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Review

Comparative effects of carrier proteins on vaccine-induced immune response

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ABSTRACT

The efficacy of vaccines against major encapsulated bacterial pathogens – *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* type b (Hib) – has been significantly enhanced by conjugating the respective polysaccharides with different carrier proteins: diphtheria toxoid; non-toxic cross-reactive material of diphtheria $toxin_{197}$, tetanus toxoid, *N. meningitidis* outer membrane protein, and non-typeable *H. influenzae*-derived protein D. Hib, meningococcal, and pneumococcal conjugate vaccines have shown good safety and immunogenicity profiles regardless of the carrier protein used, although data are conflicting as to which carrier protein is the most immunogenic. Coadministration of conjugate vaccines bearing the same carrier protein has the potential for inducing either positive or negative effects on vaccine immunogenicity (immune interference). Clinical studies on the coadministration of conjugate vaccines reveal conflicting data with respect to immune interference and vaccine efficacy.

Contents

1. Introduction

Among the major encapsulated bacterial pathogens – *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* type b (Hib) – the polysaccharide capsule is immunogenic,[1] and increases bacterial virulence. Polysaccharide vaccines were developed based on an appreciation of the importance of bactericidal anticapsular antibodies in protection against bacterial meningitis. Polysaccharide vaccines are safe and have good short-term immunogenicity in older children and adults; however, these vaccines have serious limitations that have restricted widespread use, including poor immunogenicity in young children and no durable immunologic memory [1]. Because children less than 2 years of age are unable to mount an adequate immune response to bacterial capsule polysaccharide antigens, they are the most susceptible to infection [2,3].

Conjugate vaccines have overcome the shortcomings of polysaccharide vaccines by triggering a T-cell-dependent antigen response [1]. Conjugating the capsular polysaccharide to a carrier protein led to the development of highly effective conjugate vaccines superior to that of the respective polysaccharide vaccines

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[1] With the exception of meningococcal serogroup B.

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, stimulating T helper cells that promote B-cell activation and immunological memory for longer term immunogenicity [2–4]. Although several carrier proteins, such as non-toxic cross-reactive material of diphtheria toxin$_{197}$ (CRM$_{197}$) and tetanus toxoid (TT), are commonly used for a variety of vaccines, differences have yet to be established between carrier proteins with respect to improved vaccine potency.

The aim of this review is to: (1) give a brief overview of the physicochemical differences among carrier proteins; (2) provide a critical evaluation of the relative clinical safety and efficacy of carrier proteins used in different conjugate vaccines, focusing on meningococcal, Hib, and pneumococcal vaccines; and (3) discuss whether conjugate vaccines can be differentiated based on a specific carrier protein.

2. Physicochemical characteristics of carrier proteins

Five carrier proteins have been developed, including TT, diphtheria toxoid (DT), CRM, *N. meningitidis* outer membrane protein (OMP) [2,5,6], and non-typeable *H. influenzae*-derived protein D (PD) [7]. The chemical characteristics of each are shown in Fig. 1 [2,5–8].

Each carrier protein is derived from diverse pathogens, such as *Clostridium tetani*, *Corynebacterium diphtheriae*, *N. meningitidis* serogroup B strain 11, and *H. influenzae* [5–8].

Detoxification appears to be the main feature differentiating the three carrier proteins most commonly used in meningitis vaccines. Fig. 1 shows a ribbon diagram of the CRM protein with lysine residues (purple spheres), which are potential antigen binding sites and are conserved on CRM. Unlike TT and DT, CRM does not require detoxification with formaldehyde, a chemical that can induce epitope modification through extensive cross-linking of carrier protein to accessory antigens [2,9]. This process may ultimately interfere with vaccine immunogenicity.

From a purely physicochemical perspective, size is a possible differentiating factor for the most common carrier proteins, DT, TT, and CRM. DT and CRM are diphtheria toxoid-based and are roughly the same size (62 kDa) [10]. TT is 140 kDa in size, 2.25 times larger than DT and CRM [11]. The minor carrier proteins OMP and PD are smaller, 37 kDa and 42 kDa, respectively [8,12]. However, the clinical implications of carrier protein size are unclear.

3. Safety of vaccines conjugated with different carrier proteins

Carrier proteins are generally safe and well established, as can be inferred from their use in several currently licensed conjugate vaccines. Additionally, a select number of studies have compared the safety of similar polysaccharide vaccines conjugated to various carrier proteins.

A comparative study of Hib polyribosylribitol phosphate (PRP) conjugate vaccines showed local reactions – redness, pain, and swelling – were less frequent in children receiving vaccine with carrier protein DT and CRM than OMP and TT after three doses of vaccine (Fig. 2) [13]. The study was not designed to assess statistical differences between each group, but rather to determine whether reaction rates from the groups represented the same population. Significant differences ($p \leq 0.05$) for reaction rate reporting were found between vaccine groups for pain and redness on the evening after vaccination and for redness and swelling the morning after vaccination. After the first injection, but not subsequent injections, TT showed higher rates of irritability, crying, and fever than CRM, DT, and OMP conjugate vaccines [13]. Postmarketing adverse events (AEs) associated with Hib-OMP include lymphadenopathy,

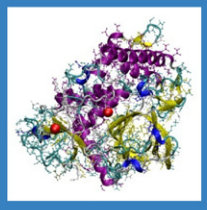
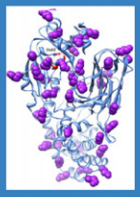
Tetanus Toxoid (TT)	Diphtheria Toxoid (DT)	Cross-Reactive Material 197 (CRM$_{197}$)	*N. meningitidis* Outer Membrane Protein (OMP)	Non-Typeable *H. influenzae* Derived Protein D (PD)
				
• Derived from *Clostridium tetani* • Inactivated with formalin • Purified with ammonium sulfate and filter sterilized prior to conjugation process	• Derived from *Corynebacterium diphtheriae* • Detoxified with formaldehyde • Purified by ammonium sulfate fractionation and diafiltration	• Enzymatically inactive, nontoxic mutant of diphtheria toxin • Requires no formaldehyde detoxification • Obtained at near 100% purity	• Outer membrane protein complex derived from *N. meningitidis* serogroup B strain 11 • Purified by detergent extraction, ultracentrifugation, diafiltration, and sterile filtration	• Antigenically conserved surface lipoprotein found in all *H. influenzae* • Used in a nonacylated, antigenically active form
• 140 kD	• 63 kD	• 63 kD	• 37 kD	• 42 kD

Fig. 1. Carrier proteins used in *Haemophilus influenzae* b, *Neisseria meningitidis*, and *Streptococcus pneumoniae* conjugate vaccines [2,5–8].

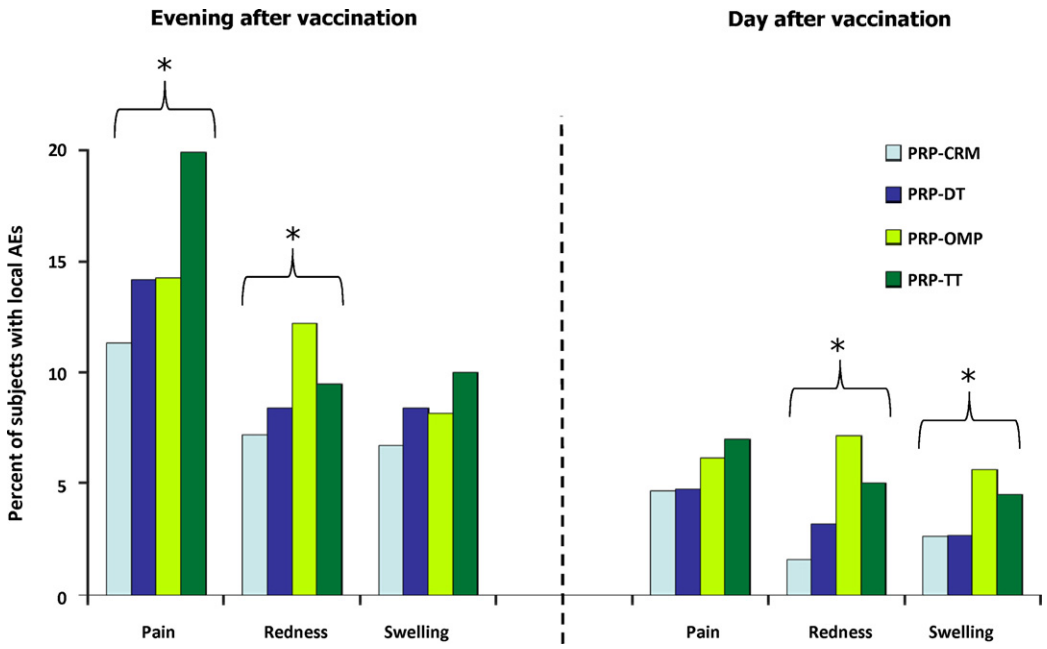


Fig. 2. Local adverse reactions to *Haemophilus influenzae b* conjugate vaccines with different carrier proteins (derived from [13]). *$p < 0.05$ when testing the null hypothesis that the four vaccine arms represent a single population whose reaction rates do not differ. The tetanus toxoid image is a generous gift from Damian Allis and Robert Doyle (Syracuse University, NY). AEs = adverse events; PRP = polyribosylribitol phosphate; PRP-CRM = conjugated with cross-reacting mutant diphtheria protein; PRP-DT = diphtheria toxoid conjugated vaccine; PRP-OMP = conjugated with outer membrane protein of *Neisseria meningitidis*; PRP-TT = tetanus toxoid conjugated vaccine.

hypersensitivity, and sterile injection site abscess, and febrile seizures [7]. Hypersensitivity to meningococcal polysaccharide C conjugated with TT vaccine (MenC-TT) and Hib-TT vaccines has also been reported, resulting in anaphylaxis (bronchospasm, facial edema, angiodema, urticaria, hypotension, or fainting) [14–16].

The comparative safety of a single muscular injection of quadrivalent meningococcal CRM_{197}-conjugated vaccine (MenACWY-CRM) or MenACWY-DT vaccines was studied in a multicenter phase III clinical study of 2170 adolescents [17]. Adverse events observed 30 min after injection showed a similar incidence between both vaccines. The most common AE was pain, experienced in 44% and 53% of MenACWY-CRM and MenACWY-DT subjects, respectively, which was mild in most subjects (>75%) in both groups. Induration and erythema were mostly mild and occurred in ≤16% of subjects for both groups. Systemic reactions were infrequent and mild and fever was uncommon (<1% subjects in both groups) [17]. This study did not include statistical analysis of safety endpoints.

The safety profile of MenACWY-TT was comparable to a licensed control, MenC-CRM, in a study of 240 toddlers 12–14 months of age. The incidence of solicited local and systemic symptoms was similar between MenACWY-TT and MenC-CRM ($p > 0.05$ for all comparisons) [18].

CRM is a well-established carrier protein used in several currently licensed conjugate vaccines with a proven safety profile with respect to both local and systemic effects (i.e., Prevnar®, VaxemHib®, Menjugate®, and Meningitec®) [13,19–23]. Recent postmarketing surveillance for Prevnar® (pneumococcal 13-valent conjugate vaccine [diphtheria CRM_{197} protein]) has shown no important safety concerns[2] for the vaccine [24].

Whether any single carrier protein can be differentiated based on overall safety of conjugate vaccines remains unclear, because AEs were mild for most carrier proteins, although hypersensitivity may be more common with Hib-OMP and Hib-TT conjugate vaccines [13].

4. Comparative clinical efficacy of vaccines conjugated with different carrier proteins

4.1. Immunogenicity

4.1.1. Meningococcal vaccines

Both CRM and TT carrier proteins conjugated to meningococcal group C (MenC) capsule polysaccharide antigens induce excellent early and sustained immune responses, as shown in the successful development and use of MenC vaccination programs in the United Kingdom and Canada [16,19,20,25–27]. Several clinical studies showed 96%, 100%, and 98% of infants responded to Meningitec® (Wyeth, Quebec, Canada), Menjugate® (Novartis Vaccines and Diagnostics, Sienna, Italy), and NeisVac-C® (Baxter International Inc., Deerfield, IL, USA/GlaxoSmithKline, Mississauga, Canada) after vaccination at 2 and 4 months, with serum bactericidal antibody (SBA) titers of 1:8 or more 1 month after primary vaccination series [16,19,20,27]. Similar responsiveness (range: 98–100% of infants) was observed when Meningitec®, Menjugate®, or NeisVac-C® was given at 2, 3, and 4 months [16,19,20].

Comparison of these three conjugate vaccines showed the highest protection was achieved with NeisVac-C® 1 month after the first vaccination at 2 months of age (97% of subjects with SBA ≥1:8), compared with Meningitec® (53% of subjects with SBA ≥1:8) and Menjugate® (80% of subjects with SBA ≥1:8) [27]. However, Meningitec® (96%) and Menjugate® (100%) produced comparable responses to NeisVac-C® (98%) after one additional dose at 4 months [27]. The study also investigated a 2-dose schedule administered at 2 and 3 months. Pooled immunogenicity data after any 2-dose schedule showed similar protective immune responses (SBA ≥1:8) were elicited for Meningitec®, Menjugate®, and NeisVac-C®

[2] National regulatory agencies provide guidelines that identify what constitutes an "important safety concern." There is currently no universally accepted definition for this language that applies across national agencies. For more information regarding the Food and Drug Administration guidance referred to in the footnoted statement above, please visit http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm092257.pdf.

(98%, 99% and 99%, respectively) [27]. The success of these three conjugate vaccines was further emphasized by the fact that each induced immunologic memory.

In a toddler study, 226 children 12–18 months old were randomized to receive one of three meningococcal serogroup C vaccines (MCC), with a C polysaccharide booster 6 months later. One month post-vaccination, 91–100% of subjects had SBA ≥1:8. MCC-TT induced higher SBA geometric mean titers (GMTs) and higher proportions with titers ≥1:8 than did MCC-CRM vaccines [28]. The decline in MenC antibodies 6 months after primary vaccination was significantly increased 7- to 11-fold for each conjugate vaccine after administration of a booster meningococcal AC polysaccharide vaccine in children, and increases in antibody avidity were observed after booster vaccination for each conjugate vaccine [28].

Menveo® (Novartis Vaccines and Diagnostics, Cambridge, Massachusetts, USA) and Menactra® (Sanofi Pasteur, Swiftwater, PA, USA) are meningococcal conjugate quadrivalent vaccines, incorporating *N. meningitidis* serogroups A, C, W-135, and Y (MenACWY) that have been developed using CRM and DT carrier proteins, respectively [6,29].

MenACWY-CRM (Menveo®) induced significant SBA titers to each serogroup in infants receiving vaccine at 2, 3, and 4 months (titers ≥1:4 in >92% of children); waning antibody responses to each meningococcal serogroup necessitated a booster dose at 12 months to confer protection [26]. MenACWY-CRM was effective in all age groups, including infants, and is approved for persons 11–55 years of age in the United States [29] and for persons ≥11 years of age elsewhere. Unlike Menveo®, the efficacy and safety of Menactra® (MenACWY-DT) has yet to be established in infants [6,29].

The comparative immunogenicity of a single injection of MenACWY-CRM and MenACWY-DT was determined in a multicenter phase III clinical study of 2170 adolescents [17]. Human complement SBA (hSBA) GMTs for each serogroup were consistently higher for MenACWY-CRM than for MenACWY-DT – the ratio of GMTs for MenACWY-CRM to MenACWY-DT ranged from 1.63 (95% CI, 1.31–2.02) for serogroup A to 2.82 (95% CI, 2.26–3.52) for serogroup Y [17].

MenACWY-TT is under development, and is currently being compared with MenACWY-DT [30].

Unlike previous quadrivalent meningococcal vaccines, MenACWY-CRM has also demonstrated immunogenicity in infants from 2 months of age onward in phase II studies [26,31]. MenACWY-DT has not demonstrated adequate immunogenicity in this age group [6,29,32]. Comparative studies with MenACWY-TT are needed to determine whether differences in immunogenicity among other conjugate vaccines also occur in infants.

Comparatively, there is a lack of broadly effective vaccines for prevention of serogroup B meningococcal disease, which accounts for >50% of all meningococcal disease cases globally. However, recombinant protein vaccines, including factor H binding protein (fHbp) and fHbp with other antigens, are in late-stage clinical development and may be effective against most serogroup B strains [33]. Thus, the prospects have never been better for developing vaccines for the prevention of meningococcal disease, including that caused by serogroup B strains.

4.1.2. Hib vaccines

CRM, TT, OMP, and DT carrier proteins have also been developed for enhancing the immunogenicity of Hib vaccines by conjugation with Hib PRP antigen [34]. In a multicenter randomized immunogenicity trial of 458 healthy infants, OMP, CRM, and TT vaccines were administered at 2, 4, and 6 months; all subjects received concomitant vaccination with diphtheria-tetanus-pertussis (DTP) and oral polio vaccine (OPV) [35]. After the first and second dose, PRP-OMP was more immunogenic than PRP-CRM and PRP-TT, as shown by a significantly higher geometric mean concentration (GMC) of anti-PRP antibody (4.0 μg/mL versus 0.5 μg/mL and 1.25 μg/mL, respectively; $p < 0.001$). After the third dose, all three PRP conjugate vaccines were equally immunogenic [35]. A similar pattern was observed when immunogenicity was assessed by the proportion of subjects with serum antibody response >1.0 μg/mL at 2, 4, and 6 months: PRP-OMP showed a high incidence of immunogenicity after the first dose (80%) that was sustained through the second and third doses (range 85–88%) [35]. In contrast, PRP-CRM and PRP-TT responses remained low (23% versus 56%) until the third dose, when the incidence increased to 90% and 97%, respectively [35].

A similar multicenter study of 252 infants compared the immunogenicity of PRP-DT, PRP-OMP, PRP-TT, and PRP-CRM vaccines after immunization at 2, 4, and 6 months of age, in which all subjects received concomitant vaccination with DTP and OPV [13]. The anti-PRP geometric mean titer (GMT) was significantly higher for PRP-OMP than for PRP-CRM, PRP-TT, and PRP-DT after one immunization (0.83 μg/mL versus 0.09 μg/mL, 0.05 μg/mL, and 0.06 μg/mL; $p \leq 0.05$), which was sustained after the second immunization, in agreement with the study by Granoff and colleagues [35]. However, the superior immunogenicity of PRP-OMP was not sustained after the third immunization [13]. When assessing the proportion of subjects with a titer of ≥1 μg/mL after the third immunization, anti-PRP antibody was higher in the PRP-TT and PRP-CRM groups (83% and 75%) than in the PRP-OMP and PRP-DT groups (55% and 29%), suggesting the CRM and TT carrier proteins conferred the highest degree of protection [13].

In conclusion, given the similarity between both study protocols, it is impossible to determine which carrier protein confers optimal immunogenicity for Hib vaccination; although CRM and TT showed consistently high immunogenicity after three immunizations in both studies, DT is generally poorly immunogenic in the infant age group. Data from six clinical studies using PRP-TT conjugate vaccine suggested little difference in immunogenicity after either two or three doses of conjugate vaccine in a primary series of PRP-TT vaccination in infants [36]. Because both doses conferred acceptable responses above those recommended by the World Health Organization, the number of PRP-TT vaccine doses could be reduced for effective primary vaccination [36]. PRP-OMP showed good immunogenicity when given in only two doses as a monovalent PRP-OMP vaccine, or as PRP-OMP combined with hepatitis B [36]. The claim that PRP-OMP evokes a more rapid immune response needs to be validated by further comparative studies with PRP-carrier proteins.

4.1.3. Pneumococcal vaccines

Multivalent pneumococcal (Pnc) conjugate vaccines containing the CRM carrier protein are highly immunogenic. The efficacy of Pnc7-CRM was demonstrated in a multicenter, randomized, double-blind clinical trial of 37,868 infants. Healthy infants received one or more doses of Pnc7-CRM ($N = 18{,}927$) or MenC-CRM (control group; $N = 18{,}941$) at 2, 4, 6, and 12–15 months of age. Routine childhood vaccines (e.g., diphtheria, tetanus, whole cell pertussis [DTwP]; diphtheria, tetanus, acellular pertussis [DTaP]; OPV; inactivated poliovirus [IPV]; Hib; hepatitis B virus [HBV]), and measles-mumps-rubella, were given as recommended [37]. Substantial antibody responses to each of the 7 polysaccharide antigens were elicited by Pnc7-CRM after the third dose, ranging from GMCs of 0.086–0.629 μg/mL predose to 1.207–5.041 μg/mL after dose 3. GMCs for all but the 6B and 14 serotypes fell by 1 μg/mL prior to boosting (predose 4). Reverse distribution accumulation analyses after dose 3 showed antibody concentrations ≥0.5 μg/mL in >80% of subjects receiving Pnc7-CRM, compared with antibody concentrations ≤0.5 μg/mL in >90% of control recipients [37]. For the fully vaccinated group, invasive disease occurred in only one subject in the Pnc7-CRM vaccine group, compared with 39 subjects in the control group, indicating significant efficacy for

Pnc7-CRM of 97.4% (95% CI, 82.7–99.9%; $p < 0.0001$) [37]. Analysis for all cases of pneumococcal disease, including those unrelated to the Pnc7-CRM serotypes, revealed a significant reduction in the total invasive pneumococcal disease burden of 81.9% (95% CI, 73.7–95.85%; $p < 0.001$) of children receiving one or more doses of Pnc7-CRM vaccine [37].

The multivalent pneumococcal vaccine, Pnc11, has also demonstrated improved immunogenicity and protection against otitis media when conjugated to PD (Pnc11-PD). In a randomized, double-blind study of 4968 infants 6 weeks to 5 months of age, the efficacy of Pnc11-PD was compared with control vaccine. Subjects received either Pnc11-PD ($n = 2455$) or hepatitis A vaccine (control group; $n = 2452$) at around 3, 4, 5, and 12–15 months. All subjects received concomitant DTaP-HBV-IPV/Hib. A subset of subjects was used to determine immunogenicity. Pnc11-PD contained 1 μg capsular polysaccharide of the pneumococcal serotypes 1, 3, 4, 5, 6B, 7F, 9 V, 14, 18 C, 19F, and 23F, each of which was conjugated to PD [38].

Antibody concentrations ≥0.2 μg/mL were observed 1 month after the third dose in >96% of infants for all pneumococcal serotypes (except 6B and 23F), compared with 0–10.2% of infants in the control group (except for serogroup 14, for which the incidence was 24%). After primary vaccination, most infants had antibodies to PD in the Pnc11-PD group, compared with 23% in the control group [38]. When measured against the first episode of acute otitis media due to pneumococcal serotypes, or non-typeable *H. influenzae*, Pnc11-PD vaccine efficacy was 52.6% ($p < 0.02$) and 31.1%, respectively, and lasted for at least 18 months [38].

In conclusion, both Pnc7-CRM and Pnc11-PD demonstrated good immunogenicity and protection against pneumococcal disease. These studies suggest that both CRM and PD offer similar advantages for development of multivalent pneumococcal vaccines.

The immunogenicity of Pnc4-TT and Pnc4-DT was tested in a double-blind, randomized, placebo-controlled clinical study of 75 infants 6–10 weeks of age. The Pnc4 vaccines contained serotypes 6B, 14, 19F, and 23F. Subjects received Pnc4-TT, or Pnc4-DT, in addition to DTP-IPV-PRP-TT at 2, 4, and 6 months of age, and OPV at 4 and 6 months ($n = 25$ for each group). A 23-valent nonconjugated pneumococcal polysaccharide vaccine was administered at 12 months (booster) with DTP-IPV-PRP-TT plus OPV. In the control group phosphate-buffered saline was substituted for Pnc4-TT ($n = 25$) [39].

The antibody GMCs for each serotype were significantly higher in the Pnc4-TT and Pnc4-DT groups after 4 and 6 months, compared with the control group ($p < 0.02$). The proportion of subjects with GMC ≥1 μg/mL was also significantly greater for Pnc4-TT and Pnc4-DT than for placebo ($p < 0.05$). Antibody levels tended to be higher after Pnc4-TT than after Pnc4-DT. Administration of nonconjugated pneumococcal polysaccharide vaccine at 12 months elicited a significant booster response to each serotype in both the Pnc4-TT and Pnc4-DT groups, compared with placebo ($p < 0.001$). The prebooster to postbooster GMC ratios ranged from 1.9 to 4.2 μg/mL for Pnc4-TT and 2.8 to 5.9 μg/mL for Pnc4-DT; however, antibody GMCs tended to be higher for Pnc4-TT than for Pnc4-DT [39].

In conclusion, although Pnc4-TT was slightly more immunogenic than Pnc4-DT – in agreement with previous studies on Hib-TT and Hib-DT conjugate vaccines – neither could be differentiated based on the type of carrier protein used. Both Pnc4 conjugate vaccines offer the potential for protection against pneumococcal disease in that they are immunogenic for four major serotypes responsible for >50% of invasive pneumococcal disease in infants [39].

Direct comparison between multivalent Pnc conjugate vaccines with different carrier proteins is needed to determine whether they differ in immunogenicity or protection against pneumococcal infection.

4.1.4. Summary

In summary, carrier proteins have made a major contribution to increasing the immunogenicity of numerous plain polysaccharide vaccines [25–28]. However, immunogenicity data for carrier proteins used in some conjugate vaccines is inconsistent and often conflicting, especially among the commonly used Hib conjugates. Consequently, it is difficult to predict which carrier protein offers the best clinical response to a given conjugate vaccine based on immunogenicity alone.

4.2. Immune response interference among carrier protein conjugate vaccines

Increased immunization of infants against a growing number of infectious agents led to the need for administration of several different vaccines simultaneously, or in the form of a combination vaccine [3]. There is some concern that concomitant vaccine administration or combination vaccines may alter the immunogenicity of vaccine antigens through immune interference, especially when consisting of multiple antigens with the same carrier protein [3,40]. Clinical data suggest immune response interference may occur after simultaneous administration of certain conjugate vaccines, but is not predicted by any specific carrier protein.

Proposed mechanisms by which immune interference may be accomplished include the following: increased carrier protein-specific T helper cell activity, leading to enhanced immunogenicity of a concomitantly administered conjugate vaccine with the same carrier protein; carrier-induced epitopic suppression (CIES), in which preexisting immunity to a given carrier protein suppresses the immune response to a polysaccharide antigen conjugated to the same carrier; and bystander interference, which may be induced by competition among different carrier proteins in coadministered vaccines for immune cells and mediators within the lymph nodes, and changes in T helper cell subtypes with possible modulation of T-cell regulation [41].

4.2.1. Conjugate vaccines using TT, CRM, and DT carrier proteins

Several clinical studies have shown that carrier proteins may influence the immune response to conjugate vaccines through positive or negative immune interference (summarized in Table 1). Carrier-specific immune interference was demonstrated in two parallel, placebo-controlled studies of 275 2-month-old infants after administration of Pnc4-TT vaccine with PRP-TT vaccines. Studies were conducted in Israel ($n = 75$) and Finland ($n = 200$), and subjects received the following vaccines at 2, 4, and 6 months of age: Pnc4-TT combined with DTP-IPV-PRP-TT (Israel study) or with DTP-PRP-TT (Finland study). Control groups received phosphate-buffered saline (placebo), or Pnc4-DT, plus the respective DTP-PRP-TT vaccine [42].

In the Israel study the GMC anti-PRP antibody response was significantly lower in the Pnc4-TT groups, compared with the Pnc4-DT and control groups, at 4 and 6 months ($p < 0.001$); a similar trend was observed in the Finland study. After the third dose a lower percentage of subjects had antibody ≥1 μg/mL in the Pnc4-TT group (83%) than in the Pnc4-DT (91%) or placebo (96%) groups in the Israel study, with a similar response in the Finland study. In the Finland cohort, reduced anti-PRP antibody response correlated with increases in the Pnc4-TT vaccine TT content. Reduced anti-TT antibody response also correlated with Pnc4-TT vaccine TT content [42].

The authors concluded that decreased anti-PRP antibody levels were related to TT carrier protein overload and immune interference, possibly through diminished antibody responses to TT carrier

Table 1
Carrier protein immune interference on anti-Hib (PRP) and anti-MenC antibodies: Summary of clinical studies [42–48].

Carrier protein interaction	Subject (dose months)	Vaccine combination	Control/comparator	Other vaccines	Change Hib antibody	Change MenC antibody	Change Pnc antibody
TT-TT [39]	Infant (2, 4, 6)	Pnc4-TT +DTP-IPV-PRP-TT	Pnc4-DT +DTP-PRP-TT	N/A	↓ GMC ↓ % ≥1 μg/mL	N/A	N/A
TT-TT [39]	Infant (2, 4, 6)	Pnc4-TT +DTP-PRP-TT	Pnc4-DT +DTP-PRP-TT	N/A	↓ GMC[a] ↓ % ≥1 μg/mL	N/A	N/A
TT-TT [41]	Infant (2, 3, 6)	MenC-TT +DTaP-IPV-Hib-TT	MenC-CRM +DTaP-IPV-Hib-TT	N/A	↑ GMC ↑ % ≥0.5 μg/mL ↑% ≥1 μg/mL	↓ GMT	N/A
TT-TT [41]	Infant (2, 3, 6)	MenC-TT +DTwP-OPV-Hib-TT	MenC-CRM +DTwP-OPV-Hib-TT	N/A	↑ GMC ↑ % ≥1 μg/mL	↑ GMT	N/A
TT-CRM [42]	Infant (2, 3, 4)	MenC-CRM +DTaP-Hib-TT +OPV	Prevaccination	N/A	↑ GMC [b]	N/A	N/A
TT-TT [43]	Infant (2, 3, 4)	MenC-TT +DTaP-HBV-IPV/Hib	MenC-CRM +DTaP-HBV-IPV/Hib-TT	N/A	↑ GMC	↓ GMT	N/A
CRM-CRM [44]	Infant (3, 4, 5, 6)	Pcn9-MenC-CRM+ HbOC	HbOC	DTaP+OPV	↑ GMC ↑ % ≥1 μg/mL	↑ % ≥2 μg/mL	N/A
CRM-CRM [45]	Infant (2, 3, 4)	Pcn9-MenC-CRM+ MenC-CRM	MenC-CRM	DTwP+PRP-TT+OPV	↓ GMC	↓ GMT ↓ % 1:8 ↓1:128	N/A
TT-TT [40]	Infant (2, 4, 6)	Pnc11-DT/TT+ DTaP/IPV/PRP-TT	Pnc11-DT/TT+ DTwP/IPV/PRP -TT	OPV, MMR	N/A	N/A	↓ GMC[c] ↓ % ≥1 μg/mL

Data from: Refs. [42–48].
aP = acellular pertussis; CRM = cross-reactive material of diphtheria toxin$_{197}$; DTP = diphtheria, tetanus, pertussis; GMC = geometric mean concentration; GMT = geometric mean titer; HbOC = Hib-CRM; HBV = hepatitis B virus; Hib = *Haemophilus influenzae* type b; IPV = inactivated polio virus; MenC = meningococcal group C; MMR = measles, mumps, and rubella; N/A = not applicable; OPV = oral polio virus; Pnc = pneumococcal; PRP = polyribosylribitol phosphate; TT = tetanus toxoid; wP = whole-cell pertussis.
[a] Finnish study: inverse correlation between anti-PRP antibodies and vaccine TT content.
[b] Compared with previous studies.
[c] Represents overall changes for the 7 anti-Pnc serotypes conjugated with TT.

protein and Hib polysaccharide epitopes through CIES [41,42]. Although anti-PRP antibody levels did not fall below protective levels, there is a danger that increasing the number of pneumococcal serotypes, and thus increasing TT content, may decrease anti-PRP antibodies below protective levels [42].

Dagan and colleagues showed that the efficacy of carrier proteins may be dependent on the type of vaccine coadministered with the conjugate vaccine [43]. Three groups of infants, 2 months of age, were studied, in which groups 1 (n = 63) and 2 (n = 70) received DTwP vaccine combinations, and group 3 (n = 54) received DTaP vaccine combinations. Each group received DT, TT, and Hib (PRP-TT) at 2, 4, 6, and 12 months, with IPV or OPV at various time points. An 11-valent Pnc vaccine, in which 7 serotypes were conjugated with TT and 4 serotypes were conjugated with DT (Pnc11-DT/TT), was coadministered to each group [43].

After primary or booster vaccination PncD/T11 with the combined vaccine containing whole-cell pertussis (wP) (DTwP/IPV/PRP-T), there were satisfactory responses to the pneumococcal polysaccharides. In contrast, when PncD/T11 was coadministered with an acellular pertussis (aP)-containing combination (DTaP/IPV/PRP-T), significantly lower antipneumococcal antibody GMCs were observed to pneumococcal polysaccharides conjugated to TT. The antipneumococcal antibody GMCs for serotypes conjugated to DT were not significantly decreased [43].

These data show that substitution of aP for wP had an inhibitory effect on the immunogenicity of pneumococcal polysaccharides conjugated to TT carrier protein, but not DT, emphasizing the difficulties in identifying effective conjugate vaccines with different carrier proteins when coadministered with multiple vaccines [43].

The effect of complex conjugate vaccine combinations on the immune response was further underscored in a study of the effects of combining MenC-TT or MenC-CRM with Hib conjugate vaccines on anti-PRP and anti-MenC antibody levels. A total of 241 infants received different conjugate vaccine combinations administered at 2, 3, and 4 months of age [44]. One group received DTaP-IPV-Hib-TT (Pediacel®; sanofi pasteur, Swiftwater, PA, USA) with MenC-TT or MenC-CRM, and the second group received DTwP-Hib-TT+OPV with MenC-TT or MenC-CRM [44].

The results are summarized in Fig. 3. At 4–6 weeks after the third dose of MenC-TT, combined with Pediacel®, or DTwP-Hib-TT+OPV, caused greater increases in anti-PRP antibody titers (GMCs) than did the combinations with MenC-CRM (Fig. 3). The combination of MenC-TT with Pediacel® reduced anti-MenC antibody GMTs below those found after combination of Pediacel® with MenC-CRM (Fig. 3). Conversely, the combination of MenC-TT with DTwP-Hib-TT+OPV increased anti-MenC antibody GMTs above those observed after combination of MenC-CRM with DTwP-Hib-TT+OPV (Fig. 3) [44]. These data suggest the Hib and MenC antibody responses were influenced by the MenC conjugate vaccine carrier protein – TT or CRM – when combined with Pediacel® or DTwP-Hib-TT+OPV. Contrary to the previous study by Dagan and colleagues [43], the TT carrier protein had the opposite effect on Hib immunity, increasing the anti-PRP antibody levels of the PRP-TT conjugate vaccine, an effect that was independent of the DTaP or DTwP vaccine components (Fig. 3) [42,44]. Possible explanations for these conjugate vaccine interactions are speculative, and include CIES, modulation of antigen presentation, and deficient T helper cell activity [44].

Interaction among TT carrier protein vaccines was further explored by Southern and colleagues [45]. Infants immunized with DTaP-Hib-TT and OPV plus MenC-TT at 2, 3, and 4 months showed progressive, significant increases in anti-Hib antibody GMCs, rising from 0.14 to 2.04 μg/mL after the first and third doses, respectively [45]. Anti-Hib antibody levels after the third vaccination were higher than previously observed in a study of DTaP-Hib-TT given alone or with MenC-CRM, suggesting that the Hib-TT conjugate vaccine enhanced the immune response to Hib [45]. This study was limited in that the conclusions were reached by comparison with historical studies. Also, the anti-Hib response measured Hib-specific IgG (enzyme-linked immunosorbent assay [ELISA]), not Hib anti-PRP antibody [45].

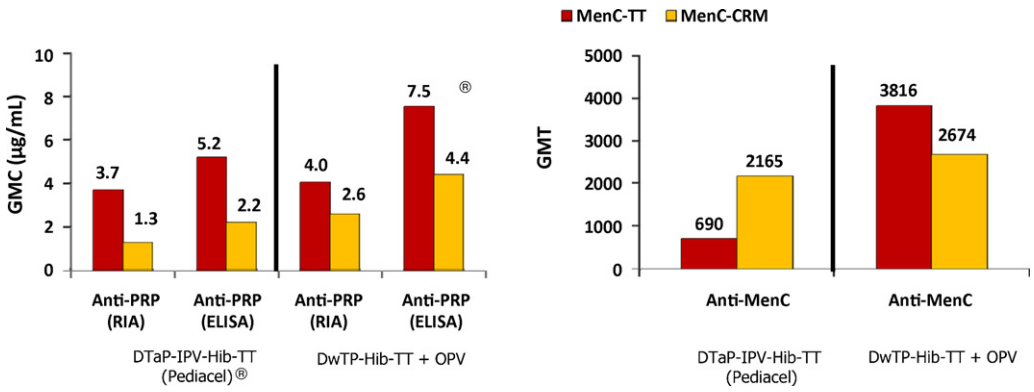


Fig. 3. Effects of TT and CRM carrier protein interactions on Hib (anti-PRP) and MenC (anti-MenC) immune responses [44]. DTaP = diphtheria, tetanus, acellular pertussis; DTwP = diphtheria, tetanus, whole cell pertussis; ELISA = enzyme-linked immunosorbent assay; GMC = geometric mean concentration; GMT = geometric mean titer; Hib = *Haemophilus influenzae* type b; IPV = inactivated polio virus; MenC-CRM = meningococcal polysaccharide C conjugated with cross-reactive material of diphtheria toxin$_{197}$; MenC-TT = meningococcal polysaccharide C conjugated with tetanus toxoid vaccine; OPV = oral polio virus; PRP = polyribosylribitol phosphate; RIA = radioimmunoassay; TT = tetanus toxoid.

The potential effects of carrier protein interactions among different conjugate vaccines on anti-PRP and anti-MenC antibody responses were explored in an open, randomized multicenter study of 520 healthy infants (mean age 11.3 weeks), vaccinated at 2, 3, and 4 months of age with MenC-TT, or MenC-CRM (control) [46]. The anti-MenC and PRP antibody responses were assessed in two treatment groups: MenC-TT given with a Hib-TT vaccine, Infanrix® hexa (DTaP-HBV-IPV/Hib; GlaxoSmithKline); and MenC-CRM given with a Hib-TT vaccine, Infanrix® hexa, as a control group [46]. MenC antibody GMTs were significantly lower for the MenC-TT vaccine combination group, compared with the MenC-CRM vaccine combination control group, after the second and third doses (no *p* values given). Anti-PRP antibody GMCs were significantly higher for the MenC-TT group, compared with the MenC-CRM control group, after the second and third doses (no *p* values given) [46].

The data show the TT carrier protein, used in a combination of TT conjugate vaccines, may exert both negative and positive immune interference on anti-MenC and anti-PRP antibody responses, respectively. The positive effects of TT conjugate vaccines on anti-PRP antibody responses to Hib-TT conjugate vaccines are in accord with the findings of Kitchin et al. and Southern et al., and may be associated with the common use of DTaP in the vaccine combinations [44–46]. However, this hypothesis is unclear, because coadministration of Pnc11-DT/TT with DTaP reduced the PRP-TT antibody response, compared with DTwP, in accord with the hypothesis that wP has an adjuvant effect on the TT immune response.

The negative effects of TT conjugate vaccines on MenC antibody titers after combination of MenC-CRM with Hib-TT conjugate vaccine are also similar to those found in the study by Kitchin et al., where DTaP was a common factor in the Hib-TT vaccines [44].

Studies in which a pneumococcal-MenC-CRM vaccine and Hib-CRM vaccine were administered simultaneously unexpectedly failed to reduce the Hib conjugate vaccine anti-PRP antibody response. In a phase II randomized control clinical trial, 213 healthy children 56–112 days of age received 9-valent pneumococcal MenC-CRM (Pnc9-MenC-CRM) plus *Haemophilus influenzae* oligosaccharide-CRM$_{197}$ (HbOC; total CRM 64 μg), or HbOC alone (total CRM 25 μg). Both groups were dosed at 3, 4, 5, 6, and 12–15 months of age and received concomitant DTaP and OPV [47].

After the third dose in infants anti-PRP antibody GMCs in the Pnc9-MenC-CRM plus HbOC group (6.72 μg/mL [95% CI, 4.99–9.05]) were twice as high as those in the HbOC alone group (3.26 μg/mL [95% CI, 2.21–4.81; $p = 0.004$]) [47]. A significantly greater proportion of subjects had anti-PRP antibody levels ≥1.0 μg/mL in the Pnc9-MenC-CRM plus HbOC group, compared with the HbOC group (91.3% versus 74.2%, respectively; $p < 0.005$) [47]. The immune response to MenC was greater in the Pnc9-MenC-CRM plus HbOC group than in the HbOC group (100% versus 1.1% attained ≥2 μg/mL anti-MenC antibody, respectively) [47]. Antibodies to other vaccine antigens – diphtheria, tetanus, and pertussis – were mostly unchanged [47].

The authors concluded that CRM had a positive effect on the immune response, in that Pnc9-MenC-CRM plus HbOC actually increased the HbOC anti-PRP antibody response after the 3-week dose, although this effect was not sustained after a booster dose [47].

In contrast, evidence of negative immune interference by CRM was demonstrated by a decrease in the proportion of subjects with >2.0 μg/mL anti-MenC antibody following administration of Pnc9-MenC-CRM plus MenC-CRM, compared to MenC-CRM [48]. In a phase II randomized controlled clinical trial, 240 healthy 7- to 11-week-old infants received either Pnc9-MenC-CRM, or MenC-CRM at 2, 3, and 4 months; all subjects received concomitant DTwP, PRP-TT (ActHIB®; sanofi pasteur, Swiftwater, PA, USA), and OPV [48].

One month after the 3-dose immunization, immunity to MenC was significantly reduced in the Pnc9-MenC-CRM group compared with the MenC-CRM group (SBA GMT 179 [95% CI, 133–243] versus 808 [95% CI, 630–1037; $p < 0.001$], respectively) [48]. Significantly lower proportions of subjects had SBA titers > 1:128 in the Pnc9-MenC-CRM group, compared with the MenC-CRM group (81% versus 94%, respectively; $p = 0.002$). Similar changes were observed for SBA titers >1:8 ($p = 0.05$). The immune response to Hib antibodies and diphtheria was also significantly decreased ($p < 0.05$) [48].

These data illustrate the unpredictability of immune responses when combining the same carrier protein with different vaccines, which alone are highly immunogenic [48]. The unexpected low MenC-CRM immunogenicity of the Pnc9-MenC-CRM vaccine could be explained by CIES, possibly due to the relatively high concentration of CRM in the multivalent Pnc9-MenC-CRM vaccine (38 μg) compared with the MenC-CRM vaccine (10 μg) [48]. However, it is difficult to reconcile these findings with those of Usonis and colleagues [47], because both studies followed virtually identical protocols utilizing similar conjugate vaccines. Moreover, the relatively higher CRM concentration in the Pnc9-MenC-CRM vaccine might be expected to decrease antibody responses through CRM carrier protein overload, as described for Pnc4-TT [42].

These studies highlight the difficulties in comparing carrier protein efficacy among conjugate vaccines, especially those with multiple antigens.

The effects of PD and CRM carrier protein on immune interference were tested by comparing the immunogenicity of routine childhood vaccines following coadministration with multivalent pneumococcal vaccines conjugated with either PD (10-valent pneumococcal vaccine [Pnc10-PD]) or CRM (Pnc7-CRM) [49]. These studies claimed lack of immune interference by Pnc10-PD compared with Pnc7-CRM [49]. However, validating the relative effects of PD and CRM is difficult because, unlike Pnc7-CRM, Pnc10-PD contained a mixture of carrier proteins: 8 of the 10 serotypes contained PD, serotype 18C contained TT, and serotype 19F contained DT [49].

In conclusion, data from these studies clearly show that interactions among vaccines containing carrier proteins, such as CRM and TT, administered concomitantly or in combination are complex, and may cause positive or negative effects on the immune response [41]. Data on anti-PRP and anti-MenC antibody responses from different studies in infants after coadministration of conjugate vaccines with the same or different carrier proteins are often conflicting. Although several mechanisms have been proposed to explain these effects, few have been validated [41]. The relative effects of different carrier proteins on conjugate vaccine efficacy is hard to predict, especially in the context of combination conjugate vaccine therapy, and should be tested on an empirical basis.

5. Discussion

Several conjugate vaccines – notably those directed against disease caused by meningococcal, Hib, and pneumococcal infections – are safe and effective when given to subjects of various ages, including infants, and have provided significant advances over the respective polysaccharide vaccines. Currently available clinical data suggest there is little evidence differentiating one carrier protein from another with respect to conjugate vaccine efficacy and safety, with the exception of Hib and MenACWY vaccines conjugated with DT carrier protein, the efficacy of which has not been demonstrated in infants and young infants, respectively [6,13,29,32,50].

According to immunogenicity studies, the use of CRM and TT in the development of MenC vaccines (Menjugate®, Meningitec®, and NeisVac-C®) is well established [16,19,20,25–27]. The higher initial immune response of infants receiving the first dose of MenC-TT (NeisVac-C®) at 2 months suggested TT offers advantages over CRM, in that infants may benefit from a more rapid onset of immunity to MenC infection [27]. However, after the second dose, the CRM vaccine elicited higher overall GMTs at 4 months.

It was thought that the early heightened antibody response to MenC-TT could be explained by acquisition of immunity to the tetanus antigen of coadministered DTP.

However, this explanation is questionable since two studies on Hib conjugate vaccines did not show differences among the onset of immunity with vaccines containing CRM, TT, or DT carrier proteins despite concomitant DTP [13,35]. Data from these clinical studies suggested OMP, rather than TT, provided the most effective Hib vaccine by conferring the highest rate of immunogenicity after the first and second doses of vaccine [13,35]. These discrepancies are hard to explain given that all studies used similar dose regimens and were completed in infants of about the same age. Interference by other vaccines offers an unlikely explanation, because infants in each study received concomitant DTP and polio vaccines. After a third dose of vaccine in the MenC and Hib conjugate vaccination studies, the high degree of immunogenicity in infants was indistinguishable between the respective carrier proteins used, suggesting CRM, TT, OMP, and DT are comparably immunogenic [13,35].

Data from recently developed quadrivalent MenACWY vaccines showed the superior immunogenicity of MenACWY-CRM conjugate vaccine over MenACWY-DT [17]. These studies were performed in adolescents, but not in infants, providing added confusion over the issue of carrier protein preference [17].

Pnc4-TT and Pnc4-DT demonstrated similar immunogenicity in infants. According to antibody GMCs, the TT carrier protein offered no significant advantage over the DT carrier protein, as has been observed for Hib-TT and Hib-DT [39]. These observations are important in that the pneumococcal conjugate vaccines were compared in the same clinical study, and provide a more accurate assessment of the relative merits of TT versus DT carrier protein in pneumococcal vaccination. Studies on larger multivalent pneumococcal vaccines – Pnc7 and Pnc11 – showed CRM and PD carrier proteins afforded good immunogenicity and protection against infant pneumococcal disease [38]. That conjugate vaccines could be differentiated by their safety profiles has been suggested in head-to-head comparison studies. One study reported that the Hib conjugate vaccines, PRP-OMP and PRP-TT, had a higher incidence of local AEs than PRP-DT and PRP-CRM after each of three doses [13]. PRP-TT was associated with a significantly greater incidence of irritability and crying that evening ($p = 0.05$) and of crying and fever $\geq$38.3 °C ($p < 0.05$) the next morning than the other PRP-conjugate vaccines only after the first dose [10]. Both PRP-OMP and, to a greater degree, PRP-TT, were associated with allergic reactions and the potential for bronchospasm and hypotension [7,14–16]. The safety of MenC-CRM conjugate is well established, and clinical data indicate that MenACWY-CRM, MenACWY-DT, and the multivalent pneumococcal conjugate vaccine, Pnc7-CRM, have comparable safety profiles [17,37]. These findings argue against the superiority of TT and OMP conjugate vaccines, regardless of their perceived, although questionable, immunogenic superiority over CRM and DT.

Immune interference with specific vaccines has been observed in infants in whom the immune response may be either increased or decreased by coadministration of different conjugate vaccines with a common carrier protein. For example, Pnc4-TT lowered the anti-PRP antibody response of DTP-IPV-PRP-TT [42]. An inverse relationship between anti-PRP antibody response and TT content of the Pnc4 vaccine suggested that this negative effect on the immune response was associated with TT antigen overload [41,42]. According to Dagan and colleagues the immune suppressive effect of TT overload may be related to competition for TT T helper cells, and argues for the use of multiple carrier proteins in multivalent vaccines [41,42].

Conversely, combination of conjugate vaccines with TT carrier proteins has been shown to increase anti-PRP antibody responses to Hib vaccines in infants [44–46]. Interpretation of the data is complicated by the use of different Hib-TT vaccine combinations coadministered with MenC-TT in these studies (e.g., DTaP-IPV-Hib-TT [Pediacel®]; DTwP-OPV-Hib-TT; DTaP-Hib-TT; DTaP-HBV-IPV/Hib-TT [Infanrix® hexa]). The positive effects of TT conjugate vaccines on anti-PRP antibody responses to Hib-TT conjugate vaccines may be associated with the common use of DTaP in the vaccine combinations [44–46]. However, this explanation is in conflict with the hypothesis that wP has an adjuvant effect on TT immune response, as suggested by Dagan and colleagues [41,43].

The negative effects of TT conjugate vaccines on anti-MenC antibody titers after combination of Hib-TT with MenC-CRM conjugate vaccine were observed by Kitchin et al. and Schmitt et al., where DTaP was a common factor in the Hib-TT vaccine [44,46]. Thus, DTaP may play a role in TT carrier protein immune interference. In this context it is of interest that substituting DTwP for DTaP in the Hib-TT vaccine prevented or reversed the negative effects of MenC-TT on anti-MenC antibody response in the Kitchin study (Fig. 3) [44]. However, substitution of DTwP for DTaP in the Hib-TT vaccine administered with MenC-TT did not reduce the positive effects on anti-PRP responses (Fig. 3) [44].

Usonis and colleagues found that coadministration of conjugate vaccines Pnc9-MenC-CRM plus HbOC enhanced the Hib

anti-PRP antibody response to HbOC given alone; the anti-MenC antibody response was unexpectedly increased. Immune responses to other coadministered vaccines (diphtheria, tetanus, and pertussis) remained unchanged, suggesting immune interference by CRM carrier protein was not related to a general effect on the immune response [47]. The unpredictable effects of carrier proteins on immune responses were further emphasized by a similar study in which the opposite effect was seen: anti-MenC immune response was significantly lower in subjects receiving Pnc9-MenC-CRM than in patients receiving MenC-CRM. It was of interest that Hib and diphtheria immune responses, but not immune responses to TT, pertussis, and OPV, were also lower after Pnc9-MenC-CRM vaccination [42,48]. The relatively high dose of CRM in the multivalent Pnc9-MenC-CRM vaccine (38 μg) compared with the MenC-CRM vaccine (10 μg) was suggested to have caused reduced epitope suppression and antigen hyporesponsiveness, similar to that described for TT carrier protein in the Pnc4 conjugate vaccine by Dagan and colleagues [42,48]. However, this suggestion adds more confusion, because it does not explain the immunoenhancing effect of Pnc9-MenC-CRM described by Usonis and colleagues [47]. Another cofounding factor is the use of different additional vaccines in these studies: DTaP+OPV was used in the study showing increased anti-PRP and anti-MenC antibody responses, whereas in the study using DTwP+PRP-TT+OPV these antibodies were diminished (Table 1).

In conclusion, close scrutiny of the literature reveals contradictory findings on carrier proteins with respect to the safety and immune response of conjugate vaccines. Clinical studies on immune interference and the immunogenic potential of conjugate vaccines are complex and conflicting, precluding a valid comparison of different carrier proteins. Regardless of the possible explanations responsible for these discrepancies, studies have yet to be published suggesting the proven superiority of one carrier protein over another. However, it should be noted that polysaccharide-carrier protein conjugate vaccines, in general, are safe and immunogenic; and represent a significant step toward reducing infant and child mortality against potentially fatal infectious agents, such as *N. meningitidis*, *S. pneumoniae,* and *H. influenzae* type b [41].

Identifying the true class effects of carrier proteins, if such effects exist, is a difficult task. Some of the larger clinical reports that assessed vaccines against infectious diseases, the largest of which included >150,000 subjects, were powered to determine vaccine effectiveness at the population level [37,51,52]. Multiple cohorts of these sizes would be needed for a prospective clinical trial to conclude with a reasonable amount of statistical certainty what main effects exist for carrier proteins in conjugate vaccines. The likelihood of such a large trial is low, due to the enormous amount of time, money, and logistic planning and execution that would be required. There is, however, a more feasible alternative that may be fast approaching.

In 2005, the World Health Assembly (WHA) adopted the revised International Health Regulations (IHR), requiring 194 States Parties, including all Member States of the World Health Organization (WHO), to develop core capacities to detect, assess, report, and respond to public health threats [53]. The WHO is working with public and private sector partners globally to help countries develop the IHR-mandated core capacities by 2012. National laboratory core capacities include data management (Laboratory Information Systems) and procedures for reporting to public health authorities [53]. Specter and colleagues recently reviewed the progress being made toward establishing/expanding laboratory capacities to process the expected workloads brought on by these recommendations [53]. With the expansion and integration of the global infectious disease surveillance network, along with digitization and sharing of medical records among national and large corporate insurance health care databases, the possibility of collecting data useful for retrospective analysis of carrier protein main effects may be closer than ever before.

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