

Elevated levels of maternal anti-tetanus toxin antibodies do not suppress the immune response to a *Haemophilus influenzae* type b polyribosylphosphate–tetanus toxoid conjugate vaccine

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Reported are the effects of elevated levels of anti-tetanus antibodies on the safety and immune response to a *Haemophilus influenzae* type b polyribosylphosphate (PRP)–tetanus toxoid conjugate (PRP–T) vaccine. A group of Thai infants ($n = 177$) born to women immunized against tetanus during pregnancy were vaccinated with either a combined diphtheria–tetanus–pertussis (DTP) PRP–T vaccine or DTP and a PRP-conjugate vaccine using *Neisseria meningitidis* group B outer-membrane proteins as a carrier (PedVax HIB). Although most infants possessed high titres (>1 IU/ml) of anti-tetanus antibodies, the DTP–PRP–T combined vaccine engendered an excellent antibody response to all vaccine components. In both vaccine groups $>98\%$ of infants attained anti-PRP antibody titres ≥ 0.15 $\mu\text{g/ml}$. The geometric mean anti-PRP antibody titres were 5.41 $\mu\text{g/ml}$ and 2.1 $\mu\text{g/ml}$ for infants immunized with three doses of PRP–T versus two doses of PedVax HIB vaccines, respectively ($P < 0.005$). Similarly, the proportion of infants who achieved titres ≥ 1 $\mu\text{g/ml}$ was higher in the PRP–T group (87.8%) than in the group immunized with PedVax HIB (74.2%) ($P = 0.036$). A subgroup analysis showed that there was no significant difference in the anti-PRP antibody response for infants exhibiting either <1 IU of anti-tetanus antibody per millilitre or ≥ 1 IU/ml at baseline. These findings indicate that pre-existing anti-carrier antibody does not diminish the immune response to the PRP moiety. All infants possessed protective levels of anti-D and anti-T antibody levels after immunization.

Keywords: diphtheria–tetanus–pertussis vaccine, antagonists and inhibitors; drug antagonism; *Haemophilus influenzae* type B, immunology; *Haemophilus* vaccines, antagonists and inhibitors; tetanus immunology, tetanus toxoid, antagonists and inhibitors; Thailand.

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Introduction

Haemophilus influenzae type B (Hib) is a leading cause of invasive diseases such as meningitis, bacteraemia, epiglottitis, and pneumonia in early childhood (1–5). Vaccine-engendered antibody directed to the polyribosylphosphate (PRP) capsular polysaccharide has been shown to confer a high level of protection (6). The original purified PRP vaccines have been replaced by a variety of conjugate vaccines capable of eliciting a protective immune response in infants (4, 7–9). The safety and immunogenicity of these

conjugate vaccines are well established, and their routine use in infants has resulted in a dramatic decline in the incidence of Hib disease. Recently, Hib conjugates have been combined with diphtheria–tetanus–pertussis (DTP) vaccines and administered simultaneously in a single-dose form (10, 11). This has greatly facilitated routine immunization programmes.

The vast majority of studies evaluating Hib vaccines have been conducted in developed countries. While long felt to be a pathogen primarily of temperate climates, Hib has been found to display a similar epidemiology in tropical and subtropical areas of the world (1, 12, 13). Therefore, evaluation of Hib vaccines, especially in combination with other vaccines routinely administered to infants, is warranted in developing countries where unique factors may influence the immune response to such vaccines. For example, race has been found to modulate the immune response to PRP-containing vaccines (12). Furthermore, the routine immunization of most mothers in developing countries against tetanus

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during pregnancy in an effort to prevent neonatal tetanus may have an impact on use of Hib conjugate vaccines. The infants of such mothers possess elevated levels of anti-tetanus toxin antibodies, which may reduce the anti-PRP antibody response via epitopic suppression.

In Thailand, >80% of Hib meningitis occurs within the first 2 years of life (14). Many Thai children within this age group do not possess protective levels of anti-PRP antibody at this age; however, by 4 years of age, the vast majority of them have attained protective levels of antibody, indicating that Hib circulates within this age group. Immunization of this high-risk group is clearly warranted.

The present study was conducted to determine the safety and immunogenicity of DTP vaccine combined with a PRP–tetanus toxoid (PRP–T) conjugate vaccine among 2-month-old infants born to mothers immunized against tetanus during pregnancy. We were specifically interested in determining whether high levels of pre-existing maternal anti-tetanus antibody would adversely affect the immune response to the PRP conjugate component (15). We therefore immunized a second group of infants with a licensed Hib conjugate vaccine that uses a carrier protein other than tetanus toxoid (outer-membrane proteins from group B meningococcus) and these infants served as controls.

Materials and methods

Subjects

The study was conducted at the well-baby clinic, a paediatric outpatient unit of the Udonthani Hospital, in rural north-eastern Thailand. Healthy infants aged approximately 2 months (range, 1.4–2.9 months), with no prior history of immunization against DTP or Hib were eligible for enrolment. All infants were born to mothers who were immunized against tetanus during pregnancy; the infants underwent a routine physical examination and a medical history was taken. Written informed consent was obtained from the parents. Exclusion criteria included the following: acute febrile illness, neurological or developmental disorder, history of allergies, previous immunization against DTP or Hib, treatment with immunosuppressive drugs, immunodeficiency, significant systemic illness, receipt of immunoglobulins (Igs), plasma, or whole blood since birth, or participation in another clinical trial.

Vaccines

The following vaccines licensed in Thailand were used: DiTePer Anatoxal Berna vaccine (Swiss Serum and Vaccine Institute, Berne, Switzerland) combined diphtheria (25 Lf), tetanus (10 Lf), and pertussis whole cell (>4 IU) vaccine adsorbed on aluminium phosphate; and PedVax HIB (Merck, Sharp and Dohme, West Point, PN, USA) containing 15 µg PRP coupled to 250 µg *Neisseria meningitidis* outer-membrane proteins adsorbed to aluminium hy-

droxide. The experimental combined DTP and DTP–PRP–T conjugate (Swiss Serum and Vaccine Institute, Berne, Switzerland) comprised the following: diphtheria toxoid (25 Lf), tetanus toxoid (5 Lf), pertussis whole cells (>4 IU), and 10 µg PRP coupled to approximately 10 Lf of tetanus toxoid adsorbed on aluminium phosphate. Hib PRP was purified essentially as described elsewhere (16) and allowed to react with cyanogen bromide. The activated PRP was coupled to adipic acid dihydrazide (ADH), and the PRP–ADH complex was linked to tetanus toxoid in the presence of carbodiimide. The ratio of PRP to tetanus toxoid was approximately 1:2 (w/w).

Serological assays

All serological assays were performed in a blinded manner. Total anti-PRP antibody was quantified using a Farr-type radioimmunoassay (17) with intrinsically labelled [³H]PRP supplied by the University of Rochester, NIAID Reference Laboratory, Rochester, NY. The results are expressed as micrograms anti-PRP antibody per ml. A reference serum (calibrated against a reference standard supplied by the Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA) with a known amount of anti-PRP antibody was included as a control for each series of samples run.

Anti-diphtheria toxin, anti-tetanus toxin, anti-pertussis toxin, and anti-filamentous haemagglutinin antibodies were determined using an enzyme-linked immunosorbent assay (ELISA). Briefly, 100 µl of 1 µg/ml antigen solution in phosphate-buffered saline (PBS) was used to coat each microtitration well (72 h, 4 °C). The solution was removed and the wells blocked with a casein solution (2 mg/ml in PBS) for 1 h at 37 °C. The plates were then washed with PBS containing 0.05% Tween-20 (PBS-T). Twofold serially diluted sera (100 µl) in PBS containing 2 mg/ml casein and 0.05% Tween-20 (PBS-T-C) was added to each well. After incubation for 3 h at room temperature, the plates were washed three times with PBS-T. Anti-human IgG (γ) (Kirkegaard & Perry, Gaithersburg, MD, USA) diluted 1:2500 in PBS-T-C was added (100 µl/well). After incubation for 2 h at 22 °C, the wells were washed with PBS-T, 100 µl 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) substrate (Boehringer Mannheim, Mannheim, Germany) was added, and the colour was allowed to develop for 30 min. The absorbance at 405 nm (A_{405}) was measured using an ELISA reader (Dynatech, MR5000, Embrach, Switzerland). A standard antiserum containing a known quantity of specific antibody was run in parallel, and the antibody values of the test samples were determined. Tetanus and diphtheria antibody levels were reported as IU/ml. Anti-pertussis toxin and anti-filamentous haemagglutinin (FHA) IgG antibody levels were reported as µg/ml.

Bordetella pertussis whole cell agglutinating antibody was determined as follows. Lyophilized

B. pertussis strain 460 (200 U.S. opacity units/ml) was reconstituted and diluted 1:10 in saline containing 0.01% (w/w) thimerosal to yield an A_{530} of 1.2–1.4. Prediluted test sera (50 μ l, 1:20) were serially diluted (twofold) using sterile saline in U-bottomed micro-titration plates. 50 μ l *B. pertussis* suspension was added to each well, and the plates were shaken vigorously for 1 min (AM69 Microshaker; available from: Cooke Microtiter System, Dynatech, Embrach, Switzerland). The plates were sealed with plastic foil and incubated at 35 °C for approximately 18 h. The agglutinating titre was defined as the reciprocal of the highest dilution of serum which results in a thin sheet of cells with a slight button. A human reference serum was run in parallel.

Study design

The study protocol was reviewed and approved by the Thai Ministry of Public Health Ethical Committee. One group of infants was randomized to receive either three doses of DTP vaccine combined with PRP-T in the same syringe administered simultaneously at approximately 2, 4, and 6 months of age. The second group of infants received DTP vaccine in one leg at 2, 4, and 6 months of age and PedVax HIB vaccine at 2 and 4 months of age in the other leg as specified by the manufacturer. All vaccines were given intramuscularly in the area of the upper thigh muscle. The infants were observed for 30 min following each immunization to monitor for immediate type reactions. Adverse reactions were recorded by the parents for 7 days after each immunization on a standard adverse reaction report form. Parents were asked to monitor the following symptoms: redness and induration at the injection site; swelling (>1 cm); fever (>37.5 °C); increased crying; refusal to feed; vomiting; apparent convulsions; and other symptoms that could be associated with immunization. Each child was examined by a study physician, and the parents were questioned concerning adverse reactions noted prior to subsequent doses of vaccine being given. Samples of venous blood were obtained just prior to the first immunization and 1 month after the third dose of vaccine had been administered. The serum was collected and stored at -20 °C. Each serum tube was labelled with the date of collection and a study subject code number. Children also received the following vaccines as part of their routine immunization programme: BCG at birth; hepatitis B at birth, 1, and 6 months of age; and oral poliovirus vaccine (OPV) at 2, 4, and 6 months of age.

Statistical analysis

Differences in the rates of adverse reactions between groups were determined using χ^2 tests, while the significance between geometric mean titres (GMTs) was analysed using Student's *t*-test (two-tailed). Significant differences in the percentage of infants who attained ≥ 0.15 μ g or ≥ 1 μ g anti-PRP antibody per ml at 7 months of age were determined by χ^2 test.

Results

A total of 177 infants aged about 2 months were enrolled in the study. Of these, 102 were allocated to receive the DTP-PRP-T combined vaccine, whereas the remaining 75 were immunized with three doses of DTP vaccine together with two doses of PedVax HIB. The mean age at enrolment, ratio of male to female participants, or mean body weight at the time of the initial immunization did not differ significantly between the two groups. The number of infants completing follow-up after the first, second, and third immunization was 177 (100%), 163 (92%), and 155 (88%), respectively. The number of infants who completed the immunization schedule and who contributed two blood samples was 84.3% for the group immunized with the DTP-PRP-T vaccine and 92% for those immunized with DTP plus two doses of PedVax HIB. The main reason for not completing the protocol was immunization with DTP vaccine at a local health clinic. There were no dropouts due to adverse reactions.

Adverse reactions reported after receipt of all three doses of vaccines are shown in Table 1. No immediate type or anaphylactic reactions were noted. Local reactions of all types were common in both groups. Redness, swelling, and induration occurred more frequently ($P < 0.05$) in the group immunized with the DTP-PRP-T combined vaccine after the first dose. This was largely because one study nurse inadvertently administered the first dose of the combined vaccine subcutaneously to 27 infants, virtually all of whom exhibited local reactions. If these infants are excluded from the analysis, only the rate at which induration appeared remained significantly higher in the combined vaccine group. Induration was significantly higher ($P < 0.05$) for the combined vaccines group after the second but not the third immunization. The vast majority of local reactions were classified as mild and resolved spontaneously without treatment.

The frequency of systemic reactions temporally associated with vaccination were comparable in both groups (Table 1). Again, most reactions were considered to be mild and transitory. Five episodes of what could be classified as febrile convulsions, based upon the parents' descriptions, were reported, three of which occurred in the group receiving DTP-PRP-T, and two in the group immunized with DTP + PedVax HIB. However, none of these infants was brought in for treatment or observation, and there was no evidence of sequelae upon subsequent routine examination.

Paired (baseline and 7 months) serum samples were obtained from 148 of the 155 (95.5%) infants who completed the course of immunization. The immune response engendered by the DTP vaccine components is presented in Table 2. At baseline, nearly all (>92.5%) of the infants showed serum anti-tetanus IgG antibody levels ≥ 0.1 IU/ml, an amount 10-fold higher than what is generally believed to be the minimum protective level. The anti-tetanus IgG

Table 1. Adverse reactions reported after immunization with DTP-PRP-T or DTP + PedVax HIB vaccines

Adverse reactions	First dose				Second dose				Third dose			
	DTP-PRP-T		DTP + PedVax HIB		DTP-PRP-T		DTP + PedVax HIB		DTP-PRP-T		DTP	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Local												
Redness	58/82 ^a	71	37/68	54	47/82	57	40/68	59	54/82	66	37/68	54
Swelling												
>1 cm	39/82 ^a	49	22/68	32	30/82	37	20/68	29	33/82	40	25/68	37
Induration	62/82 ^a	76	34/68	50	60/82 ^a	73	37/68	54	59/82	72	42/68	62
Systemic												
Fever												
(>37.5 °C)	22/82	27	29/68	43	28/82	34	31/68	46	35/82	43	19/68	28
Increased crying												
	53/82	65	41/68	60	49/82	60	47/68	69	52/82	63	34/68	50
Irritability												
	45/82	55	42/68	62	50/82	61	48/68	71	53/82	65	39/68	57
Refusal to feed												
	23/82	28	14/68	21	26/82	32	28/68	41	26/82	32	21/68	31
Vomiting												
	11/82	13	12/68	18	10/82	12	14/68	21	13/82	16	9/68	13
Febrile convulsion												
	1/82	1	0/68	0	1/82	1	1/68	2	1/82	1	0/68	0

^a $P = < 0.05$ between DTP-PRP-T and DTP + PedVax HIB.

 Table 2. Anti-diphtheria toxin, anti-tetanus toxin, anti-pertussis toxin, anti-filamentous haemagglutinin, and *Bordetella pertussis* whole cell agglutinating antibody response after vaccination with DTP-PRP-T or DTP + PedVax HIB vaccines

Antigen	Vaccine	Baseline (pre-dose 1)		7 months (1 month after third dose)		
		GMT ^a	% ≥ 0.1 IU/ml	GMT	% ≥ 0.1 IU/ml	% \geq fourfold rise in titre
Diphtheria antitoxin (IU/ml)		0.06	31.7	2.87	100	NA ^b
	DTP + PedVax HIB	0.06	21.2	3.04	100	NA
Tetanus antitoxin (IU/ml)		1.43	95	6.87	100	NA
	DTP-PRP-T	1.12	92.4	7.13	100	NA
Pertussis antitoxin (μ g IgG/ml)	DTP-PRP-T	2.06	NA	8.75	NA	40
	DTP + PedVax HIB	1.34	NA	6.24	NA	50
Anti-FHA IgG (μ g IgG/ml)	DTP-PRP-T	0.55	NA	1.53 ^c	NA	33
	DTP + PedVax HIB	0.52	NA	0.91	NA	23
Pertussis agglutinating antibody titre	DTP-PRP-T	10.3	NA	234	NA	98.8
	DTP + PedVax HIB	10.4	NA	262	NA	98.5

^a GMT = geometric mean titre.

^b NA = not applicable.

^c $P < 0.005$.

GMT was >1 IU/ml serum at baseline in both groups. There was no statistically significant difference between the two groups in terms of either the proportion of subjects with >1 IU of anti-tetanus IgG antibody per ml or the GMT value. Such high titres reflect the fact that nearly all mothers were vaccinated against tetanus at least once during

pregnancy. In contrast, only 25% of infants possessed baseline titres of anti-diphtheria toxin IgG antibody ≥ 0.1 IU. After completing the three-dose immunization regimen, all infants achieved titres of tetanus and diphtheria anti-toxin ≥ 0.1 IU/ml. The GMTs increased approximately 50-fold and 5-fold after immunization for diphtheria and tetanus,

respectively. Most infants had significant amounts of anti-pertussis toxin, anti-FHA, and pertussis agglutinating antibody at baseline. After immunization there was a significant ($P < 0.05$) increase in the GMTs to all three pertussis antigens in both groups. The anti-FHA GMT was significantly ($P < 0.005$) higher for the group that was vaccinated with the DTP-PRP-T combined vaccine. No significant difference was observed for anti-pertussis toxin, anti-diphtheria toxin, or pertussis agglutinating titres.

The anti-PRP antibody response is shown in Table 3. Prior to immunization about 40% of all infants possessed ≥ 0.15 μg anti-PRP antibodies/ml serum, whereas few (6%) had levels of ≥ 1 $\mu\text{g}/\text{ml}$. There were no significant differences between the groups at baseline in terms of anti-PRP antibody titres. After immunization, both groups achieved >20-fold rise in geometric mean anti-PRP antibody titres over baseline ($P < 0.001$). The GMT for the group that was immunized with the DTP-PRP-T combined vaccine was significantly ($P < 0.005$) higher than that for the group that received two doses of PedVax HIB vaccine. In both groups, more than 98% of infants achieved titres of anti-PRP antibody >0.15 μg at 7 months. The proportion of infants who attained levels of anti-PRP antibodies >1 $\mu\text{g}/\text{ml}$ were 87.8% and 74.2%, respectively, following immunization with DTP-PRP-T combined vaccine or PedVax HIB vaccine ($P = 0.036$).

The results presented above indicated that high baseline levels of anti-tetanus toxin antibodies did not suppress the anti-PRP antibody response engendered by the PRP-T conjugate vaccine. In an attempt to confirm this finding, we analysed the anti-PRP-T immune response in subgroups of subjects who possessed either lower (<1 IU/ml) or higher (≥ 1 IU/ml) levels of anti-tetanus antibodies at baseline (Table 4). For recipients of the DTP-PRP-T vaccine, there was approximately a 10-fold difference in anti-tetanus GMT at baseline. However, no significant difference was observed between the two subgroups as gauged by either post-immunization GMT, or the percentage of infants achieving titres ≥ 0.15 μg or ≥ 1 $\mu\text{g}/\text{ml}$. As would be expected, similar results were seen for the group which was immunized with DTP + PedVax HIB.

Discussion

The routine use of Hib conjugate vaccines in neonates has proved to be an extremely effective method of preventing invasive Hib disease (4, 9, 18). Several previous studies have demonstrated that Hib conjugates can be combined with DTP vaccines and administered simultaneously without compromising either safety or immunogenicity (10, 19, 20). Such an approach greatly facilitates neonatal immunization programmes especially if additional vaccines, such as

Table 3. Anti-PRP antibody response following immunization either with DTP-Hib or DTP + PedVax HIB^a vaccine

Vaccine	Baseline (pre-dose 1)			7 months		
	GMT ($\mu\text{g}/\text{ml}$)	% ≥ 0.15 $\mu\text{g}/\text{ml}$	% ≥ 1 $\mu\text{g}/\text{ml}$	GMT ($\mu\text{g}/\text{ml}$)	% ≥ 0.15 $\mu\text{g}/\text{ml}$	% ≥ 1 $\mu\text{g}/\text{ml}$
DTP-PRP-T	0.15	41.5	6.1	5.41 ^b	98.8	87.8 ^c
DTP+PedVax HIB	0.14	37.9	6	2.1	98.5	74.2

^a Two doses of PedVax HIB were administered versus three doses of PRP-T.

^b $P < 0.005$ versus DTP + PedVax HIB group.

^c $P = 0.036$ versus DTP + PedVax HIB group.

Table 4. Anti-PRP antibody response in subgroups possessing high or low levels of anti-tetanus antibody at baseline

Vaccine	Baseline anti-tetanus anti-body levels	Anti-tetanus GMT (IU/ml)	Anti-PRP antibody response at 7 months of age			
			GMT ($\mu\text{g}/\text{ml}$)	% ≥ 0.15 $\mu\text{g}/\text{ml}$	% ≥ 1 $\mu\text{g}/\text{ml}$	
DTP-PRP-T	<1 IU/ml (32) ^a	0.39	4.33	97	81	$P = 0.279$
	>1 IU/ml (50)	3.31	6.24	100	92	
DTP + PedVax Hib	<1 IU/ml (28)	0.36	2.06	96	75	$P = 0.932$
	>1 IU/ml (38)	2.54	2.12	100	74	

^a Figures in parentheses are number of subjects per group.

hepatitis B or parenterally administered inactivated polio vaccines need to be incorporated. The majority of studies evaluating the safety, immunogenicity, and efficacy of monovalent Hib vaccines or Hib conjugates combined with DTP have been conducted in developed countries (5). Recent epidemiological studies have shown that Hib is also a leading pathogen during early life in developing areas of the world (12, 13). Therefore, routine immunization of infants against this pathogen may be warranted in such countries.

The goal of the current study was to evaluate the safety and immunogenicity of a combined DTP-Hib-T vaccine administered to infants born to women vaccinated against tetanus during pregnancy. We were interested in determining whether high levels of maternal anti-tetanus toxin antibodies would diminish the immune responses to the PRP moiety of a conjugate which utilized tetanus toxoid as a carrier protein via epitopic suppression (21, 22). For comparison, we immunized a group of infants with an Hib conjugate vaccine where a different carrier protein was used, in this case outer-membrane proteins from *N. meningitidis*. While it would have been preferable to use an Hib conjugate that follows a three-dose immunization schedule, as did the PRP-T conjugate, and which could be combined with DTP vaccine to avoid possible biases, PedVax HIB was the only Hib conjugate vaccine licensed in Thailand when this trial was conducted. Similarly, since virtually all Thai women are immunized against tetanus during pregnancy, it was not feasible to have a study group of infants with very low levels of anti-tetanus antibody levels.

We found both Hib vaccines to be safe. The type, frequency, and severity of systemic and local reactions were comparable to those previously reported for similar vaccines (19, 20, 23). Undoubtedly, the majority of reactions seen with the DTP-PRP-T vaccine could be attributable to the pertussis whole cell component. The addition of the PRP-T conjugate to DTP vaccine may have modestly increased the rate at which induration at the injection site occurred.

The vast majority of neonates enrolled possessed high levels of maternal anti-tetanus toxin antibodies at 2 months of age, and fully 92.5% had titres >0.1 IU/ml. This is the result of an aggressive campaign to vaccinate all pregnant women with tetanus toxoid to prevent neonatal tetanus, which continues to be a leading cause of mortality among neonates in developing countries. In contrast, few North American or European infants possess such levels shortly after birth (15, 23). Elevated tetanus antitoxin titres did not appear to diminish the immune response to the PRP component of a PRP-T conjugate vaccine combined with DTP as was previously reported in a Danish study (15). More than 98% of children who received either Hib conjugate vaccine attained what is considered to be a protective level of anti-PRP antibody (titre ≥ 0.15 $\mu\text{g/ml}$). Significantly, the PRP-T conjugate evoked a superior anti-PRP response, as gauged by both the final GMT attained and the proportion of infants who achieved an anti-PRP antibody level ≥ 1 $\mu\text{g/ml}$ compared with infants receiving two doses of PedVax HIB vaccine. A recent study has found that priming infants shortly after birth with tetanus toxoid produces an enhanced anti-PRP immune response subsequent to immunization with a PRP-T conjugate vaccine (15, 24). However, maternally acquired anti-tetanus antibodies did exert a modest suppressive effect on the anti-PRP response seen in the above-mentioned Danish infants as judged by the proportion attaining an anti-PRP antibody titre ≥ 1 $\mu\text{g/ml}$ (15). It is important to note, however, that only two doses of PRP-T vaccine were administered in the Danish study at 4 and 6 months of age.

In summary, the present findings indicate that PRP-T conjugate vaccines are highly immunogenic regardless of the levels of anti-tetanus antibodies possessed by infants when vaccination against Hib is initiated. This would indicate that PRP-T conjugate vaccines can be used in developing areas of the world where pregnant women are routinely vaccinated with tetanus toxoid to prevent neonatal tetanus. ■

Résumé

Des taux élevés d'anticorps maternels contre la toxine tétanique ne suppriment pas la réponse immunitaire à un vaccin conjugué PRP de *Haemophilus influenzae* type b-anatoxine tétanique

Dans le monde entier, *Haemophilus influenzae* type b (Hib) est une cause majeure de morbidité et de mortalité chez l'enfant. Les vaccins conjugués contenant le polyribosylphosphate (PRP) de Hib, un polyside capsulaire couplé par covalence à diverses protéines porteuses, se sont montrés hautement protecteurs chez le nourrisson. Leur utilisation en routine a pratiquement éliminé la forme invasive de l'infection à Hib. La plupart des vaccins de ce type ont été évalués dans des pays développés. Leur efficacité dans les pays en développement peut cependant être influencée par des facteurs

ethniques et par les politiques vaccinales. Ainsi, dans de nombreux pays les femmes enceintes sont vaccinées par l'anatoxine tétanique dans le but d'empêcher le tétanos néonatal. Cette pratique peut modifier l'immunogénicité des vaccins conjugués anti-Hib qui utilisent l'anatoxine tétanique comme support. Pour déterminer si des taux élevés d'anticorps antitétaniques sont susceptibles de réduire la réponse immunitaire à un vaccin conjugué PRP-anatoxine tétanique (PRP-T), nous avons réalisé une étude sur des nourrissons thaïlandais vaccinés soit par un vaccin PRP-T soit par un vaccin conjugué anti-Hib

utilisant des protéines de la membrane externe de *Neisseria meningitidis* groupe B comme support (PedVax HIB). Dans les deux groupes, plus de 98 % des nourrissons ont présenté des titres d'anticorps anti-PRP $\geq 0,15 \mu\text{g/ml}$. La proportion de nourrissons présentant des titres $\geq 1 \mu\text{g/ml}$ était plus élevée dans le groupe ayant reçu trois doses de PRP-T (87,8 %) que dans le groupe ayant reçu deux doses de PedVax HIB (74,2 %; $p = 0,036$). La moyenne géométrique de la réponse en anticorps anti-PRP chez les nourrissons

vaccinés avec le PRP-T (5,41 $\mu\text{g/ml}$) était significativement plus élevée ($p < 0,005$) que dans le groupe vacciné avec le PedVax HIB (2,1 $\mu\text{g/ml}$). Une analyse des sous-groupes a montré que l'intensité de la réponse en anticorps anti-PRP n'était pas significativement influencée par le taux initial d'anticorps antitétaniques. Par conséquent, les programmes qui visent à réduire le tétanos néonatal par vaccination des femmes enceintes n'empêchent pas d'utiliser le PRP-T chez les nourrissons nés de ces femmes.

Resumen

Los niveles elevados de anticuerpos maternos contra la toxina tetánica no suprimen la respuesta inmunitaria a una vacuna conjugada de HibPRP-anatoxina tetánica

Haemophilus influenzae tipo b (Hib) es una de las principales causas de morbilidad y mortalidad en la infancia en todo el mundo. Se ha demostrado que las vacunas conjugadas del polisacárido capsular de polirribosil-fosfato (PRP) de Hib unido covalentemente a varias proteínas portadoras tienen un alto efecto protector cuando se administran a lactantes. Su uso sistemático ha permitido eliminar prácticamente la enfermedad invasiva por Hib. La mayoría de las vacunas de este tipo han sido evaluadas en países desarrollados. Sin embargo, la utilidad de las vacunas conjugadas contra Hib en los países en desarrollo puede verse afectada por la raza y por las políticas de inmunización. Así, en muchas de esas zonas las mujeres embarazadas son vacunadas a menudo con anatoxina tetánica para prevenir el tétanos neonatal, y eso puede alterar la inmunogenicidad de los conjugados contra Hib que emplean esa anatoxina como portador. Por ello, con objeto de determinar si unos niveles elevados de anticuerpos antitétanos reducirían la respuesta inmunitaria a una vacuna de PRP-anatoxina tetánica (PRP-AT), llevamos a cabo un estudio con lactantes tailandeses a

los que se inmunizó ya fuese con una vacuna PRP-AT o con una vacuna conjugada anti-Hib cuyo agente portador eran proteínas de la membrana externa de *Neisseria meningitidis* del grupo B (PedVax HIB). En más del 98% de los lactantes se detectaron títulos de anticuerpos anti-PRP $\geq 0,15 \mu\text{g/ml}$ en los dos grupos vacunados. La proporción de lactantes cuyos títulos alcanzaron valores $\geq 1 \mu\text{g/ml}$ fue mayor en el grupo al que se administraron tres dosis de PRP-AT (87,8%) que en los inmunizados con dos dosis de PedVax HIB (74,2%, $P = 0,036$). La media geométrica de la respuesta de producción de anticuerpos anti-PRP en los lactantes inmunizados con PRP-AT (5,41 $\mu\text{g/ml}$) fue significativamente superior ($P < 0,005$) a la observada con PedVax HIB (2,1 $\mu\text{g/ml}$). El análisis por subgrupos reveló que la magnitud de la respuesta de producción de anticuerpos anti-PRP no se veía influida significativamente por los niveles basales de anticuerpos antitétanos. Así pues, los programas de reducción del tétanos neonatal mediante inmunización de las mujeres embarazadas no son impedimento para la administración de PRP-AT a sus lactantes.

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