

Th2-Associated Local Reactions to the Acellular Diphtheria-Tetanus-Pertussis Vaccine in 4- to 6-Year-Old Children

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Acellular vaccines against diphtheria-tetanus-pertussis (acellular pertussis) (DTaP) are being progressively introduced into vaccination programs worldwide, with the aim of reducing T-helper 1 (Th1)-associated reactivity associated with the cellular diphtheria-tetanus-pertussis (whole-cell pertussis) (DTwP) vaccine. The DTaP vaccine has an improved safety profile in infants, but little information is available concerning the nature of the ensuing immunological memory in older children and how this may affect the reactivity of DTaP booster doses. We have addressed this question in the present study by assessing polyclonal and vaccine antigen-specific humoral and cellular immune responses to boosting with DTaP in 4- to 6-year-old children primed during infancy with DTaP ($n = 30$) or DTwP ($n = 16$) and by correlating these parameters, in particular cytokine responses, with expression of local side effects at the injection site. Large local reactions (≥ 50 -mm diameter) 24 to 72 h after receiving the DTaP booster occurred in 43% of exclusively DTaP-primed children, in contrast to 6% of children primed with DTwP. These reactions were associated with vigorous T helper 2 (Th2)-polarized memory responses to vaccine antigen exemplified by interleukin 5 (IL-5), IL-6, and IL-13 production and log-scale boosting of tetanus-specific immunoglobulin E and occurred most frequently among children who are intrinsically “high Th2 responders” as detected by *in vitro* responsiveness to polyclonal mitogen. Our findings suggest that priming during infancy with DTaP promotes stable, boostable Th2-polarized immunity against vaccine antigens, which in a significant subset of children is subsequently associated with local reactions at the booster site. The time course of these reactions suggests that the underlying mechanism involves reactivation of Th2-polarized cellular immune memory.

Morbidity and mortality to infectious diseases are maximal during early infancy, and as a consequence, the pressure to develop an increasingly broad range of vaccines in progressively younger age groups is increasing. An archetypal example is the diphtheria-tetanus-pertussis (DTP) vaccine, which provides protection against pathogens responsible for severe illness and death in millions of children worldwide, and initiatives to introduce versions of this and other vaccines for use in neonates to provide protection during the crucial first months of life are well advanced in many centers (3, 11, 17, 29).

The primary consideration underpinning research and development in this area continues to be that of safety, and the first major shift in formulation of the DTP vaccine from the original combination of whole-cell pertussis combined with tetanus and diphtheria toxoids (DTwP) to the new-generation acellular version (DTaP) was driven precisely by this imperative. Notably, the occurrence of febrile reactions in a small proportion of DTwP-vaccinated children (6, 8, 13) which are likely to be associated with cell-mediated responses to the vaccine has provided the impetus for development of the second-generation DTaP, and large-scale

international trials have demonstrated efficacy with a significantly improved safety profile with this vaccine in infants (7, 16). However, more recent studies in older children suggest that the DTaP booster dose given at the end of the preschool years, given alone or in combination with inactivated polio vaccine, is relatively frequently associated with local side effects of redness and swelling at the injection site (2, 14, 19, 26). The mechanism(s) underlying these local reactions is incompletely understood and has been variously suggested to result from the large amount of diphtheria toxoid in the vaccine (27) or a combination of vaccine antigen-specific immunoglobulin E (IgE) antibody (20, 24) and T helper 2 (Th2) cytokine production (24). The present study provided us with the opportunity to directly compare local side effects to the preschool DTaP booster with humoral and cellular immune response profiles of individual children to vaccine antigens, contrasting subjects who were primed in infancy with either DTaP vaccine or the original DTwP vaccine. Our results suggest a possible association between injection site reactions to the DTaP booster and Th2-polarized memory against vaccine antigens which was selectively primed in infancy by the DTaP vaccine.

MATERIALS AND METHODS

Subjects. The study complied with guidelines set by the National Health and Medical Research Council of Australia and was approved by our institutional human ethics committee. The study cohort consisted of 46 healthy children between 4 and 6 years of age (median age, 54 months; age range, 48 to 74

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months). They were recruited from one of the centers (Perth) involved in an Australia-wide randomized multicenter phase IIIb clinical trial designed to compare the immunogenicity and reactogenicity of the combined Infanrix-IPV (inactivated poliomyelitis virus) vaccine given in one arm, with Infanrix and IPV given in separate arms. All children additionally received the measles-mumps-rubella (MMR) vaccine. At the end of the study, examination of the infant vaccination history of the study subjects revealed unforeseen heterogeneity with respect to DTP priming. Notably, while all 46 of the subjects had received four DTP vaccinations at 2, 4, 6, and 18 months, 30 were found to have received exclusively (acellular) DTaP, whereas the priming schedules of 16 in the group included one or more injections of the (whole-cell) DTwP. This information was employed to stratify the population for subsequent analyses, as detailed below.

As part of the multicenter study protocol, an initial questionnaire was employed to collect demographic characteristics, such as age, gender, vaccination history, doctor diagnosis of asthma, hay fever, eczema, or food allergy, as well as atopic family history. Additionally, the incidence of local reactions at the site of the DTaP booster dose (pain, redness, and swelling) were recorded during the first 4 days postvaccination, together with any general (fever, irritability, and drowsiness) symptoms. Using this information, the individuals were grouped on the basis of the incidence of local reactions at the DTaP injection site. In the analyses shown below, the children primed exclusively with DTaP were divided into two subgroups of similar size: DTaP-reactive (DTaP-R), defined as the children with large local reactions comprising redness with a diameter of ≥ 50 mm, and DTaP-nonreactive (DTaP-NR) designating those with smaller or no reactions. The selection of a 50-mm cutoff defining large reactions was based on findings from a previous publication (28). In the DTwP group who had been given the whole-cell vaccine during infant priming, only 1 of 16 experienced a large local reaction upon preschool boosting.

Peripheral blood was collected prior to (prebleed) and 4 to 6 weeks after vaccination (postbooster), either into an equal volume of RPMI 1640 (Cytosystems) containing preservative-free heparin or separately for serum preparation. Peripheral blood mononuclear cells (PBMC) were isolated from diluted blood and cryopreserved for later batch analysis, employing methodology that has been successfully utilized in a variety of prospective cohort study settings (9, 18, 21–23).

Vaccines. Both DTaP (Infanrix; GlaxoSmithKline [GSK] Biologicals; injected into the left deltoid) and DTaP-IPV (Infanrix-IPV; GSK Biologicals; left deltoid) contained 25 limes flocculation doses (Lf) of diphtheria toxoid, 10 Lf of tetanus toxoid (TT), 25 μ g of pertussis toxoid, 25 μ g of filamentous hemagglutinin, and 8 μ g of pertactin adsorbed on 0.5 mg of aluminum salts, and both DTaP-IPV and IPV (IPOL; Aventis Pasteur MSD's IPV; injected subcutaneously into the lower right deltoid), contained 40 D-antigen (D-Ag) units of poliovirus type 1, 8 D-Ag units of poliovirus type 2, and 32 D-Ag units of poliovirus type 3.

In addition, all children in this study were vaccinated with the MMR vaccine (PRIORIX; GSK Biologicals; injected subcutaneously into the upper right deltoid), which contained ≥ 103.0 50% tissue culture infectious doses (TCID₅₀) of Schwarz measles strain, ≥ 103.7 TCID₅₀ of RIT 4385 mumps strain, and ≥ 103.0 TCID₅₀ of RA 27/3 rubella strain.

Antibody assays. For the purpose of this study, the TT antigen component was selected as representative of the DTaP vaccine, and associations are reported below between aspects of TT-specific immunity and the most frequent local reaction (redness) at the injection site.

TT-specific IgG was assayed as previously described (23a) with a reading of greater than 0.1 IU/ml being considered protective.

Total IgE, IgE specific for both TT (RC 208) and house dust mite (*Dermatophagoides pteronyssinus*), Phadiatop test, and FX5 (food mix containing egg white, cow's milk, cod fish, wheat flour, peanut, and soya bean) were assayed via the Pharmacia UniCAP 100 system per the manufacturer's instructions.

Cell culture. As described elsewhere (23), cryobanked PBMC were cultured on a batch analysis basis over a short time period, using the same reagents throughout, with pre- and postvaccination samples from each individual being processed together. PBMC were resuspended at 1×10^6 cells/ml in either RPMI 1640 (Cytosystems) supplemented with 5% pooled human AB serum (for cultures with vaccine antigens) or AIM-V serum-free medium (Gibco Life Technologies) supplemented with 2-mercaptoethanol (4×10^{-5} M final concentration; Sigma) for cultures with phytohemagglutinin (PHA). Aliquots of 0.5×10^6 cells were cultured for 96 h alone or together with TT to measure responses to the DTaP vaccine (0.5 Lf/ml; CSL) or with measles lysate (ML) or control rhesus monkey kidney cell lysate (1 in 240 dilution; generous gift from S. Wesselingh, Macfarlane Burnet Institute for Medical Research and Public Health) to measure responses to the MMR vaccine or for 48 h alone or together with PHA (1 μ g/ml; Murex Biotech).

TABLE 1. Summary of local reactions to the DTaP booster in the 46 children

Group (n)	% of group with the following local reaction (diameter [mm]):							
	Redness				Swelling			
	≥ 50	20–49	3–19	≤ 2	≥ 50	≥ 20 –49	3–19	≤ 2
DTwP (16)	6	13	0	81	0	0	6	94
DTaP exclusively (30)	43	7	0	50	30	7	3	60

Detection of cytokine protein. The levels of interleukin 5 (IL-5), IL-6, IL-10, IL-13, and gamma interferon (IFN- γ) in PBMC culture supernatants were measured by in-house time-resolved fluorometry assays as described previously (21).

Statistical analysis. The Kruskal-Wallis test was used to compare the differences between vaccine-specific antibody and cytokine responses between the three symptomatology groups. Where significant differences were seen, the Mann-Whitney U test was used to do pairwise testing between the three groups.

Wilcoxon matched-pair signed-rank test was used for analysis between responses pre- versus postvaccination for each individual. Spearman's ρ test was used to analyze the correlations between the size of local reactions and cytokine responses, and the chi-square test was used to assess differences between the incidence of large local reactions and IgE boosting in DTaP-primed children given different versions of IPV. Stepwise logistic regression modeling was used to further examine associations between reactivity at the booster site and immunological indices. The statistical package SPSS (SPSS, Inc., Chicago, Ill.) was used for all analyses.

RESULTS

The local reactions to the DTaP booster in the 46 4- to 6-year-old children reported in this study are illustrated in Table 1. Laboratory assays (detailed below) were conducted in a blind manner as to the clinical outcome. Of the 16 children who had been given the DTwP vaccine during infant priming, only 1 individual experienced a large local reaction to the booster. In contrast, 13 of the 30 who were primed exclusively with DTaP developed large reddening (erythema) reactions at the injection site (DTaP > DTwP group; $P < 0.009$), and among this reactive subgroup the reddening was highly correlated with swelling ($P < 0.001$; 0.774 by Spearman's ρ).

For subsequent analyses, the DTaP-primed group was stratified into "reactors" (DTaP-R; $n = 13$) versus "nonreactors" (DTaP-NR; $n = 17$), and these children were compared separately with the DTwP-primed children. TT-specific IgE and IgG titers did not differ significantly in prebleed serum samples from these subgroups (Fig. 1). TT-specific IgG titers boosted equivalently in all three subgroups. However, the exclusively DTaP-primed children with large local reactions (DTaP-R) also displayed markedly (in some cases, log-scale) elevated TT-specific IgE responses postboosting (Fig. 1) which were essentially restricted to this subgroup.

Analysis of TT-specific T-cell responses in the groups revealed a pattern of Th2-polarized vaccine-specific cytokine responses, which is consistent with the TT-specific IgE antibody data. Notably, the DTaP-R group displayed relatively elevated TT-specific IL-5, IL-6, and IL-13 responses at the time of boosting (Fig. 2), which in the case of the IL-5 component was further amplified by the booster and remained relatively elevated thereafter ($P = 0.025$; not shown).

Table 2 illustrates Spearman correlations between TT-specific humoral and cellular immune responses and the size of

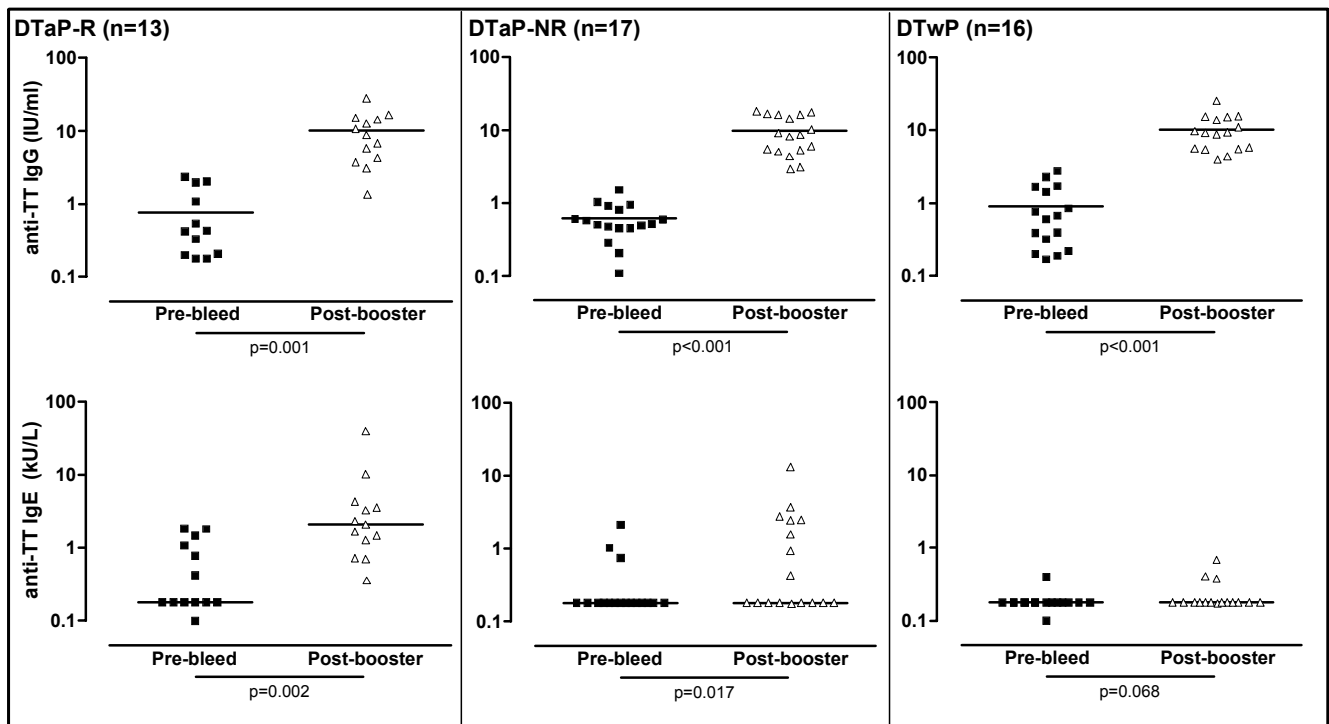


FIG. 1. Anti-TT IgG and IgE in DTaP-R-, DTaP-NR-, and DTwP-primed groups. Serum samples taken from 4- to 6-year-old children at the time of (prebleed) and 4 weeks after (postbooster) vaccination with DTaP were assayed for TT-specific IgG (in 10^3 units per liter [kU/L]) and IgE (IU/ml). The children have been classified as DTaP-primed reactive (DTaP-R) (those with a ≥ 50 -mm-diameter redness at the site of the vaccine), DTaP-primed nonreactive (DTaP-NR) (those with smaller or no reaction), and DTwP-primed (DTwP). Data are expressed as scatter plots, with the solid line representing the median value. Below each graph, *P* values are shown, which were determined by the Wilcoxon matched-pair signed-rank test.

local reactions to the booster among exclusively DTaP-primed children. Strong associations are apparent with TT-specific Th2 cytokine responses involving IL-5, IL-6, and IL-13 and a weaker association with IFN- γ . It is additionally noteworthy that production levels of the Th2 cytokines IL-5 and IL-13 in response to the polyclonal T-cell mitogen PHA also correlated with reaction size, suggesting that susceptible children are part

of a subgroup who are intrinsically high Th2 responders. This did not in turn associate with atopy expression (data not shown), although the sample numbers may be insufficient to detect such relationships, particularly at this early age when allergic manifestations are often of low intensity. In this regard, 11 of the 19 DTaP-primed children had a doctor diagnosis of atopy or asthma, and the reaction frequency was not different

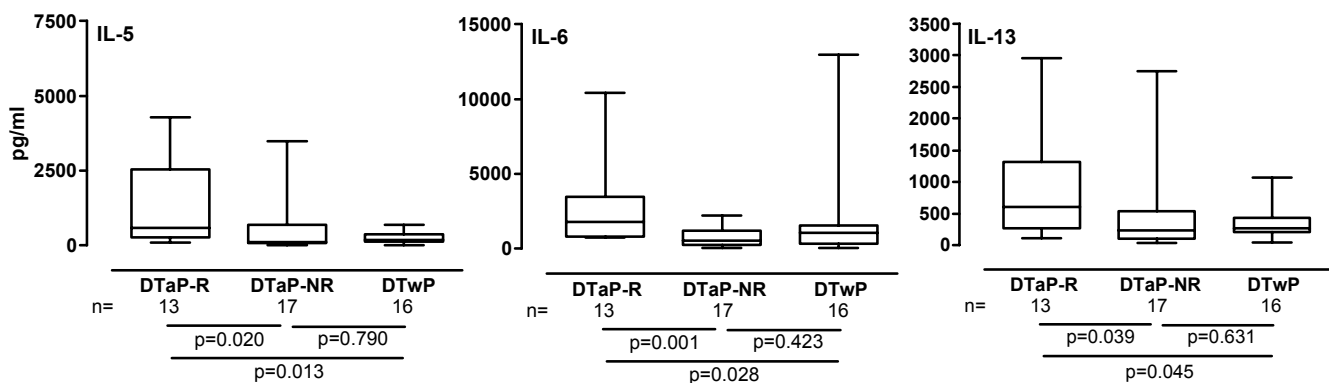


FIG. 2. TT-specific cytokine production by PBMC. PBMC isolated from children at the time of (prebleed) vaccination with DTaP were cultured for 96 h alone or together with TT (0.5 Lf/ml). Supernatant levels of IL-5, IL-6, IL-10, IL-13, and IFN- γ were determined by time-resolved fluorometry, and IL-5, IL-6 and IL-13 data are shown here as picograms per milliliter. The change in values (treatment control) for each individual was calculated, and the pooled data were then expressed as box plots for DTaP-R-, DTaP-NR-, and DTwP-primed groups described in the legend to Fig. 1. The limits of the boxes represent the 25th and 75th percentiles of the results, the black line in the box represents the median (50th percentile), and the whiskers represent the 5th and 95th percentiles. Significant differences between each of the groups are shown and were determined by the Mann-Whitney U test for unpaired responses.

TABLE 2. Correlations between immune response and local reaction size

Statistic	Cellular or humoral response										TT IgE	TT IgG
	IL-5		IL-6		IL-10		IL-13		IFN- γ			
	TT	PHA	TT	PHA	TT	PHA	TT	PHA	TT	PHA		
Spearman's ρ	0.496	0.566	0.588	0.148	0.222	0.062	0.464	0.690	0.405	0.065	0.240	-0.036
<i>P</i>	0.005	0.003	0.001	0.480	0.239	0.767	0.010	<0.001	0.026	0.758	0.201	0.812

between those with and without this diagnosis ($P = 0.183$ by chi-square test).

The degree to which DTaP-induced boosting of vaccine-specific Th2 immunity influenced systemic Th2 immunity to other antigens was separately analyzed. We did not detect statistically significant differences between groups (or between bleeds) in titers of total or house dust mite-specific IgE or in titers of IgE to mixtures of inhalant (Phadiatop test) or food allergens (FX5 test). Moreover, cytokine responses to the measles component of the MMR vaccine given at the time of the DTaP boosting was comparable in all groups (data not shown).

As noted in Table 2, the polyclonal IL-5 and IL-13 response capacity measured at the time of DTaP boosting also correlated with the magnitude of the red reaction at the site of the DTaP booster dose within the exclusively DTaP-primed group. Stratification of this group prior to analysis revealed enhanced overall Th2 competence, as evidenced by elevated polyclonal IL-5 and IL-13 reactivity in the DTaP-R subgroup manifesting the strongest local reactions (DTaP-R > DTaP-NR; $P = 0.008$ for IL-5 and $P = 0.001$ for IL-13; data not shown).

Stepwise logistic regression was used to further examine associations between reactivity at the booster site (as a binary variable, reactivity defined as redness ≥ 50 mm) and immunological indices at the prebleed (cellular immune responses and antibody titers), adjusting for infant vaccination status and IPV vaccination status at the time of the booster as potential confounding factors. As shown in Table 3, the magnitude of the Th memory response to tetanus antigen exemplified by IL-6 production and exclusive priming in infancy with DTaP are positively associated with reactivity to the booster. In contrast, covaccination with IPV admixed with the DTaP booster was negatively associated with reactivity. It is of interest to note in this context that anti-TT IgG levels were boosted to a significantly higher degree in children receiving the combined DTaP-IPV relative to those given the vaccines in opposite arms (13.7 ± 2.3 versus 7.6 ± 1.2 IU/ml; $P = 0.031$), and moreover, a significantly lower proportion of this subgroup (3/16 children

versus 9/14 children; $P = 0.011$) displayed ≥ 4 -fold rise in TT-specific IgE titers following the booster.

DISCUSSION

As detailed above, the results presented here stem from a post hoc analysis of immunological data collected on a group of local 4- to 6-year-old children participating in a multicenter trial on the reactogenicity of DTaP-IPV vaccine. The analysis was prompted by the unexpected observation of an apparently strong association between the intensity of injection site reactions postboosting and the type of DTP vaccine given earlier to the children during infancy. Notably, large reactions were essentially restricted to children primed with the acellular version of the DTP vaccine.

While this is a small study with insufficient statistical power to permit firm conclusions to be drawn, there are nevertheless sufficient internal consistencies within the data set and with the published literature, outlined below, to suggest that the observations reported merit systematic follow-up in other (and larger) study populations.

Firstly, the overall results of this study are consistent with a series of previous reports (2, 14, 19, 26) indicating relatively frequent local reactions to the preschool booster in children primed in infancy exclusively with the DTaP vaccine. Moreover, the findings are consistent with earlier reports suggesting that the preschool DTaP booster selectively increases levels of Th2-associated responsiveness to vaccine antigen, including both humoral (specific IgE antibody) and cellular immunity (24), and also with data suggesting that susceptibility to the Th2-trophic effects of DTaP is highest among children who are intrinsically "high Th2 responders" (4). The salient new finding from this study is the apparent association between preexisting Th2-polarized cellular immune memory to DTaP vaccine antigen prior to administration of the preschool booster and the subsequent expression of local reactions at the injection site over the 24 to 72 h following administration of the booster. The time course of these reactions reflects a delayed type as opposed to immediate response and is hence consistent with a cellular (as opposed to humoral) immune etiology. Consistent with this thesis, there was no association detected between local reactions to the DTaP booster and circulating vaccine-specific IgE antibody which was universally low prior to boosting, although production of this antibody was triggered by the booster.

The apparent association between Th memory and injection site reactions suggests that susceptibility to local side effects of the DTaP booster in preschoolers is programmed earlier during the initial priming process in infancy. In this regard, we (22,

TABLE 3. Relationship between large local reactions (≥ 50 mm) at preschool booster vaccination site and immune response variables in children determined at the time of boosting^a

Variable	Odds ratios	95% CI ^b	<i>P</i> value
DTaP exclusively in infancy	21.926	1.682-285.871	0.018
Combined DTaP-IPV	0.136	0.022-0.858	0.034
Prebleed TT-specific IL-6	27.676	2.204-347.530	0.010

^a Relationship determined by logistic regression modelling.

^b 95% CI, 95% confidence interval.

23) and others (4, 25) have previously demonstrated that T-cell memory responses in infants to the DTaP vaccine are initially highly Th2 polarized relative to those observed with the more Th1-trophic DTwP vaccine, presumably reflecting a combination of the intrinsic (developmental) Th2 bias of the immature human immune system (1, 5, 10) and the relative paucity of Th1-stimulatory cell wall-derived microbial “danger” signals in the DTaP vaccine (12). It appears from these and other studies (24) that rechallenge with DTaP vaccine antigens 2 to 3 years after priming recalls immune responses with a comparable Th2 bias, leading to the further amplification of this component of the immune response, including stimulation of vaccine-specific IgE production. It should be stressed that these DTaP-induced Th2 recall responses do not spill over significantly into the systemic compartment, as there is no evidence from this or previous studies (24) that the preschool booster (or the earlier priming doses of DTaP) promote environmental allergen-specific IgE antibody production.

The significance of these findings in relation to the responsiveness of DTaP-vaccinated children to subsequent encounters with antigens within the DTaP mixture cannot be gauged with certainty, and it is for this reason that we believe follow-up studies in preschool children and older age groups should be undertaken. For example, it is a matter of potential concern that a significant subset of exclusively DTaP-vaccinated children exhibited marked increases in Th2-polarized vaccine-specific immunity exemplified by TT-specific IgE levels following the DTaP booster. While the overall sample size of DTaP-primed children is admittedly small ($n = 30$), the increase in specific IgE occurred in 60% of these subjects, but more importantly, this increase was seen in 12 of the 13 children in the subgroup manifesting large local reactions. The time frame over which vaccine-specific Th2 immunity remains elevated postboosting, conceivably during which the risk for further (potentially more-intense) Th2-mediated responses to exposure to vaccine antigens is high, is currently unknown and merits more-detailed investigation of larger study populations. In this context, components of the DTaP vaccine, such as TT, are increasingly being used as carrier proteins to enhance antibody responses to polysaccharide conjugate vaccines, such as meningococcal C conjugate vaccines which are being administered to preschool and school-age children as part of mass immunization campaigns in a number of countries. If the Th2 component of memory induced by the DTaP booster proves to be persistent in a subset of these children, this may have unforeseen effects on the carrier functions of DTaP-associated proteins and hence on the efficacy and/or reactogenicity of the vaccines in which they are used.

An additional issue which merits further investigation involves potential interactions between the DTaP and IPV vaccines at the booster site. In particular, it is possible that analogous to the early phase of Th memory development in the mouse (15), Th2-polarized DTaP-specific memory in 4- to 6-year-old children resulting from infant priming may remain at least partially “plastic” and thus susceptible to immune deviation via exposure to Th1-trophic stimuli. This possibility was indirectly tested via the analyses in Table 3. All the children in this study had been primed during infancy with live (oral) polio vaccine, which promotes development of T-cell memory comprising low-level IFN- γ production in the absence of IL-5 or IL-13, indicative of overall Th1 polariza-

tion (30). The findings in Table 3 and accompanying data on titers of TT-specific antibody demonstrate that if the DTaP booster is accompanied by concomitant IPV boosting at the same site, ensuring Th2-associated local reactions and the accompanying boosting of TT-specific IgE are significantly reduced, while IgG boosting improves. This suggests the possibility that reactivation of Th1-polarized poliovirus-specific memory cells by IPV may provide “bystander” feedback inhibition of the Th2 component of DTaP-specific memory, both locally and in the draining lymph node. This possibility merits more-detailed investigation employing larger study groups, including subjects primed during infancy with IPV, which is now used in many countries in preference to oral poliovirus vaccine. It should additionally be noted that all the children in this study also received the MMR vaccine, but as this was common to all groups, it is unlikely to have contributed to the observed effects.

In conclusion, the results of this small study point to the possible existence of a series of underlying complexities relating to the performance of the DTaP vaccine in young children, which become evident only when underlying DTaP-primed memory is systematically analyzed. Several of the relevant issues raised here can be partially addressed by retrospective analysis of existing reactogenicity data from preschool booster studies, combined with analyses in cryobanked sera of TT-specific IgE antibody. However, our results also add further weight to growing arguments which stress the necessity for parallel monitoring of cellular immune indices in assessment of the safety and efficacy of infant vaccines, as opposed to the current practice of almost complete reliance upon markers of humoral immunity.

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