78 Taylor CE, Bright R. T-cell modulation of the antibody response to bacterial polysaccharide antigens. *Infect Immun* 1989; **57:** 180–85.

79 Muller E, Apicella MA. T-cell modulation of the murine antibody response to *Neisseria meningitidis* group A capsular polysaccharide. *Infect Immun* 1988; **56:** 259–66.