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REVIEW

Carbohydrate–protein conjugate vaccines

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Various pathogenic bacteria have coats of polysaccharide, many with repeating epitopes. Though polysaccharide vaccines have been available for some time, they induce mainly IgM production, and are only moderately protective in adults and ineffective in young children. It was originally shown in 1931 that the immunogenicity of polysaccharides could be enhanced by conjugating to a protein. The last two decades have witnessed the production and clinical testing of polysaccharide–protein conjugates specific for at least four different bacteria which normally cause considerable mortality and morbidity, especially in young children. In some cases, immunizing children from 4 months of age, with a booster early in the second year, has resulted in remarkably high success rates in protecting them from disease. For one pathogen, *Haemophilus influenza* type b, the success rate has been sufficiently high (> 95%) to suggest that this disease might, in time, be globally controlled in this way. The results of immunization with conjugate vaccines to *Streptococcus pneumoniae*, *Neisseria meningiditis* and *Salmonella typhi* are also very encouraging. More conjugate preparations are under development.

Keywords carbohydrate, protein, vaccine, vaccination, immune response, antibody

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INTRODUCTION

Some infectious agents express polysaccharide molecules at their surface, e.g. encapsulated bacteria, or their surface proteins frequently have complex carbohydrate side-chains, e.g. viruses such as human immunodeficiency virus (HIV) and influenza. These structures serve several purposes. First, polysaccharides are less immunogenic than many proteins, and second, particularly in the case of glycoproteins, the sugar side-chain may protect susceptible peptide bonds from attack by proteases. In addition, these saccharides are sometimes involved in attachment of the agent to a susceptible cell. All these features favor the survival of the agent. For extracellular organisms in particular, the composition of a surface polysaccharide is determined solely by the organism's DNA and is therefore specific for the bacterium.

Landsteiner in 1924 [1] showed that, whereas a small hapten molecule was non-immunogenic, immunizing with the hapten linked to a protein antigen induced an antihapten antibody response. Polysaccharides were recognized as being relatively poorly immunogenic, but Avery and Goebel showed in 1929 that by conjugating a bacterial polysaccharide to a carrier protein, a stronger antibody response to the carbohydrate moiety was obtained [2]. The mechanism involved was completely unknown at that time. The discovery of T helper cells in the 1960s and their role in 'helping' B-cells make antibody was a great leap forward in our understanding; antigens could now be described as T-cell independent (TI) or T-cell dependent (TD).

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IMMUNE RESPONSES TO T-CELL-INDEPENDENT AND T-CELL-DEPENDENT ANTIGENS

With TD antigens, an immune response can occur at or shortly after birth, affinity maturation of the

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B-cell response takes place, immunologic memory occurs, adjuvants can induce an enhanced response, and there is a heterogeneous immunoglobulin response [3].

Polymers bind directly to the IgM receptor on B-cells, initiating an IgM antibody response. For example, in adult rats, the polymeric flagella of *Salmonella* induce an initial IgM response followed by a long-lasting, strong IgG response. The monomeric protein flagellin induces only an IgG response. In both cases, the IgG response is induced by interaction of the B-cell with specific activated Th2 cells.

TI antigens have been classified into two groups [4]. The first group includes B-cell mitogens such as lipopolysaccharides. The second group contains the different polysaccharides which have repeating epitopes. The immune responses induced by them have the following characteristics [4,5]:

1. Ontogeny—the response occurs 3–18 months after birth in humans, but it varies with different preparations; generally, children less than 2 years of age respond poorly.
2. There is no affinity maturation of the antibody response; no immunologic memory and no enhancement of the immune response by adjuvants occurs.
3. Mainly (>90%) IgM is produced [6] and synthesized in the spleen [7].

Polysaccharides also induce low levels of other immunoglobulin responses, such as IgG and IgA, so that they appear to have some of the characteristics of a TD antigen. In one study [8], 36 of 40 adults developed specific IgG responses (IgG_1, IgG_2 or both) to the 23-valent pneumococcal polysaccharide vaccine.

The switch to producing IgG by an IgM-secreting B-cell usually requires interaction with an activated, antigen-specific T helper (Th) lymphocyte. There is no indication that $CD4^+$ Th cells with an α,β-receptor recognize any products other than classical MHC–peptide complexes. The activation of human T-lymphocytes by lipopolysaccharide-treated macrophages was found to be non-MHC restricted but involved the co-stimulator B7 [9]. However γ,δ-T-cells may express non-classical MHC antigens and recognize non-protein antigens [10,11]. Nevertheless, there is no evidence that these cells have receptors which recognize and respond to processed polysaccharides.

Another critical step in achieving T-cell-dependent B-cell activation is the interaction between CD40 on B cells and its ligand. Using this pathway, a fungal polysaccharide can induce the proliferation of CD4 T-cells, with the secretion of interleukin-4 (IL-4) and IL-10 [12]. In another in vitro system, addition of the CD40 ligand and a cytokine, such as IL-4, has been shown to induce immunoglobulin class switching by IgM B-cells [13]. It therefore seems likely that such 'non-antigen-specific' events, including some IgM-secreting B-cells switching to produce antipolysaccharide IgG or IgA, could occur in vivo.

THE ADVANTAGES/ DISADVANTAGES OF POLYSACCHARIDE VACCINES

The major advantage of these vaccines is the relative simplicity of production of the different preparations. The main disadvantage is the lack of a protective immune response in young children, where the need is very great. The pneumococcal polysaccharide vaccine is recommended for the elderly, but here also, the efficacy is variable.

CONJUGATE VACCINES

The nature of the protein component and conjugation

Several highly immunogenic proteins have been proposed as the protein component, but, mainly, four have been used [5]: diphtheria (D) or tetanus (T) toxoids, CRM197 (a non-toxic variant of diphtheria toxin), and a complex outer-membrane protein (OMP) mixture from *Neisseria meningitidis*. The polysaccharides or an oligosaccharide are linked to the carrier, either directly or with carbon spacers. The toxoids were chosen as the carrier proteins because, apart from their inherent immunogenicity, if the recipient had been earlier immunized with the toxoid, a booster effect was expected. However, under certain circumstances (discussed later), suppressive effects can also occur.

Immunogenicity and efficacy of conjugate vaccines

A summary of the major findings on immunogenicity and efficacy for the different conjugate preparations is presented in Table 1. Many of these points are now considered in greater detail.

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Table 1 Immunogenicity and efficacy of conjugate vaccines

Organism	Protein carriers	Immunogenicity	Efficacy
Haemophilus influenzae type b	Diphtheria toxoid (modified) Tetanus toxoid *N. meningitidis* OMP	Highly immunogenic in infants and immunocompromised (far more so than polysaccharide vaccine) [3–14]	95–100% effective in eliminating all Hib disease when used in routine infant schedule [16–20,22–24]
Streptococcus pneumoniae	As for Hib	Highly immunogenic in infants and immunocompromised (far more so than polysaccharide vaccine) [25–27]	94–97% protective against all invasive disease due to serotypes in vaccine [28] 57% effective against otitis media caused by vaccine serotypes, but only 6% against all otitis media due to serotype replacement [29] Serotype 19F immunogenic, but poorly protective [29]
Neisseria meningitidis	Diphtheria toxoid (modified) Tetanus toxoid	Highly immunogenic from infancy [31,32] (conjugate far more immunogenic than polysaccharide [30–32])	92% protective in toddlers and 97% in teenagers [33]
Salmonella typhi	*Pseudomonas aeruginosa* exotoxin A	Immunogenic in humans over 2 years old [34,35]	91.5% protective in 2–5-year-old children [35]
Group B streptococcus	Tetanus toxoid Cholera toxin B (nasal)	Immunogenic in animals [36–39] and adult humans [40]	Protective in animals [36–39] No human data on protection
Staphylococcus aureus	*Pseudomonas aeruginosa* exotoxin A	Immunogenic in animals [41,42] and humans	A single dose had 57% efficacy over 40 weeks in patients receiving hemodialysis [42]

The differences between conjugate and polysaccharide vaccines are most marked for infants and for the immunocompromised. Infants mount a poor response to polysaccharides: this explains the incidence of meningitis due to organisms with a polysaccharide outer capsule being highest in the first 2 years of life. Immunocompromised patients, including those with HIV infection, may also respond poorly to polysaccharides, and again conjugate vaccines have given a stronger immune response [14].

Haemophilus influenzae type b (Hib)

Protection against Hib has long been known to correlate with antibodies to the outer polysaccharide type b capsule [15], made of polyribosyl ribitol phosphate (PRP). The natural immune response to PRP is poor under 18–24 months of age, and this is the age of highest incidence of disease in non-immunized populations. Early studies in Finland, in which purified PRP was used as a vaccine, showed a strong antibody response in children aged 2–6 years, an intermediate response from 18 to 24 months of age, and no response at all under 18 months of age [16]. The introduction of the PRP vaccine resulted in a reduction in infections in children over 2 years of age, but no reduction in younger children [17].

When the Hib conjugate vaccines became available, one typical immunization schedule for infants involved injections at 2, 4 and 6 months, with a booster dose at between 9 and 15 months. Four different *H. influenzae* conjugate preparations were compared in four trials. PRP was conjugated as follows: PRP–OMP, PRP–CRM197 and PRP–T. An oligosaccharide–D (HbOC) preparation was also made. PRP–OMP was found to be the most potent; a response occurred after the first administration, and antibody titers >10 mg/L were achieved. HbOC was the least potent [4]. Antibody titers waned after the third dose, but responses to all the preparations were significantly boosted after the final dose. All preparations except HbOC were licensed for use in the USA.

There is evidence that the conjugate vaccine induces a substantial B-cell memory response, as judged by a large increase in the level of specific IgG formed, together with a significant increase in antibody avidity after a boosting immunization. Infants were immunized with PRP–T, diphtheria–

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tetanus–acellular pertussis (DTP) and oral polio vaccine at 2, 3 and 4 months of age [18]. A booster dose of the conjugate was given at 1 year of age. Serum samples were obtained at 5, 11 and 13 months of age. Mean specific IgG levels at these three times were 6.23, 0.40 and 139.86 μg/mL. The mean avidity index of 0.28 at 5 months had increased to 0.52 at 13 months. It is, of course, too early to know whether the protection achieved by this immunization schedule persists for many years, but the results are encouraging.

The introduction of conjugate vaccines led to a rapid and sustained reduction in all Hib infections [17,19]. This reduction was more precipitate than would have been expected from the vaccine coverage, and further studies [20,21] showed that the Hib conjugate vaccines induced a local immune response, which resulted in reduced nasal carriage of Hib, and thus to reduced exposure of susceptible organisms. Thus, there was a herd immune effect of Hib immunization. In populations where one of the Hib conjugate vaccines has been introduced as a universal infant vaccine, including the USA, the UK, Finland, Iceland and Australasia, the reduction in all Hib infections has been between 95% and 100% [3,17,19,22–24]. Effective vaccines have used tetanus toxoid, CRM197 or OMPs of *N. meningiditis* as carrier proteins. When tetanus toxoid is used as the carrier protein and the infant has been primed with a prior dose of tetanus toxoid, the antibody response to PRP is enhanced ('carrier priming'). In the UK, infants aged 3–11 months were highly protected within 1 week of their first dose of PRP–T vaccine [22]. *H. influenzae* disease has the great advantage of being caused predominantly by a single serotype, Hib.

Streptococcus pneumoniae

In contrast to Hib, there are 90 serotypes with different capsular polysaccharides, and seven of these together cause about 85% of invasive pneumococcal infections in Finnish, Israeli and US children [25]. Pneumococcal polysaccharide vaccines (PPVs) are available which contain the 23 most common pneumococcal serotypes. Children under 2 years of age respond poorly to all serotypes, and even respond less well than adults to some less immunogenic serotypes (6B, 14, 19F, 23F) up to 5–10 years of age [26,27].

Pneumococcal conjugate vaccines require the individual conjugation of each serotype to a carrier protein. Most pneumococcal conjugate vaccines have involved the same carrier proteins as used for Hib, although novel carriers are under investigation.

A heptavalent pneumococcal conjugate vaccine, using CRM197 as carrier, has been used in two double-blind control trials. In the first study [28], comprising 37 868 infants immunized at 2, 4, 6 and 12 months, the vaccine was 97.4% protective against invasive disease caused by vaccine serotypes (93.9% on an intention-to-treat analysis). In the second study, which looked at acute otitis media [29], the same vaccine reduced the number of culture-confirmed pneumococcal cases of otitis media by 34% and episodes due to vaccine serotypes by 57%. The overall number of episodes of acute otitis media, however, was only reduced by 6% (95% CI 4–16%), due to serotype replacement.

Neisseria meningiditis

The serogroup B meningococcal polysaccharide, a homopolymer of sialic acid residues, is poorly immunogenic at any age. This may be because of structural similarities to brain glycoproteins. Purified capsular polysaccharides from serogroups A, C, W135 and Y have been used in vaccines. Serogroup C polysaccharide is not immunogenic in children under 2 years of age, although serogroup A polysaccharide is unusual in eliciting an antibody response and priming in infants as young as 3 months of age [30].

Conjugate vaccines using A and/or C polysaccharides are safe and immunogenic [31,32]. In the Gambia, however, while the C conjugate vaccine induced memory, the A conjugate did not [31]. The reasons for the different behaviors of A and C polysaccharides and conjugates are still being elucidated.

A meningococcal serogroup C conjugate vaccine, with CRM197 as the carrier, was introduced in the UK for infants, toddlers and teenagers [33]. The estimated short-term efficacy of the vaccine, based on surveillance data, was 97% for teenagers and 92% for toddlers (aged 1–2 years); the data for protection are not yet available.

Salmonella typhi

The capsular polysaccharide of *Salmonella typhi*, Vi, is an essential virulence factor, and is protective in children over 5 years of age. There is also a live attenuated vaccine available but it also is not very effective in young children. Conjugates have been developed using a *Pseudomonas aeruginosa*

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recombinant exoprotein A (rEPA) as a carrier. This vaccine is safe and immunogenic in children as young as 2 years of age [34], and proved to be 91.5% protective against infection in 2–5-year-old Vietnamese children [35].

Group B streptococcus

Group B streptococci (GBS) represent the leading cause of neonatal septicemia and meningitis in industrial countries. The response of normal adults to GBS polysaccharide vaccines is suboptimal [36]. Conjugate vaccines are immunogenic in laboratory animals [37–39], and a tetanus toxoid-based conjugate of type II was immunogenic in healthy women [40].

Others

A conjugate vaccine which is immunogenic in animals has been developed against *Staphylococcus aureus* [41,42] and is undergoing clinical trials. The safety, immunogenicity and efficacy of the vaccine with *S. aureus* types 5 and 8 capsular polysaccharides conjugated to rEPA from *P. aeruginosa* was assessed in patients with end-stage renal disease, who frequently have staphylococcal infections [43]. A single dose conferred partial immunity (57% efficacy) for approximately 40 weeks.

Escherichia coli polysaccharide conjugated to rEPA from *P. aeruginosa* [44] and a conjugate preparation from *Shigella* spp. [3] are also being developed.

Safety

Polysaccharide vaccines have been in use for several decades and have proved to be very safe. There has been less experience with conjugate vaccines but, to date, the safety record is also very good.

Suppressive effects—administration with other vaccines

There are now numerous reports (reviewed in [45]) that the vaccine diphtheria tetanus acellular pertussis (DTaP) administered in the same syringe with PRP–T induces significant suppression of the PRP and tetanus antibody responses, an effect considered to be due to antigenic (T) competition. This effect was not significant if the preparations were administered separately.

Comparison of the pneumococcal polysaccharide and conjugate vaccines

It is expensive to make one vaccine containing many conjugate preparations, each with a different polysaccharide specificity, to control a single disease caused by one agent, e.g. the 23 different specificities in the pneumococcal polysaccharide vaccine. Because of this, there is still some interest in looking for a way of making a polysaccharide vaccine more protective for the young, such as increasing the extent of interaction between the polysaccharide-binding B-cell and activated, non-antigen-specific T-cells.

Because of the complex immunization schedule, it would be advantageous to combine the conjugate vaccines with other vaccines. Suggested combinations include Hib/DTP(DTaP) and Hib/DTP/HepB.

The major advantage of conjugate vaccines is their ability to induce protective levels of immunity in very young children, thus meeting a major need. The Hib conjugate in particular has achieved this goal so effectively that there is now great interest in including this vaccine in global vaccination programs.

Will the advent of conjugate vaccines result in the phasing out of polysaccharide vaccines? Immunologically, there is reason to believe that the conjugate vaccines will be equally and very likely more effective than the corresponding polysaccharide vaccines in all age groups. This will happen over time, one exception being the 23-valent pneumococcal vaccine given, in particular, to the elderly. However, as administration of polysaccharide vaccines can boost the earlier response to the conjugate vaccine, boosting an earlier response to the heptavalent conjugate pneumococcal vaccine with the 23-valent polysaccharide vaccine would be rational. It could be adopted as a more effective way to protect the elderly against a wide range of pneumococcal strains.

CONCLUSION

There is no other example where the introduction of a new approach to vaccine development has had such a rapid and positive effect in preventing infections by a series of human pathogens. Live, attenuated viral vaccines have been highly successful in most cases, but their development has occurred over a prolonged period. The current

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progress with conjugate vaccines has occurred over two decades, and for a group of bacterial pathogens for which it had previously proved difficult to develop highly effective vaccines, especially for young children.

REFERENCES

1. Landsteiner K. *The specificity of serological reactions*. Cambridge: Harvard University Press, 1945.
2. Avery OT, Goebel WF. Chemo-immunological studies on conjugated carbohydrate proteins. II. Immunological specificity of synthetic sugar protein antigen. *J Exp Med* 1929; 50: 533–50.
3. Lindberg AA. Glycoprotein conjugate vaccines. *Vaccine* 1999; 17: S28–36.
4. Stein KE. Thymus-independent and thymus-dependent response to polysaccharide antigens. *J Infect Dis* 1992; 165(suppl 1): S49–52.
5. Ward JI, Zangwill KM. *Haemophilus influenzae* vaccines. In: Plotkin SA, Orenstein WA, eds. *Vaccines*, 3rd edn. Philadelphia: WB Saunders, 1999: 183–221.
6. Baker PJ. T cell regulation of the antibody response to bacterial polysaccharide antigens: an examination of some general characteristics and their implications. *J Infect Dis* 1992; 165(suppl): S44–8.
7. Breukels MA, Zandvoort A, Van Den Dobblesteen GP *et al.* Pneumococcal conjugate vaccines overcome splenic dependency of antibody response to pneumococcal polysaccharides. *Infect Immun* 2001; 69: 7583–7.
8. Rodrigo M-J, Miravitlles M, Cruz M-J *et al.* Characterization of specific immunoglobulin G (IgG) and its subclasses (IgG1 and IgG2) against the 23-valent pneumococcal vaccine in a healthy adult population: proposal for response criteria. *Clin Diagn Lab Immunol* 1997; 4: 168–72.
9. Mattern TF, Hans-Dieter L, Brade ET *et al.* Stimulation of human T lymphocytes by LPS is MHC-non-restricted but strongly dependent on B7 interactions. *J Immunol* 1998; 160: 3412–16.
10. Born W, Cady C, Jones-Carson J *et al.* Immunoregulatory functions of γ,δT cells. *Adv Immunol* 1999; 71: 77–124.
11. Hayday AC. γ,δ Cells: a right time and a right place for a conserved third way of protection. *Annu Rev Immunol* 2000; 18: 975–1026.
12. Noelle RJ, Ledbetter JA, Aruffo A. CD40 and its ligand, an essential ligand-receptor pair for thymus-dependent B cell activation. *Immunol Today* 1992; 13: 431–3.
13. Hasbold J, Lyons AB, Kehry MR, Hodgkin PD. Cell division number regulates IgG1 and IgE switching of B cells following stimulation by CD40 ligand and IL-4. *Eur J Immunol* 1998; 28: 1040–51.
14. Weinberg GA, Granoff DM. Immunogenicity to *Haemophilus influenzae* type b conjugate vaccines in children with conditions associated with impaired antibody responses to type b polysaccharide vaccine. *Pediatrics* 1990; 85: 654–61.
15. Kayhty H, Peltola H, Karanlo V, Makela PH. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. *J Infect Dis* 1983; 47: 1100–1.
16. Peltola H, Kayhty H, Sivonen A. *Haemophilus influenzae* type b capsular polysaccharide vaccine in children: a double-blind field trial of 100,000 vaccinees 3 months to 5 years of age in Finland. *Pediatrics* 1977; 60: 730–7.
17. Adams WG, Deaver KA, Cochi SL *et al.* Decline of childhood *Haemophilus influenzae* type b (Hib) disease in the Hib vaccine era. *JAMA* 1993; 269: 221–6.
18. Goldblatt D, Pinto Vaz AJPM, Miller E. Antibody avidity as a surrogate marker of successful priming by *Haemophilus influenzae* type b conjugate vaccines following infant immunization. *J Infect Dis* 1998; 177: 1112–15.
19. Peltola H, Kilpi T, Anttila M. Rapid disappearance of *Haemophilus influenzae* type b meningitis after routine childhood immunisation with conjugate vaccines. *Lancet* 1992; 340: 592–4.
20. Schuchat A, Robinson K, Wenger JD *et al.* Bacterial meningitis in the United States in 1995. *N Engl J Med* 1997; 337: 970–6.
21. Takala AK, Escola J, Lievonen M. Reduction of oropharyngeal carriage of the *Haemophilus influenzae* type b (Hib) in children immunised with an Hib conjugate vaccine. *J Infect Dis* 1991; 164: 982–6.
22. Barbour ML, Mayon-White RT, Coles C, Crook DWM, Moxon ER. The impact of conjugate vaccine on carriage of *Haemophilus influenzae* type b. *J Infect Dis* 1995; 171: 93–8.
23. Booy R, Heath PT, Slack MPE, Begg N, Moxon ER. Vaccine failures after primary immunisation with *Haemophilus influenzae* type b. *Lancet* 1997; 349: 1197–202.
24. Jonsdotir KE, Steingrimsson O, Olafsson O. Immunisation of infants in Iceland against *Haemophilus influenzae* type b. *Lancet* 1992; 340: 252–3.
25. Eskola J, Kayhty H. Pneumococcal vaccines. *Clin Paediatr* 1997; 5: 101–20.
26. Robbins JB, Austrian R, Lee CJ. Consideration for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. *J Infect Dis* 1983; 148: 1136–59.
27. Lievonen M, Sakkinen A, Kalliokoshi R. Antibody response to pneumococcal capsular polysaccharide vaccine in pre-school age children. *Pediatr Infect Dis* 1986; 5: 39–44.
28. Black S, Shinefeld H, Fireman B *et al.* Efficacy, safety and immunogenicity of heptavalent pneu-

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mococcal conjugate vaccine. *Pediatr Infect Dis J* 2000; 19: 187–95.
29. Eskola J, Kilpi T, Palmu A *et al.* Efficacy of a pneumococcal conjugate vaccine against otitis media. *N Engl J Med* 2001; 344: 403–9.
30. Makela PH, Peltola H, Kayhty H. Polysaccharide vaccines of group A *Neisseria meningitidis* and *Haemophilus influenzae* type b: a field trial in Finland. *J Infect Dis* 1996; 174: 1360–3.
31. Leach A, Twumasi PA, Kumah S *et al.* 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: 200–4.
32. 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: 1685–9.
33. Ramsay ME, Andrews N, Kaczmarski EB, Miller E. Efficacy of meningococcal serogroup C conjugate vaccine in teenagers and toddlers in England. *Lancet* 2001; 357: 195–6.
34. Kossaczkz Z, Lin FY, Ho VA *et al.* Safety and immunogenicity of Vi conjugate vaccines for typhoid fever in adults, teenagers, and 2- to 4-year-old children in Vietnam. *Infect Immun* 1999; 67: 5806–10.
35. Lin FYC, Vo AH, Khiem HB *et al.* The efficacy of a *Salmonella typhi* Vi conjugate vaccine in two- to five-year-old children. *N Engl J Med* 2001; 344: 1263–9.
36. Baker CJ, Rench MA, Edwards MS *et al.* Immunisation of pregnant women with a polysaccharide vaccine of group B streptococcus. *N Engl J Med* 1988; 319: 1180–5.
37. Wessels MR, Paoletti LC, Guttormsen HK *et al.* Structural properties of group B streptococcal type III polysaccharide conjugate vaccines that influence immunogenicity and efficacy. *Infect Immun* 1998; 66: 2186–92.
38. Shen X, Lagergard T, Yang Y *et al.* Preparation and preclinical evaluation of experimental group B streptococcus type III polysaccharide–cholera toxin B subunit conjugate vaccine for intranasal immunization. *Vaccine* 2000; 19: 850–61.
39. Marques MB, Kasper DI, Shroff A *et al.* Functional antibodies to the group B streptococci elicited by a polysaccharide–protein conjugate vaccine. *Infect Immun* 1994; 62: 1593–9.
40. Baker CJ, Paoletti LC, Rench MA *et al.* Use of capsular polysaccharide–tetanus toxoid conjugate vaccine for type II group B streptococcus in healthy women. *J Infect Dis* 2000; 182: 1129–38.
41. Fattom AI, Sarwar J, Basham L *et al.* Antigenic determinants of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharide vaccines. *Infect Immun* 1998; 66: 4588–92.
42. Fattom A, Li X, Cho YH *et al.* Effect of conjugation methodology, carrier protein and adjuvants on the immune response to *Staphylococcus aureus* capsular polysaccharides. *Vaccine* 1995; 13: 1288–93.
43. Shinefield H, Black S, Fattom A *et al.* Use of a *Staphylococcus aureus* conjugate vaccine in patients receiving hemodialysis. *N Engl J Med* 2002; 346: 491–6.
44. Cryz SR Jr, Que JO, Cross S, Furer E. Synthesis and characterization of a polyvalent *Escherichia coli* O-polysaccharide–toxin A conjugate vaccine. *Vaccine* 1995; 13: 449–53.
45. Daum RS, Zenko CE, Given GZ *et al.* Magnitude of interference after diphtheria–tetanus–acellular pertussis/*H. influenzae* type b capsular polysaccharide–tetanus vaccination is related to the number of doses administered. *J Infect Dis* 2001; 184: 1293–9.

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