

Pertussis IgE and atopic disease

Nilsson L, Grüber C, Granström M, Björkstén B, Kjellman N-IM. Pertussis IgE and atopic disease.

Allergy 1998; 53: 1195–1201. © Munksgaard 1998.

Background Pertussis toxin (PT) stimulates IgE production in animals, and pertussis vaccination and whooping cough may have similar effects in man.

Methods We analyzed IgE responses to PT (PT-IgE) in sera from children primarily immunized with three doses of either an acellular 2- or 5-component vaccine, or a whole-cell (Wc) pertussis vaccine, and in children after whooping cough. The study comprised 50 children with both atopic disease and positive skin prick test, 99 nonatopic controls, and 40 children with verified pertussis.

Results Immunoglobulin E antibodies against PT were demonstrated in 19% and 24% of sera from vaccinated children at 7 and 12 months, respectively, and in 9% at 2.5 years. At 7 months, PT-IgE was more common after vaccination with acellular (24%) than with the Wc vaccine (3%, $P=0.02$). PT-IgE was also more common ($P=0.001$) after vaccination in children classified as atopic (36%) than in the control group (10%). Thirty percent of the children with pertussis had PT-IgE, more often so in atopic than nonatopic children ($P=0.02$).

Conclusions Transient production of PT-IgE seems to be common after primary pertussis immunization with acellular vaccines, and after whooping cough, particularly in atopic subjects.

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Key words: bronchial asthma; hypersensitivity; IgE antibodies; pertussis.

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Accepted for publication 4 August 1998

Pertussis toxin (PT) is widely used in animal experiments as an adjuvant to enhance sensitization to simultaneously administered antigens (1–3). Similarly, moderate increases of serum IgE and sensitization to unrelated allergens have been demonstrated in children after whooping cough (4, 5). Furthermore, circulating IgE antibodies to PT (PT-IgE) are commonly detected after pertussis immunization (6–8).

It was recently claimed that pertussis vaccination in infancy may increase the risk of asthma by up to fivefold; i.e., from 2% to 11% (9). We could not confirm this in a prospective study comprising 669 infants who were immunized either with one of two acellular pertussis vaccines, or a whole-cell (Wc) vaccine, employing a control group of diphtheria and tetanus (DT)-vaccinated infants (10). We here report the incidence and levels of PT-IgE after immunization with one of three pertussis vaccines and after pertussis infection. The main hypothesis was that children with atopic disease and IgE to common food or inhaled allergens are more prone than nonatopic children to produce PT-IgE antibodies. We also assessed the incidence of local side-

effects after vaccination in relation to the presence of PT-IgE in children who did or did not develop atopic disease up to 2.5 years of age.

Material and methods

A double-blind, randomized study comprising 9829 children was performed at 14 centers in Sweden comparing an experimental two-component (2-c; SmithKline Beecham, Rixensart, Belgium), and a five-component (5-c; Connaught Ltd, Toronto, Canada) acellular pertussis vaccine, as well as whole-cell pertussis vaccine (Wc; Connaught Laboratory, Inc., Swiftwater, PA, USA), with diphtheria and tetanus vaccine (DT; Swedish National Bacteriological Laboratory, Stockholm, Sweden) as placebo (11). The children received three vaccinations at 2, 4, and 6 months of age. Blood samples were drawn at 7 and 12 months and at 2.5 years of age.

The study population in Linköping, Sweden, comprised 788 infants, 711 of whom took part in a study on development of allergy (12). Of these, 669 children were given three vaccine doses and

Table 1. Questions asked as part of structured interview relating to bronchial asthma. Boldface denotes responses supporting diagnosis

1.	Has your child <i>ever</i> had wheezing or whistling in the chest? No/ Yes
2.	Has your child had wheezing or whistling in the chest at any time during the last 12 months? No/ Yes
3.	How many episodes with wheezing has your child had during the last 12 months? None/1-3/ 4-12 / > 12
4.	During the last 12 months, how often, on average, has your child been disturbed by wheezing? Never/Less than one night/week/ 1 or more nights/week
5.	During the last 12 months, has the wheezing of your child ever been so severe that it could only say 1-2 words between the breathings? No/ Yes
6.	During the last 12 months, has your child had wheezing in the chest during or after exercise? No/ Yes
7.	During the last 12 months, has your child had dry cough in the nights without having a cold or an infection? No/ Yes
8.	Has your child ever had wheezing at any time after 2 years of age? No/ Yes
9.	Has your child ever had three diagnosed episodes of bronchitis before 2 years of age? No/ Yes
10.	Has your child ever had treatment with inhaled "Lomudal" (sodium cromoglycate) or inhaled steroids? No/ Yes
11.	Has your child ever had a diagnosis of bronchial asthma according to a physician? No/ Yes

Cumulative diagnosis of "bronchial asthma" by 2.5 years of age in 10 children was based on affirmative answer to question 1 and:

- affirmative answer to at least one of questions 3-7 and 8 and/or 9 ($n=5$)
- affirmative answer to question 11 and confirmed in medical records ($n=4$)
- affirmative answer to at least one of questions 8-10 and confirmed in medical records ($n=1$)

participated in the follow-up until 2.5 years of age (10).

The children were assessed at 2 months (before the first vaccine dose), 7 months (1 month after the third vaccine dose), and 2.5 years of age (27-32 months). In addition, the families were contacted by telephone every 6 weeks and asked to report possible symptoms of pertussis. They were also instructed to call if their child had had 7 days of cough. Blood samples for serologic analyses and nasopharyngeal cultures for *Bordetella pertussis* were obtained after 7 days of coughing. Questionnaires on smoke exposure of their child, home dampness, pets in the home, type of feeding, and possible allergic symptoms in the child were completed by the parents. The diagnosis of allergy was based on questionnaires completed at 2.5 years, the clinical findings on the follow-up, and information in medical records. The investigation was blinded regarding which vaccine the child had received until the diagnoses were established in all the children.

Diagnostic criteria

Atopic diseases. Atopy was defined as at least one positive skin prick test (SPT) to a common allergen. The diagnoses were established from questionnaires and clinical evaluation by specially trained research nurses. Standardized questions were used; e.g., for bronchial asthma (Table 1). Atopic dermatitis was defined as persistent or recurring itching eczema for 6 months or more (13). A child was regarded as having allergic rhinoconjunctivitis if answering affirmatively both of the following questions: "Has your child suffered from sneezing, rhinorrhea, or blocked nose without having a cold during the last 12 months?" and "Have the nose complaints been accompanied by itching or running eyes during the last 12 months?" Urticaria was

defined as allergic if it appeared after exposure to a particular allergen and with a positive SPT to the same allergen. The diagnostic criteria for food allergy included diarrhea, vomiting, urticaria, or Quincke's edema after ingestion of a specific food at least once, and demonstrable IgE antibodies to the same food.

Whooping cough. The diagnosis of pertussis was based on the criteria established by the World Health Organization (14). The families were contacted every 6 weeks. Blood sampling for serologic analyses and nasopharyngeal cultures for *B. pertussis* were done after 7 days of coughing. A case was defined as the presence of paroxysmal cough for at least 21 consecutive days, *plus* one of the following criteria: isolation of *B. pertussis* in culture, an increase of 100% or more in IgG or IgA antibodies against pertussis toxin, an increase of 100% or more in IgG or IgA antibodies against filamentous hemagglutinin (in the absence of positive results for *B. parapertussis* on culture or polymerase chain reaction), or documented contact with an infected household member with culture-confirmed *B. pertussis* infection who began to cough within 28 days before or after the onset of cough in the study child.

SPT

SPT were performed in duplicate according to the guidelines of the European Academy of Allergy and Clinical Immunology (15) at 7 months and 2.5 years. Pasteurized low-fat cow's milk; natural egg white; and extracts of cat, timothy, birch, *Dermatophagoides pteronyssinus*, and *D. farinae* (10 HEP, ALK, Hørsholm, Denmark) were used. Skin wheals with a mean diameter of 3 mm or more after 15 min were regarded as positive. Histamine

dihydrochloride (10 mg/ml) and blank lancets were included as positive and negative controls. No antihistamine should have been used for 3 days preceding the SPT. The results of the SPT at 7 months of age showed seasonal variation and have been published elsewhere (12).

Study groups

Blood samples from three groups of children among the participants in the allergy study (10) were chosen for the purpose of this study. Atopic disease developed in 182 of 669 children, SPT were positive once or more to foods or inhalant allergens in 120 of the 669 children, and whooping cough was diagnosed in 47 of the 669 children.

Group 1. Group 1 consisted of all the 50 children with atopic disease and a positive SPT to food and/or inhaled allergens but no whooping cough during the study period. Among the 50 children who were classified as atopic, 18 had two or more atopic diseases. Atopic dermatitis occurred in 34 of the children, and urticaria in 12. Ten had bronchial asthma, eight food allergy, and six allergic rhinoconjunctivitis. Forty of the 50 group 1 children were sensitized to egg and/or milk, and 23 had a positive SPT to inhalant allergens. Blood samples were available from 47, 48, and 48 children at 7 months, 12 months, and 2.5 years of age; in all, 143 blood samples were available for analysis from group 1.

Group 2. Group 2 included 99 children who were selected at random from among 183 children with no clinical manifestations of atopic disease, negative SPT, no pertussis infection, and no parental history of allergy. In all, 228 blood samples obtained at 7 months ($n=93$), 12 months ($n=70$), and 2.5 years ($n=65$) were available for analysis.

Group 3. In group 3, blood samples were available for analysis from 40/47 children with a verified pertussis infection between 7 months and 2.5 years of age, irrespective of any atopic disease and parental history of allergy. The children had been vaccinated with Wc ($n=8$), 2-c ($n=11$), 5-c ($n=1$), and placebo ($n=20$).

Antibodies against pertussis toxin

IgG antibodies against pertussis toxin were analyzed by an enzyme-linked immunosorbent assay (ELISA), as described previously (11).

The levels of IgE antibodies to pertussis toxin were analyzed by the Magic Lite® System (ALK,

Hørsholm, Denmark), an immunochemiluminometric assay that uses paramagnetic particles as a solid phase. Paramagnetic particles were coupled to pertussis toxin, as described below. The solid phase and serum were incubated overnight at room temperature for higher sensitivity. The detection limit was 0.5 SU/ml. We used a cutoff level for a positive test of 1.43 SU/ml, the cutoff level of the commercially available Magic Lite method, when not stated otherwise.

Pertussis toxin was purified from a culture of *B. pertussis*, strain D 382/83, which was grown for 5 days at 36°C in 4 l of Stainer-Scholte PE-medium. The cells were removed by centrifugation at 8300 g. The supernatant was collected, and pH was adjusted to 6.0 and then applied to a column of hydroxyapatite (Biorad SA, Ivry sur Seine, Cedex, France) and equilibrated with 0.01 phosphate buffer at pH 6.0. After washing of the column with 400 ml 0.01 M phosphate buffer, pH 7.0, and 400 ml 0.1 M phosphate buffer, pH 7.0, the bound material was eluted with 0.1 M phosphate buffer, pH 7.0, containing 0.5 M sodium chloride. The protein fractions were pooled and diluted one in two in distilled water, applied to a fetuin-Sepharose 4B (Pharmacia, Uppsala, Sweden) column, and equilibrated with 0.1 M NaHCO₃, pH 8.3, containing 0.5 M sodium chloride. After washing of the column with 50 ml 0.1 M NaAc, pH 7.0, containing 0.5 M sodium chloride, PT was eluted with 0.1 M NaAc, pH 7.0, containing 3 M NaSCN. The eluate was dialyzed overnight against 25 mM phosphate buffer, pH 9.0, containing 0.5 M sodium chloride. Analysis of the SDS gel showed only the five typical bands of the toxin subunits.

To ensure proper solution for the coupling process, medium of solubilized pertussis toxin was replaced with ammonium bicarbonate 0.005 M by membrane diffusion and freeze-dried. Protein was reconstituted with PBS, pH 7.4, immediately before coupling to a concentration of 238 g/l.

Resolubilized pertussis toxin was coupled to paramagnetic particles with the Magic Lite® SQ Allergy System Coupling Kit, according to the instructions of the manufacturer (ALK, Hørsholm, Denmark). Coupling efficiency was high, as no protein could be recovered from the buffer used for the coupling process. There was no nonspecific binding, as no signal was detected when the PT solid phase was incubated with negative control serum or diluent only.

Statistics

The chi-square test (with Yates' correction when $N < 100$) was employed for categorical variables, and Fisher's exact test was used when the expected

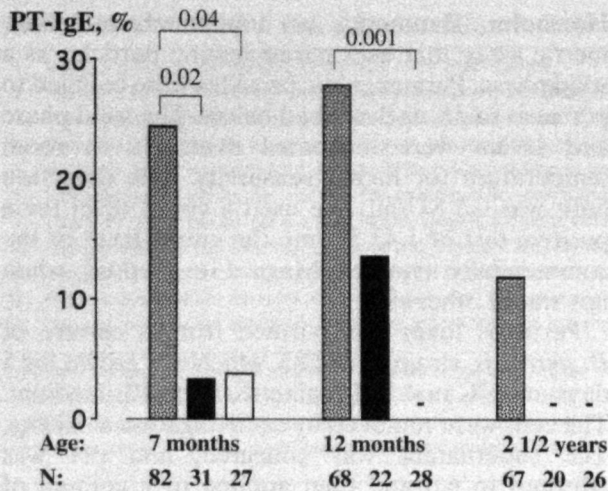


Fig. 1. Prevalence of pertussis IgE (PT-IgE) of ≥ 1.43 SU/ml at 7 and 12 months and 2.5 years in 149 children immunized with either acellular (two- or five-component) pertussis vaccines (stippled), whole-cell pertussis vaccine (filled columns), or diphtheria/tetanus vaccine (open columns) at 2, 4, and 6 months (groups 1 and 2).

frequency for any cell was less than 5. The Kolmogorov-Smirnov test (16) was used for the nonparametric evaluation of antibody levels, and the Wilcoxon signed rank test for changes in antibody levels in the same child. The Spearman

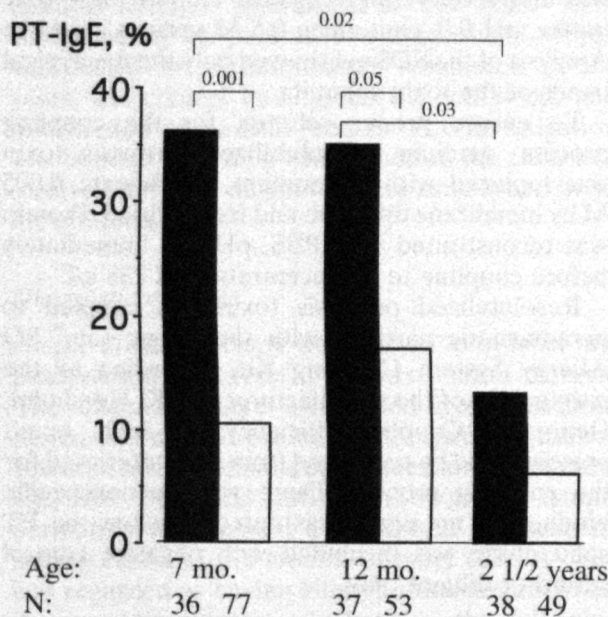


Fig. 2. Prevalence of PT-IgE (%) of ≥ 1.43 SU/ml at 7 and 12 months and 2.5 years in 39 children with atopic disease and positive skin prick test (filled columns) and 81 nonatopic (open columns) children after vaccination with acellular (two- or five-component) or whole-cell pertussis vaccine at 2, 4, and 6 months of age.

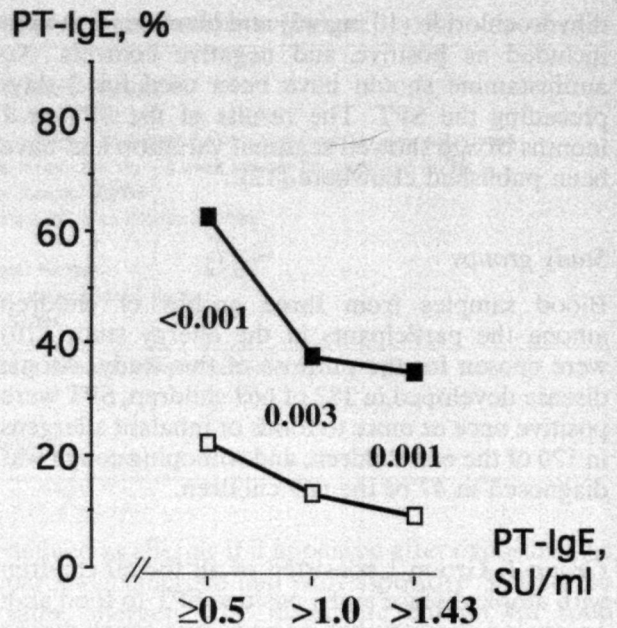


Fig. 3. Proportion of children 7 months of age with PT-IgE (%), employing different cutoff levels, i.e., ≥ 0.5 , 1.0, and 1.43 SU/ml, in 36 children with (filled squares) and 77 without (open squares) atopic manifestations who were pertussis vaccinated at 2, 4, and 6 months of age (groups 1 and 2, DT group excluded).

rank correlation test, corrected for ties, was employed for analysis of correlations.

Ethics

The parents of all participating children had consented to pertussis-related serologic studies and studies of development of allergies in relation to vaccination. The study was approved by the Human Research Ethics Committee of the medical faculty at Linköping University.

Results

IgE antibodies against PT were detected in 19%, 24%, and 9% (extended chi-square, $P=0.05$) of the pertussis-vaccinated children (groups 1 and 2) at 7 months, 12 months, and 2.5 years of age. The prevalence of PT-IgE was similar in children who were vaccinated with 2-c and 5-c acellular vaccines (data not shown). Children immunized with Wc vaccine developed PT-IgE less often than those receiving acellular vaccine; i.e., 3% vs 24% at 7 months ($P=0.02$, Fig. 1), 14% vs 28% at 12 months ($P=NS$), and none vs 12% at 2.5 years (NS).

The atopic (group 1) more often had PT-IgE than the control children (group 2) both at 7 months (36% vs 10%, $P=0.001$) and at 12 months (35% vs 17%, $P=0.05$) (Fig. 2). The corresponding prevalences at 2.5 years were 13% (5/38) and 6% (3/49), respectively (NS).

PT-IgE, %

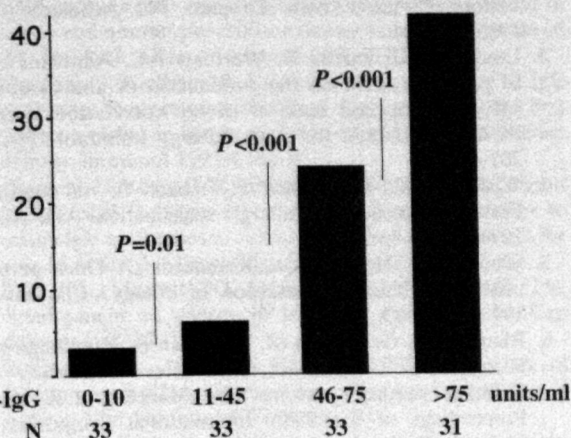


Fig. 4. Prevalence of PT-IgE (≥ 1.43 SU/ml) in relation to PT-IgG levels in 130 children at 7 months who were immunized with one of three pertussis vaccines at 2, 4, and 6 months. Statistical significance (chi-square test) is shown for association between PT-IgG levels and proportion of children with PT-IgE, employing various PT-IgG cutoff levels.

The rate of PT-IgE at 7 months (Fig. 3), 12 months, and 2.5 years (data not shown) was also associated with the cumulative incidence of atopic disease, independent of the chosen cutoff limit. Among those with detectable PT-IgE antibodies, the geometric mean value increased slightly from 7 to 12 months of age from 0.8 to 0.9 SU/ml ($P=0.02$) and was higher in atopic than in nonatopic children (1.1 vs 0.7 SU/ml at 7 months, $P<0.001$ and 1.2 vs 0.8 SU/ml at 12 months, $P=0.02$).

The only statistically significant relationship between PT-IgE at 7 months of age and side-effects was a nodule appearing at the site of the first vaccine injection at 2 months of age in 25 of 140 (18%) children without and in none of 28 children with PT-IgE at 7 months of age ($P=0.02$).

Twelve of 40 children (30%) with whooping cough (group 3) had PT-IgE more than 1.43 SU/ml at 2.5 years of age. PT-IgE more than 1.43 SU/ml was present in 9/20 DT vaccinated, and in 3/11 children vaccinated with the two-component vaccine but in none of the eight children who had received Wc vaccine, nor in the only child in this group who was vaccinated with the five-component vaccine. A PT-IgE of ≥ 1.43 SU/ml was significantly more often found in DT-vaccinated children with verified pertussis (9/20) than in pertussis-vaccinated children (Fig. 1, $P<0.01$) at 7 months of age.

Five of the 12 PT-IgE positive children in group 3 also had a positive SPT at 7 months, as compared with only two among the 28 PT-IgE negative children in this group ($P=0.02$). At 2.5 years, PT-IgE was detected in 22% (4/18) of children who had whooping cough between 7 months and 1.5 years of

age, and in 36% (8/22) with the disease after that age (NS).

There was a moderate correlation between the PT-IgE and PT-IgG levels at 7 months, 12 months, and 2.5 years ($\rho=0.53$, 0.48, and 0.35, respectively, $P<0.001$, for all comparisons) (Fig. 4).

Discussion

Infants immunized with three doses of either of two acellular pertussis vaccines more often developed PT-IgE than children vaccinated with a Wc vaccine. Furthermore, the levels of PT-IgE were higher after acellular than after Wc pertussis vaccine. The presence of PT-IgE was mostly transient, and 2 years after the primary immunization, the prevalence of PT-IgE was low in all the pertussis-vaccinated groups, but not in those who had a confirmed diagnosis of whooping cough. Moderate PT-IgE responses have previously been shown after pertussis vaccination (6-8, 17-19). Similarly, IgE antibodies to diphtheria and tetanus toxoids were common after booster vaccination with DT vaccines (20), while the levels of IgE to tetanus toxoid were low in children 12 months after primary immunization with tetanus vaccine (21).

Children were classified as atopic (group 1) only if they had the combination of symptoms suggesting a diagnosis of atopic disease and a positive SPT to common food and/or inhalant allergens. Transient low levels of IgE antibodies are common in healthy children (22), while higher levels early in life and particularly if found at repeated investigations are associated with atopic disease later in childhood (22-24).

Injection of antigens from a stinging insect may induce IgE antibody production in both atopic and nonatopic subjects (25). The association between IgE and IgG antibodies to pertussis toxin, which has been seen also for IgE and IgG to tetanus and diphtheria toxoids (20, 21), may indicate that a transient IgE response after vaccine injection could be a normal consequence of an injection of proteins. The similar prevalence of allergic symptoms at 2.5 years in a study comprising 9600 children who were immunized with a pertussis-containing vaccine or only diphtheria/tetanus indicates that the appearance of PT-IgE in itself was not necessarily associated with an increased risk of allergic manifestations (26). This is indicated even more clearly by our recent prospective allergy study of 669 children, in which none of the pertussis-containing vaccines were associated with a significantly increased incidence of either atopic disease or positive SPT (10).

The higher prevalence of IgE responses in children immunized with the acellular vaccines

than with the Wc pertussis vaccine could be due to a higher amount of PT injected in the former (27) or to the adjuvant effects of other components in Wc pertussis vaccine such as pertussis lipopolysaccharide, which can induce a Th1-like response (28). In fact, mononuclear cells from children immunized with Wc pertussis vaccine and from children who have had pertussis infection secrete interferon-gamma (IFN- γ), but not interleukin (IL)-5, when stimulated with pertussis antigens *in vitro* (29, 30). In contrast, mononuclear cells from children immunized with acellular pertussis vaccines secrete both IFN- γ and IL-5, indicating a mixed immune response (29).

Circulating PT-IgE at 2.5 years of age was detected in one-third of the children with verified pertussis, indicating a Th2-like response. Furthermore, we recently reported that asthma appeared twice as often in the children who had pertussis as in the children with no such infection (10). Only 2/9 children with a diagnosis of asthma by 2.5 years of age had positive PT-IgE, however, and none had a positive SPT, indicating that most of these subjects had only transient bronchial irritability after whooping cough (31).

The two- and five-component acellular pertussis vaccines gave better protection (11) and fewer local and general side-effects (10), but also a higher frequency of PT-IgE than the Wc pertussis vaccine. However, this was not associated with an increased risk of atopic manifestations; i.e., positive SPT, atopic symptoms, or diagnosed atopic diseases (10). Thus, the temporary PT-IgE responses to pertussis vaccination, particularly after acellular vaccines and in atopic subjects, appear to be a common consequence of the vaccination, and not an indication of an increased risk of atopic disease.

Acknowledgments

We are grateful for valuable advice from Rafael Ferrándiz on IgE-coupling procedures; technical assistance by Kristina Wahrstedt, Kerstin Hagersten, and Carina Bengtsson; scientific discussions with Ulrich Wahn; and statistical advice from Anders Magnusson. Financial support from the Queen Silvia's Jubilee Fund for research on children and handicaps, the Medical Research Fund of the County of Östergötland, Deutscher Akademischer Austauschdienst, the Vårdal foundation, the Swedish Asthma and Allergy Association, the Swedish Medical Research Council (no. 7510), and the National Institute of Public Health is gratefully acknowledged.

References

- Munoz JJ, Arai H, Bergman RK, Sadowski PL. Biological activities of crystalline pertussigen from *Bordetella pertussis*. *Infect Immun* 1981;**33**:820-6.
- Ishizaka K, Iwata M, Akasaki M, Ishizaka T, Munoz JJ. Biochemical basis of the adjuvant effects of pertussis toxin for the IgE response. In: Sekura RD, Moss J, Vaughan N, editors. *Pertussis toxin*. Orlando, FL: Academic Press, 1985:185-204.
- Lindsay DSJ, Parton R, Wardlaw AC. Adjuvant effect of pertussis toxin on the production of anti-ovalbumin IgE in mice and lack of direct correlation between PCA and ELISA. *Int Arch Allergy Immunol* 1994;**105**:281-8.
- Wjst M, Dold S, Reitmeir P, Fritzsche C, von Mutius E. Pertussis infection and allergic sensitization. *Ann Allergy* 1994;**73**:450-4.
- Schuster A, Hofmann A, Reinhardt D. Does pertussis infection induce manifestation of allergy? *Clin Investig* 1993;**71**:208-13.
- Blennow M, Granström M, Björkstén B. Immunoglobulin E response to pertussis toxin after vaccination with acellular pertussis vaccine. In: Manclark CR, editor. *Proceedings of the Sixth International Symposium on Pertussis*. Bethesda, Maryland: Department of Health and Human Services, United States Public Health Service, DHHS Publication (FDA), 1993:184-8.
- Hedenskog S, Björkstén B, Blennow M, Granström G, Granström M. Immunoglobulin E response to pertussis toxin in whooping cough and after immunization with a whole cell and an acellular pertussis vaccine. *Int Arch Allergy Immunol* 1989;**89**:156-61.
- Odelram H, Granström M, Hedenskog S, Duchén K, Björkstén B. Immunoglobulin E and G responses to pertussis toxin after booster immunization in relation to atopy, local reactions and aluminium content of the vaccines. *Pediatr Allergy Immunol* 1994;**5**:118-23.
- Odent M, Culpin E, Kimel T. Pertussis vaccination and asthma: is there a link? [Letter]. *JAMA* 1994;**272**:591-2.
- Nilsson L, Kjellman N-IM, Björkstén B. A randomized controlled trial of the effect of pertussis vaccines on atopic disease. *Arch Pediatr Adolesc Med* 1998;**152**:734-8.
- Gustafsson L, Hallander HO, Olin P, Reizenstein E, Storsaeter J. A controlled trial of a two-component acellular, a five-component acellular, and whole-cell pertussis vaccine. *N Engl J Med* 1996;**334**:349-55.
- Nilsson L, Kjellman N-IM. Atopy and season of birth. *Allergy* 1996;**51**:138-9.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1980;**92**:44-7.
- WHO meeting on case definition of pertussis: Geneva, 10-11 January 1991. Geneva: World Health Organization (Issue no. MIN/EPI/PERT/91.1): 1991, vols. 4-5).
- Dreborg S, Frew A. Allergen standardization and skin tests. *EAACI Position paper. Allergy* 1993;**48** Suppl 14:48-82.
- Kirkwood B. *Essentials of medical statistics*. Oxford: Blackwell Scientific, 1988:148.
- Haus M, Weinberg EG, Malherbe D. Specific IgE antibodies to *Bordetella pertussis* after immunisation in infancy. *Lancet* 1988;**1**:711.
- Warner JO. Specific IgE antibodies to *Bordetella pertussis* after immunisation in infancy. *Lancet* 1988;**1**:1058.
- Duchén K, Granström M, Hedenskog S, Blennow M, Björkstén B. Immunoglobulin E and G responses to pertussis toxin in children immunised with adsorbed and non-adsorbed whole cell pertussis vaccines. *Vaccine* 1997;**15**:1558-61.
- Mark A, Björkstén B, Granström M. Immunoglobulin E and G antibodies two years after a booster dose of an aluminium-adsorbed or a fluid DT vaccine in relation to atopy. *Pediatr Allergy Immunol* 1997;**8**:83-7.

21. Dannemann A, van Ree R, Kulig M, et al. Specific IgE and IgG4 immune responses to tetanus and diphtheria toxoid in atopic and non-atopic children during the first two years of life. *Int Arch Allergy Immunol* 1996;**111**:262-7.
22. Hattevig G, Kjellman B, Björkstén B. Appearance of IgE antibodies to ingested and inhaled allergens during the first 12 years of life in atopic and non-atopic children. *Pediatr Allergy Immunol* 1993;**4**:182-6.
23. Sigurs N, Hattevig G, Kjellman B, Kjellman N-IM, Nilsson L, Björkstén B. Appearance of atopic disease in relation to serum IgE antibodies in children followed up from birth for 4 to 15 years. *J Allergy Clin Immunol* 1994;**94**:757-63.
24. Burr ML, Merrett TG, Dunstan FD, Maguire MJ. The development of allergy in high-risk children. *Clin Exp Allergy* 1997;**27**:1247-53.
25. Settupane GA, Newstead GJ, Boyd GK. Frequency of Hymenoptera allergy in an atopic and normal population. *J Allergy Clin Immunol* 1972;**50**:146-50.
26. Nilsson L, Kjellman N-IM. Lack of association between pertussis vaccination and symptoms of asthma and allergy. [Letter]. *JAMA* 1996;**275**:760.
27. Edwards KM, Meade BD, Decker MD, et al. Comparison of 13 acellular pertussis vaccines: overview and serologic response. *Pediatrics* 1995;**96**:548-57.
28. Daugelat S, Kaufmann SHE. Role of Th1 and Th2 cells in bacterial infections. In: Romagnani S, editor. Th1 and Th2 cells in health and disease: Chem Immunol 1996;**63**:66-97.
29. Ryan M, Murphy G, Nilsson L, et al. Distinct Th cell subtypes induced with whole cell and acellular pertussis vaccines in children. *Immunology* 1998;**93**:1-10.
30. Ryan M, Storsaeter J, Nilsson L, Mills KHG. *Bordetella pertussis* respiratory infection in children is associated with preferential activation of type 1 Th cells. *J Infect Dis* 1997;**175**:1246-50.
31. Ellis EF. Acute bronchopulmonary infections. In: Bierman CW, Pearlman DS, editors. Allergic diseases from infancy to adulthood. Philadelphia: Saunders, 1988:537.

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