

Development of immunologic memory against tetanus toxoid and pertactin antigens from the diphtheria-tetanus-pertussis vaccine in atopic versus nonatopic children

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Background: Recent findings suggest that a hallmark of the atopic phenotype is reduced capacity to respond to vaccine antigens, as well as to environmental allergens, during infancy. This deficiency, which is most marked for the cytokine IFN- γ , appears transient but can result in a long-lasting imbalance within T helper cell (T_H) memory responses to allergens. Indirect evidence suggests that parallel effects may occur within immunologic memory responses against vaccine antigens in atopic children.

Objective: Our purpose was to compare vaccine antigen-specific T_H memory responses in atopic and nonatopic children.

Methods: We analyzed specific serum IgG and cytokine responses to pertactin and tetanus antigens as well as to mitogen (PHA) and house dust mite (HDM) allergen in 25 HDM-sensitized atopic and 25 nonatopic 6-year-old children who were vaccinated and boosted with diphtheria-tetanus-pertussis (DTP) vaccine.

Results: PBMCs from the atopic subjects produced higher levels of T_H1 and T_H2 cytokines to HDM allergen and PHA. Vaccine antibody titers were normal in the atopic subjects; vaccine-specific T_H2 responses were rarely detectable, yet T_H1 (IFN- γ) responses, in particular against tetanus, were frequent and higher in the atopic subjects (121.5 [SE 64.3] vs 8.0 [3.5] pg/mL culture fluid, $P = .04$). Corresponding pertactin responses were comparable in both groups.

Conclusions: At the completion of the full primer-boost DTP vaccination regimen, levels of vaccine-specific immunity in atopic 6-year-old children are at least equivalent to their nonatopic counterparts, indicating that the transient atopy-associated deficiency in T_H1 function in childhood can be successfully overcome by appropriate vaccination and boosting regimens. (*J Allergy Clin Immunol* 2000;105:1117-22.)

Key words: Atopy, children, T_H1/T_H2 , vaccines, cytokines

The nature of the immunologic defect(s) associated with genetically determined susceptibility to atopy, and how it interacts with known environmental risk factors, represents an area of intensive research. An issue of particular interest, which is becoming increasingly controversial in both the biomedical and lay press, concerns interactions between the developing immune system in children genetically susceptible to atopy and microbial stimulation through normal environmental contact or through deliberate exposure by vaccination.

This complex issue has arisen in the light of a series of recent findings on postnatal maturation of adaptive immunity in children and how these relate to the subsequent development of diseases such as atopic asthma. It has been recognized for many years that certain aspects of immune function are poorly expressed in humans at birth and do not attain adult-equivalent levels of competence for several years postnatally.¹ Recently, it has become evident that the principal functions that exhibit this maturational deficiency in early life are associated with the T helper type 1 (T_H1) arm of the immune response.² The primary function of T_H1 immunity involves antimicrobial defense, and the principal stimulus for postnatal maturation of the relevant T_H1 functions is provided by contact with microbial flora and pathogens in the extrauterine environment.^{3,4} Secondly, T_H1 immunity also provides a protective counterbalance against the development of excessive T helper type 2 (T_H2)-mediated immune responses, which through elaboration of cytokines such as IL-4, IL-5, and IL-13 can potentially produce pathogenic allergic inflammation.⁵

It has also been recently shown that key aspects of long-term immunologic memory against environmental allergens, in particular the balance within the T helper cell system between T_H1 versus T_H2 immunity, is frequently established during early childhood,^{6,7} and hence the rate at which postnatal maturation of T_H1 function(s) proceeds during this period is likely to be a significant determinant of subsequent allergen responder phenotype.⁴

In this context, we have recently demonstrated that generalized T helper cell activity in neonates and infants genetically at high risk of atopy is depressed relative to

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Abbreviations used

CMI:	Cellular immunity
DTP:	Diphtheria-tetanus-pertussis vaccine
HDM:	House dust mite
mRNA:	Messenger RNA
RT:	Reverse transcriptase
SPT:	Skin prick test
T _H 1:	T helper type 1
T _H 2:	T helper type 2

the population at large and that the rate of postnatal maturation of T helper cell functions (in particular T_H1-associated functions) is intrinsically slower in these high-risk subjects.^{7,8} Furthermore, this in turn is reflected in patterns of developing allergen-specific T-cell memory during early childhood, notably in the failure of high-risk subjects to switch off (or “immune deviate”) the T_H2 component of their responses.^{7,9} Recent studies also suggest that T-cell memory development against vaccine antigens may be compromised in high-risk children. Notably, a retrospective study on 12-year-old children has demonstrated that expression of atopy and asthma at this age was strongly associated with a low capacity to develop stable T_H1-dependent cellular immunity (CMI) to BCG vaccination during infancy¹⁰; we have shown a comparable association in a pilot study on early development of CMI to the “triple” antigen vaccine during infancy,¹¹ and a generalized deficiency has also been reported in numbers of circulating CD45⁺RO⁺ T helper memory cells in older atopic children.¹²

Collectively, these latter findings suggest that children at genetic risk of atopy, which with current figures constitute 35% to 45% of the population, may be intrinsically hyporesponsive to vaccines given during infancy, and if so this has important public health implications in relation to pediatric vaccination programs. To further complicate this picture, however, it has also been suggested on the basis of indirect epidemiologic evidence that early childhood vaccination may in some circumstances increase the risk for subsequent development of atopy.^{13,14} Such unexpected effects might conceivably derive from direct influences of components of the vaccine (eg, T_H2 “adjuvants” in *Bordetella pertussis* cell walls¹⁵) or indirectly by lowering of the overall microbial burden during childhood and thus reduction in the level of stimulation provided to the developing immune system.¹⁶

These issues are increasingly being debated in the public arena, particularly those relating to infections and vaccination programs, and the implications of this debate provide an imperative for more focused research on the underlying immunologic processes in early life. In particular, there is an urgent need for detailed comparative information on the nature of immune responses to vaccines commonly used during infancy in atopic and nonatopic children, especially in relation to T helper cell function.

The current study focused on one aspect of this question, the development of humoral and cellular immunity to the triple antigen (diphtheria-tetanus-pertussis [DTP]) vaccine in panels of atopic and nonatopic 6-year-old children who have undergone the full preschool immunization-boosting program.

METHODS

Subjects

Two groups of 25 age-matched children, respectively, atopic (mean 6.0 and range 5.8–6.3 years) and nonatopic (mean 5.9 and range 5.6–6.1 years) were selected from a nested case-control subsample from a continuing cohort study. Skin prick test (SPT) responses were assessed to a panel of inhalant and food allergens including house dust mite (HDM). The atopic subjects selected were all HDM responsive and the nonatopic subjects were unresponsive to the full panel. All subjects in the study had been vaccinated with cellular DTP vaccine (CSL, Melbourne, Australia) at 2, 4, 6, and 18 months and at 5 years. The study was carried out with the approval of the Princess Margaret Hospital for Children Ethics Committee.

Cell preparation and culture

PBMCs were cryopreserved after collection and batch analyzed, as per previous studies.^{6,7} After being thawed, the cells were resuspended at 1×10^6 viable cells per milliliter in serum-free medium supplemented with 2-mercaptoethanol, which has previously been shown to be optimal for measuring responses to allergens or mitogens, or RPMI 1640 medium (Gibco, Australia) supplemented with 5% pooled human AB serum, which is optimal for demonstrating responses to vaccine antigens.¹⁷ Aliquots of 0.5 to 1.0 mL from each subject were cultured at 37°C with 5% carbon dioxide for 48 hours in medium alone or medium containing 2 µg/mL pertactin from *B pertussis* (SmithKline Beecham, Belgium), tetanus toxoid (0.5 Lf/mL; CSL Laboratories, Australia), PHA (HA16, 1 µg/mL; Murex Australia), or HDM allergen (10 µg/mL; aqueous extract of *Dermatophagoides pteronyssinus* from CSL, Australia).

After culture the cells were centrifuged and used immediately for RNA extraction; the culture supernatants were stored at –20°C for ELISA.

Analysis of cytokine responses

Semiquantitative reverse transcriptase (RT)–PCR was used for detection in cell pellets of messenger RNA (mRNA) specific for IL-4 and IL-9 because IL-4 levels were below the limits of detection by ELISA, and reagents for IL-9 ELISA were not available to us. mRNA specific for β-actin was also determined. Details of primer sequences, conditions for RT-PCR reactions, and detection of PCR products by slot blotting were as described previously.^{6,9} Production levels of mRNA for IL-4 and IL-9 above background controls were expressed as ratios to the β-actin control as described previously.^{6,9}

Quantitation of IL-5, IL-10, IL-13 and IFN-γ protein in supernatants used ELISA assays as detailed.^{6,9} Data were expressed as picograms of cytokine protein per milliliter of culture after subtraction of backgrounds from unstimulated cultures.

Antibody assays

B pertussis IgG was measured with use of a commercial ELISA kit (PanBio, Australia) and expressed as arbitrary units per milliliter. Tetanus- and diphtheria toxoid-specific IgG was measured in the clinical immunology laboratory at Princess Margaret Hospital with use of in-house ELISA assays with World Health Organization–derived standards provided by CSL (Melbourne, Australia) and expressed as international units per milliliter.

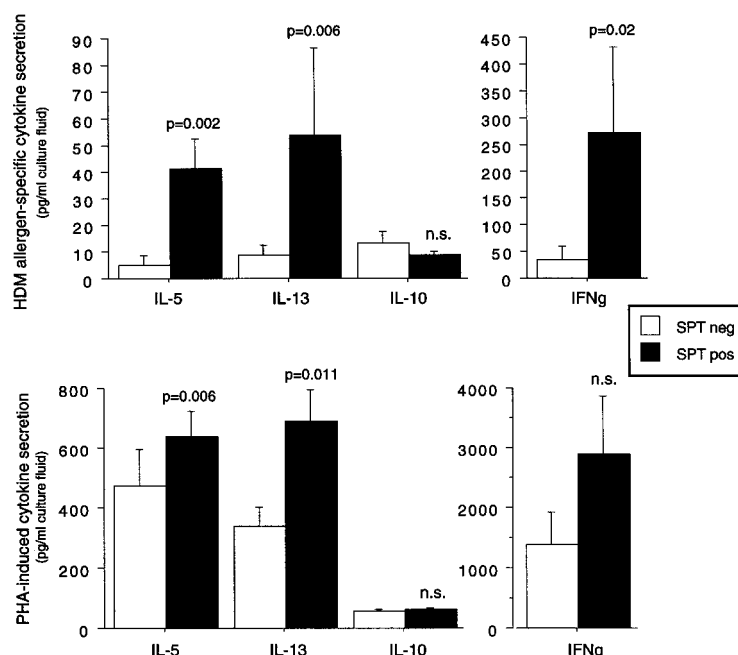


FIG 1. Cytokine responses to mitogen and allergen in PBMCs from 6-year-old children. PBMCs were stimulated with either HDM (top) or PHA (bottom) as detailed in Methods, and culture supernatants were harvested at 48 hours for ELISAs. Data shown are group means $\pm$ SE.

Statistical analyses

Data were analyzed by the Mann-Whitney *U* test with Statview (SAS Institute, Cary, NC).

RESULTS

Cytokine production patterns in atopic versus nonatopic children

Cytokine responses in cultures of HDM-stimulated PBMCs clearly distinguished between atopic populations who were responsive to HDM and their nonatopic counterparts (Fig 1, top). Notably, HDM-induced production of the T_H2 cytokines IL-5 and IL-13 and also the T_H1 cytokine IFN- γ were markedly increased in the SPT⁺ atopic group.

This pattern was mirrored in cytokine responses induced by the T-cell mitogen PHA (Fig 1, bottom). In particular, polyclonal production of IL-5 and IL-13 were significantly increased in the atopic group; mean levels of production of IFN- γ were also higher among the atopic subjects, but the differences were not statistically significant because of the large variations within the groups.

Vaccine-specific antibody production

IgG antibody to all 3 vaccine antigens was detected in all subjects, and no significant differences were observed between the 2 groups (Fig 2).

Cytokine responses to vaccine antigens

The most consistent responses detected were against the tetanus toxoid antigen. As shown in Fig 3, mean response levels within the atopic and nonatopic groups as

measured by IL-5, IL-10, or IL-13 protein or mRNA specific for IL-4 or IL-9 were comparable. IFN- γ responses against the tetanus antigen were significantly higher within the atopic group.

Cytokine responses against pertactin were less frequent and of lower magnitude. The most consistent were IFN- γ , and group mean responses were not significantly different (atopic 13.1 [SE 5.9] vs nonatopic 10.6 [SE 5.2] pg/mL culture fluid, *P* = .9).

DISCUSSION

Previous studies from our laboratory on inhalant allergen-specific T helper cell function during early life have demonstrated that responses in neonates follow the typical fetal pattern, being dominated by T_H2 cytokines, together with a very low and variable T_H1 component that is usually detectable only at the level of mRNA.¹⁸ During early infancy the contribution of T_H2 cytokines to these responses rapidly wanes in nonatopic subjects, with concomitant up-regulation of allergen-specific T_H1 reactivity as exemplified by IFN- γ .¹⁸ In contrast, allergen-specific¹⁸ and generalized T_H1 activity⁸ up-regulate more slowly in atopic subjects, who instead consolidate their fetally primed T_H2 immunity,^{7,9} possibly as a direct result of the relative paucity of cross-regulatory signals from the T_H1 arm of their responses.⁴

As noted above, there is some evidence to suggest that capacity to develop sustained vaccine immunity in response to priming during infancy may also be compromised in atopic subjects. Two recently described examples are in vivo T_H1 -dependent tuberculin responsiveness

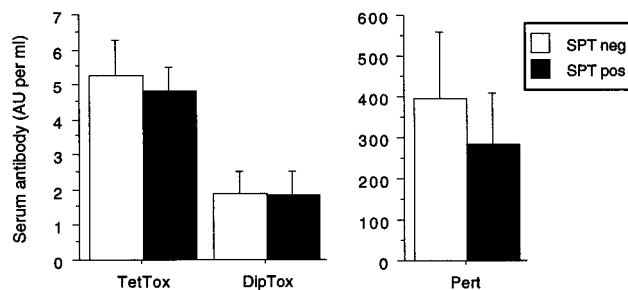


FIG 2. Vaccine antigen-specific IgG antibody in serum. Antibody levels were determined as detailed in Methods. Data shown are group means $\pm$ SE.

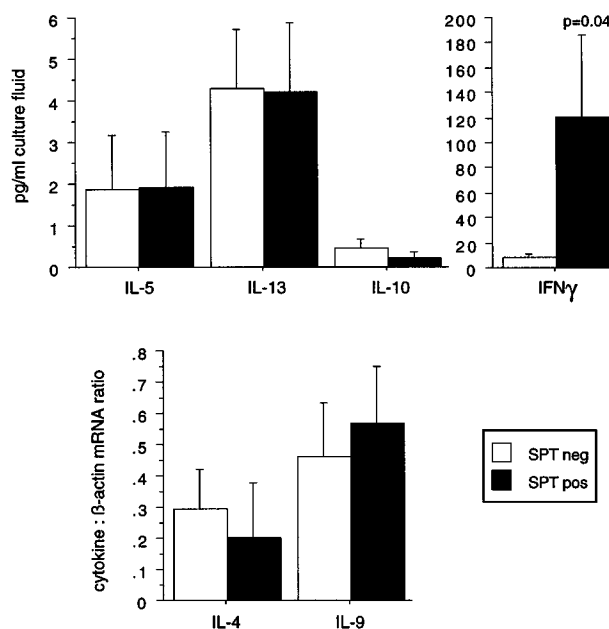


FIG 3. Cytokine responses to tetanus toxoid antigen in PBMCs from 6-year-old children. PBMCs were stimulated with tetanus toxoid antigen, and ensuing cytokine responses were determined as cytokine protein (by ELISA) or cytokine-specific mRNA relative to mRNA specific for the housekeeping gene β -actin (by semiquantitative RT-PCR), as detailed in Methods. Data shown are group means $\pm$ SE.

to BCG vaccination given at ages <3months¹⁰ and in vitro lymphoproliferation (a surrogate marker for CMI) in response to tetanus antigen measured at outcome age 2 years in children vaccinated during infancy.¹¹

In the current study we focused on panels of atopic and nonatopic 6-year-old children who had been fully vaccinated during infancy with the DTP vaccine and then boosted at age 5 years. We sought information on their specific T helper cell cytokine and antibody responses to antigens in the vaccine and additionally their overall T-cell responsiveness with use of a polyclonal T-cell stimulant.

As shown in Fig 1, at age 6 years the atopic group displayed enhanced polyclonal production of the T_H2 cytokines IL-5 and IL-13, and their IFN- γ production capacity was at least equivalent to that of nonatopic subjects. Earlier studies from several laboratories have demonstrated reduced capacity for polyclonal production

of both T_H2 and especially T_H1 cytokines in atopic neonates^{7,19-24} and infants,⁸ and the findings in Fig 1 suggest that this maturational deficiency is redressed by the age of 6 years.

Fig 1 additionally demonstrates that IFN- γ responses against HDM are also well developed in T helper memory cells from 6-year-old HDM-SPT⁺ atopic children, and in fact exceed those of nonatopic children. We have recently reported similar findings in a cohort of children of comparable age.⁶ The atopic children in this study additionally displayed vigorous production of IL-5 and IL-13 to HDM and thus exhibited the mixed (or T_H0-like) cytokine pattern typical of many atopic adults.^{25,26} The increased production levels of IFN- γ manifested by the atopic subjects at this age may be indicative of an underlying compensatory mechanism that is associated with redressing their earlier T_H1/T_H2 imbalance; additional cross-section-

al studies are required to determine how long this apparent IFN- γ overshoot persists in this group and whether it plays a role in disease pathogenesis. This latter possibility is plausible, given recent findings from human^{27,28} and experimental animal systems^{29,30} that suggest that T_H1 immunity may contribute to tissue damage at sites of allergic inflammation. In relation to the overall allergic sensitization process, these findings also imply that the delayed postnatal upsurge of T_H1 function in the atopic subjects may occur too late to provide effective feedback inhibition of the T_H2 component of their allergen-specific T helper cell memory responses in the atopic children. One plausible explanation for this failure may be that the relevant negative feedback for T_H2 cell expansion is not provided by IFN- γ itself but instead by upstream cytokines in the T_H1 pathway, in particular IL-12, which is known to play a major role in the early phase of T_H1/2 switch regulation.³¹

The question of whether this transient T_H1 deficiency in infancy has significant long-term consequences with respect to T helper cell memory generation in vaccine-specific immune responses was explored in detail in Figs 2 and 3. The data shown in Fig 2 represent IgG antibody titers to the 3 major antigens in the DTP vaccine at outcome age 6 years. Significant responses were observed in all subjects and group means were not different.

Fig 3 illustrates T helper cell responses in these subjects against tetanus toxoid as exemplary of the DTP vaccine antigens. It can be seen that, unlike the situation observed with respect to responses against allergens (Fig 1), T_H2 cytokine production against the vaccine antigen among the atopic subjects corresponds closely with the nonatopic range. Moreover, the atopic subjects displayed an enhanced IFN- γ response to this antigen, mirroring the pattern observed with use of polyclonal stimulation. The mixed T_H1/T_H2 cytokine response patterns in Fig 3 are consistent with a recent report on a multicenter trial with acellular DTP vaccine.³²

These findings suggest that, although initial immune responses to the DTP vaccine in infants at high risk of allergic disease may develop slower than in those at low risk, vaccine-specific immunologic memory is eventually expressed strongly in the former subgroup after the preschool booster. It should also be emphasized that a recent large prospective study indicates that vaccination per se is not associated with increased risk of allergy development.³³

However, the possibility remains that the initial sluggishness of T helper cell function in potentially atopic children, resulting in transiently lower levels of responsiveness to vaccination up to age 2 years,¹¹ may also equate to transiently lower levels of protection against the pathogens targeted by the vaccine. This is a potentially important question, given the frequency of children at high risk of atopy within the overall population and the growing trend for the introduction of an ever-increasing range of vaccines for early pediatric use, particularly multivalent vaccines in which the doses of individual components may be low relative to preparations current-

ly used. In addressing this issue, continuing prospective studies in our laboratory are focusing on DTP vaccine-specific T helper cell function in birth cohorts spanning the period of 3 to 18 months of age. Specific studies are also urgently required on T helper cell responses to mucosal vaccines over this age range because even less information is available on the nature of the immune responses that these induce in infants.

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