Booster response to the tetanus and diphtheria toxoid carriers of 11-valent pneumococcal conjugate vaccine in adults and toddlers

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Abstract

We measured the tetanus and diphtheria antitoxin responses after administration of one dose of a mixed carrier (tetanus and diphtheria toxoids) 11-valent pneumococcal conjugate vaccine (PncDT) in 20 Finnish adults (mean age 26.1 years) and 20 Finnish (mean age 23.2 months) and 23 Israeli (mean age 18.5 months) toddlers. The vaccinees had previously been immunised with multiple doses of vaccines containing diphtheria and tetanus toxoids. A double-antigen ELISA was used to measure the antitoxin concentrations. PncDT induced significant booster responses in both adults and toddlers to the tetanus and the diphtheria carrier proteins. Thus, the effect on the tetanus and diphtheria immunity of multivalent conjugate vaccines containing tetanus and diphtheria toxoids as carriers needs to be evaluated before such vaccines are routinely implemented. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Conjugation technology has improved the immunogenicity of polysaccharide vaccines, especially in small children. Polysaccharides conjugated to various carrier proteins, e.g. tetanus (T) or diphtheria (D) toxoids or mutant diphtheria toxin (CRM197), stimulate a T cell-dependent antibody response and induce a long-term immunological memory. The success of Haemophilus influenzae type b (Hib) conjugate vaccines has led to the development of new vaccines against other childhood diseases caused by encapsulated bacteria, such as Neisseria meningitidis and Streptococcus pneumoniae [1,2].

In one of the new pneumococcal (Pnc) conjugate vaccines (PncDT), each of the pneumococcal serotypes is conjugated to either diphtheria (PncD) or tetanus (PncT) toxoids. This results in a considerable amount of carrier protein in the final vaccine. The vaccination schedules for the conjugate vaccines coincide with or succeed diphtheria, tetanus and pertussis (DTP) vaccinations in childhood. Previous studies with early experimental lots of monovalent pneumococcal conjugate vaccines showed that adults respond to the diphtheria and tetanus carrier proteins in addition to the polysaccharide [3,4]. However, the immune responses to the carrier proteins in the multivalent conjugate vaccines administered according to different schedules and with different time intervals are not well documented.

We have reported previously that pneumococcal conjugate vaccines with tetanus and diphtheria toxoids as carriers containing as many as 11 pneumococcal serotypes, are safe in adults and toddlers [5,6]. Here we assess tetanus and diphtheria antibody responses after administration of one dose of an 11-valent pneumococcal conjugate vaccine to adults and toddlers, all previously primed with diphtheria and tetanus toxoid vaccines.

2. Materials and methods

2.1. Vaccinees and vaccination history

Twenty Finnish adults (mean age 26.1 years, range 22–35 years), 20 Finnish toddlers (mean age 23.2 months, range 21.1–24.8) and 23 Israeli toddlers (mean age 18.5 months, range 13–24.5) were enrolled in safety and immunogenicity studies of a new 11-valent PncDT vaccine in 1997 [5,6].
All the adults included in the study had a documented history of at least one tetanus–diphtheria (Td) vaccination one to 10 years ago. Records from the childhood vaccinations were not available. Since childhood immunisation coverage rates have been high (i.e. >90%) throughout years in Finland [7], it is likely that the majority had received the routine vaccinations that consisted of four doses of diphtheria–tetanus–pertussis or diphtheria–tetanus (DT) vaccines until the age of 2 years.

Prior to the study, all toddlers had received the routine vaccinations included in the Finnish and Israeli national vaccination programmes, respectively. The vaccination programmes are presented in Table 1.

Vaccines given to the Finnish toddlers before recruitment to the current study had been as follows: the diphtheria, tetanus, whole cell pertussis combination vaccine (DTP, National Public Health Institute (KTL), Finland), contained 5 Lf (approximately 15 µg/g) of purified tetanus toxoid, 19 Lf (approximately 50 µg/g) of purified diphtheria toxoid and $5 \times 10^9$ B. pertussis bacteria per dose. The H. influenzae type b vaccine (HibOc, Wyeth-Lederle, USA), contained 25 µg per dose of mutated non-toxic diphtheria CRM 197 toxin. The inactivated poliovirus vaccine (IPV) by Aventis Pasteur, Lyon, France and the MMR vaccine by Merck, Sharp & Dohme, West Point, USA. The Td vaccine (KTL, Finland) administered to adults in Finland contained 5 Lf (approximately 15 µg) of purified tetanus toxoid, 19 Lf (approximately 50 µg) of purified diphtheria toxoid and $5 \times 10^9$ B. pertussis bacteria per dose. The oral poliovirus vaccine (OPV) and the Hepatitis B virus vaccine (Hep B) were from SmithKline Beecham Biologicals, Rixensart, Belgium and the MMR vaccine from Merck, Sharp & Dohme, West Point, USA.

The total amount of tetanus and diphtheria antigens thus administered to the Finnish infants before the study was approximately 45 µg of tetanus toxoid in three injections and approximately 225 µg of diphtheria toxoid in five injections, whereas the Israeli infants had received approximately 200 µg of tetanus toxoid and 260 µg of diphtheria toxoid, both in four injections (Table 1).

2.2. Study vaccines, vaccination schedule and sampling

The pneumococcal vaccine (PncTD) from Aventis Pasteur, Lyon, France was a mixture of 11 individual polysaccharide–protein conjugates. Two vaccine formulations were used, one with aluminium hydroxide adjuvant (300 µg/L) per dose, lot number S3416) and one without (lot number S3417). The vaccine contained 1 µg per dose of types 1, 4, 5, 7F, 9V, 19F and 23F pneumococcal polysaccharides (PS) conjugated to tetanus toxoid, 3 µg per dose of types 3, 14 and 18C PSs conjugated to diphtheria toxoid and 10 µg of type 6B PS conjugated to diphtheria toxoid. The tetanus carrier protein content of the pneumococcal conjugate vaccine was approximately 12 µg per dose and the diphtheria toxoid content approximately 60 µg per dose.
The vaccines were administered once by intramuscular injection into the right upper deltoid (adults) or right upper thigh (toddlers). Blood samples were obtained before and 28 days (range ±5 days) after vaccination. The serum samples were stored frozen until analysis.

2.3. Serological analysis

A double-antigen enzyme-linked immunosorbent assay (ELISA) described by Kristiansen et al. [8] was used to measure tetanus and diphtheria antitoxins in serum samples taken on days 0 (pre) and day 28 (post). Duplicates of eight serum samples, starting with the 1:100 dilution and progressing by dilution factor of two were incubated in ELISA plate wells coated with tetanus or diphtheria toxoid. An immunoglobulin was used as an in-house standard and was calibrated against the WHO international standards for tetanus and diphtheria antitoxins. The antibody concentrations were calculated with a reference line method (Unicalc; Unisys, Stockholm, Sweden). Tetanus and diphtheria antitoxin concentrations were thus expressed in international units per millilitre (IU/ml). The lower limit of quantification was 0.007 IU/ml for both types of antibodies. The interassay coefficient of variation was 12%. A two-fold difference between the antitoxin concentrations of the pre- and post-samples was considered as a significant indication of response.

2.4. Statistical analysis

The geometric mean concentration (GMC) and 95% confidence intervals (95% CI) of tetanus and diphtheria antitoxins at days 0 (pre) and day 28 (post) in each group was calculated after logarithmic transformation of the data. The geometric mean fold rise and 95% confidence intervals (95% CI) for each group was calculated from the individual post-to-pre-vaccination concentration ratios. The logarithmic data were also used for analysis of the difference (Student’s t-test) between groups with or without adjuvant.

3. Results

Detailed safety and pneumococcal immunogenicity results have been published earlier [5,6]. Since the aluminium hydroxide adjuvant had no significant effect on the response of the adults or toddlers to the tetanus or diphtheria carrier proteins compared to the response seen with the PncTD vaccine formulation without adjuvant (data not shown, P > 0.05 for all comparisons), we combined the results of the two study vaccine groups for each age group.

3.1. Tetanus and diphtheria antitoxin responses in adults

The tetanus antitoxin concentrations in the pre-vaccination sera were all above 1 IU/ml (GMC 4.35 IU/ml) (Fig. 1). Of the 20 adults immunised with PncTD vaccine 18 (90%) responded to the tetanus carrier with a ≥two-fold increase in antitoxin concentrations. The tetanus carrier induced a mean fold rise in the antitoxin concentrations of 4, resulting in a post-vaccination tetanus GMC of 19.3 IU/ml (Table 2). The pre-vaccination diphtheria antitoxin GMC was 0.92 IU/ml. All adults had a pre-vaccination diphtheria antitoxin concentration above 0.1 IU/ml and 11 out of 20 (55%) had concentrations above 1.0 IU/ml. All vaccinees responded to the diphtheria carrier protein with a ≥two-fold increase in antitoxin concentration. The mean fold rise in the antitoxin concentrations was 29, resulting in a post-vaccination GMC of 26.6 IU/ml (Table 2).

3.2. Tetanus and diphtheria antitoxin responses in toddlers

All the Finnish and Israeli toddlers had pre-vaccination tetanus and diphtheria antitoxin concentrations above 0.1 IU/ml (Fig. 1), a level considered to provide good protection. Pre-vaccination tetanus antitoxin GMCs were 0.61 and 2.30 IU/ml for the Finnish and Israeli toddlers, respectively (Table 2). These were positively correlated to the amount of antigen and the number of doses previously received: 45 μg in three doses in Finland and 200 μg in four doses in Israel. All 20 Finnish toddlers (100%) and 18 out of 23 (78%) Israeli toddlers responded to the tetanus toxoid carrier in the PncTD vaccine with at least a two-fold increase in antitoxin concentration (Fig. 1). The highest tetanus antitoxin responses were seen among the Finnish toddlers (46-fold mean rise) resulting in a GMC of 28.1 IU/ml (Table 2). In Israeli toddlers, the rise was lower (9-fold mean rise) though the GMC was as high as 20.1 IU/ml (Table 2).

Table 2

Tetanus and diphtheria antitoxin responses in the three study groups expressed as geometric mean concentrations (GMC, IU/ml) and geometric mean fold rises (GMF) with 95% confidence intervals (95% CI).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Pre (95% CI)</th>
<th>Post (95% CI)</th>
<th>Pre (95% CI)</th>
<th>Post (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finnish adults</td>
<td>20</td>
<td>4.35 (3.18–5.95)</td>
<td>19.3 (17.0–21.8)</td>
<td>4 (3.4–6)</td>
<td>0.92 (0.53–1.60)</td>
</tr>
<tr>
<td>Finnish toddlers</td>
<td>20</td>
<td>0.61 (0.42–1.07)</td>
<td>28.1 (21.9–36.0)</td>
<td>46 (33–65)</td>
<td>2.86 (1.60–5.11)</td>
</tr>
<tr>
<td>Israeli toddlers</td>
<td>23</td>
<td>2.30 (1.27–4.17)</td>
<td>20.1 (13.0–31.2)</td>
<td>9 (4–17)</td>
<td>1.06 (0.56–2.02)</td>
</tr>
</tbody>
</table>
Fig. 1. Correlation of pre- and post-antitoxin concentrations.
Although the Israeli toddlers had previously received slightly more diphtheria toxoid than the Finnish toddlers (see Section 2), Finnish toddlers showed a higher antitoxin GMC (2.86 IU/ml) than the Israeli toddlers (1.06 IU/ml) in the pre-vaccination samples, perhaps due to the booster effect of the HibOC vaccine given alone on two occasions to Finnish toddlers.

In both adults and toddlers, the fold response was inversely proportional to the pre-vaccination antibody concentrations, but in general the final post-vaccination antitoxin concentration seemed not to correlate with the pre-vaccination antitoxin level (Fig. 1).

4. Discussion

Many of the licensed or experimental conjugate vaccines contain tetanus toxoid, diphtheria toxoid or mutated non-toxic diphtheria toxin (CRM$_{197}$) as a carrier protein. Of these proteins, tetanus and diphtheria toxoids are also included in DTP, DT and Td vaccines that are frequently used in children and adults around the world to protect them against diphtheria, tetanus and pertussis. The mutated diphtheria toxin CRM$_{197}$ is not as such included in any vaccine but it has been shown to induce neutralising antitoxin titres as high as those induced by the diphtheria toxoid [9,10].

In this study we focused on the booster responses to diphtheria and tetanus toxoids carriers included in two 11-valent pneumococcal conjugate vaccines administered to toddlers and adults with history of DTP, PRP-T or PRP-CRM$_{197}$ and Td (only adults) vaccinations. Our results clearly show that the routine diphtheria and tetanus vaccinations had primed adults and children to respond to the carrier proteins of the study vaccines. Furthermore, the magnitude of this response, in terms of post- to pre-vaccination antibody concentration ratio was negatively correlated to the pre-vaccination concentrations, though the post-vaccination concentrations were not in general dependent on the pre-vaccination concentrations. This response resembles the response to a DT or Td immunisation. Results from an unpublished study in Finland show that 11-year-old Finnish children immunised with one dose of Td vaccine (KTL, Finland) respond with a 50-fold mean increase in both tetanus (pre-GMC 0.83 IU/ml, post-GMC 41.7 IU/ml) and diphtheria (pre-GMC 0.12 IU/ml, post-GMC 6.48 IU/ml) antitoxin concentrations.

The pre-immunisation antibody concentrations correlated to the total amount of antigen and/or number of injections previously received. Administration of a non-toxic diphtheria toxoid Hib conjugate vaccine (HibOC) during the second year of life to Finnish toddlers having received three doses of DTP are likely to have further increased their diphtheria antitoxin concentrations. The differences seen between the Finnish and the Israeli toddlers pre-immunisation antitoxin concentrations may be due, not only to different amounts of the antigen given according to different schedules, but also to different commercial DTP vaccines used in the two countries. So far, polysaccharide–protein conjugate vaccines have been primarily designed to induce protection against the polysaccharide-bearing pathogens. However, it is obvious that the carrier proteins are capable of both priming [11] and boosting children and adults, as shown in this study. In addition, simultaneous administration of conjugate vaccines using D, CRM$_{197}$ or T as a carrier protein and DTP, DT or Td vaccines containing the same proteins can even reduce the response, both to the polysaccharide and to the protein part of the vaccine [12–15]. With the introduction of polysaccharide–protein conjugate vaccines, the deletion of one or two tetanus–diphtheria vaccinations could reduce the risk of negative interference and possible also reduce the risk of adverse reactions. Additionally, from a public health viewpoint, such a measure would be economically favourable. The possibility of modifying the routine DTP, DT or Td vaccine schedules should therefore be investigated when new conjugate vaccines are introduced into vaccination programmes.

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References


