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Specific IgE and IgG4 Immune Responses to Tetanus and Diphtheria Toxoid in Atopic and Nonatopic Children during the First Two Years of Life

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Abstract

Background: In order to investigate, whether atopic and nonatopic children show differences in their specific IgE and IgG4 immune responses to tetanus (T) and diphtheria (D) antigens, we studied 538 children who had been followed from birth on and from whom records had been kept of all immunizations.

Methods: The prevalence of eczema and asthma was registered at regular intervals and the cumulative incidence of symptoms was determined at 24 months of age. Total serum IgE and specific IgE to a panel of nine allergens as well as T- and D-specific IgE and IgG4 were determined from the 24-month blood samples.

Results: Our results show that both atopic and nonatopic children are capable of mounting high levels of toxoid-specific IgE antibody responses. Children with cord blood IgE > 0.9 kU/l, serum IgE 10–100 kU/l and > 100 kU/l and at least one sensitization to an allergen at 24 months of age have significantly higher IgE responses to T and D ($p < 0.001$). In contrast, specific IgG4 antibody concentrations to T and D were not significantly different in children with elevated total IgE levels at 24 months. No differences in subgroups of children with or without early symptoms of atopy were observed. **Conclusions:** Our data indicate that IgE responses to toxoids such as T and D are not limited to infants with clinical manifestations of atopy in the first 2 years of life but are related to immunological parameters of atopy.

Key Words

IgE
Immune responses
Atopy
Tetanus immunization
Diphtheria immunization

Introduction

Hypersensitivity reactions after immunization with either diphtheria (D) or tetanus (T) toxoids are occasionally observed in atopic and nonatopic individuals. In the literature, a low incidence of serious side effects after T immunization is reported [1, 2]. In young infants, preschool and school children reported side effects are mostly minor local

reactions, while systemic reactions are quite rare [3, 4, 5]. The risk of anaphylactic reactions to tetanus toxoid is extremely rare, although case reports have been published [6]. In 1952, Kuhns and Pappenheimer [7] found a significantly higher rate of immediate wheal and flare reactions to D toxoid in allergic compared to nonallergic individuals, while Facktor et al. could not confirm these findings for T toxoid [8]. Whether observed adverse reactions to T or D immuni-

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zations with vaccine combinations with D and T (DT) or D, pertussis (P) and T (DPT) are due to an unspecific irritation by the toxoids itself or by the aluminum component, has been discussed controversially [3, 4, 9].

Elevated serum IgE antibody levels to T are observed after booster immunization with aluminum-adsorbed T vaccine [10] and also in relation with DPT vaccine in 1- to 2-year-old children after the first immunization [5]. In cross-sectional studies on atopic adults as well as 4 to 17-year-old atopic children T and D IgE antibody levels were found to be higher compared to nonatopic individuals [11, 12]. Additionally, it was found, that older children with markedly elevated total serum IgE levels synthesized increased anti-D and anti-T IgE antibodies [12].

The objective of this study was to investigate the determinants of the IgE and IgG4 antibody responses to T and D toxoid in a large cohort of 2-year-old children, after receiving their primary immunization course and to study the very early IgE response to toxoid vaccination as a model in these very young children to pinpoint similarities and differences between atopic and nonatopic individuals.

Material and Methods

Study Population

538 children out of a cohort of $n = 1,314$ from an ongoing prospective non-interventional study on the natural course of atopy in early childhood (MAS-90) were included in our investigation, patient characteristics are presented in table 1. Details on this cohort were presented elsewhere [13].

Briefly, the cohort was followed regularly at 1, 3, 6, 12, 18 and 24 month of age. Parents received a structured interview about their infants' diseases, atopic symptoms, immunizations and other topics. The infants underwent a standardized physical examination by a pediatric allergist. According to history and physical findings, an obvious or probable atopic eczema and wheezing was diagnosed using the following criteria: 'obvious wheezing' required at least two wheezing episodes with shortness of breath, 'obvious eczema', a combination of at least three typical morphological features at three typical sites.

Cohort children were included in the study if they had received at least two immunizations against D and T, before their second birthday if the records of immunization including the date of each injection were complete and if the serum volume was sufficient. Since IgG4 analysis was performed at a 1:40 dilution, more material was available for this assay than for the IgE analysis working with undiluted serum. If serum availability was limiting, tetanus assays were given priority. The number of sera in each test and the status of vaccination of the children in each panel are given in table 2.

Total and Allergen-Specific Serum IgE

Blood samples were taken at birth (cord blood-IgE) and at 12 and 24 months for determining total IgE and specific IgE antibodies to a panel of common food and inhalant allergens (milk, egg, soy, wheat, grass, birch, *Dermatophagoides pteronyssinus*, cats, dogs). All IgE

Table 1. Patient characteristics

Male, %	53.3
Elevated cord blood IgE (≥ 0.9 kU/l), %	17.3
Total serum IgE (at 2 years of age)	
< 10 kU/l, %	35.7
10–100 kU/l, %	52.1
> 100 kU/l, %	12.3
Specific sensitization ($\geq$ CAP class 1), %	26.0
Obvious atopic eczema, %	19.4
Recurrent wheezing (≥ 2 episodes), %	8.7

Table 2. Number of immunizations against D and T per antibody assay group

Number of immunizations	D IgE	D IgG4	T IgE	T IgG4
2	76	111	128	135
3	172	237	270	280
4	55	72	85	90
5	3	5	7	6
total	306	425	490	511
0 ¹	1	2	2	2
1 ¹	19	20	24	23

¹ Not included in the analysis.

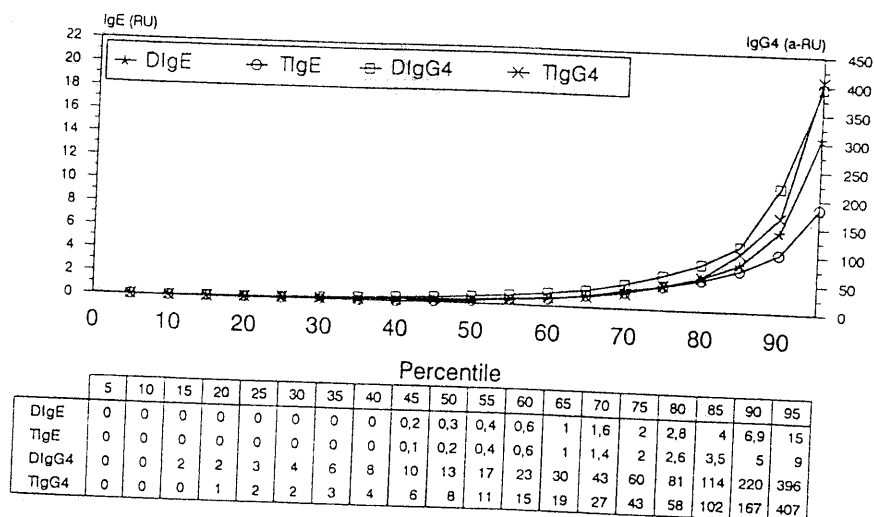
determinations were done by the CAP-fluorescence enzyme immunoassay (FEIA, Pharmacia). Children were considered sensitized, when the serum IgE concentration against at least 1 of the 9 allergens was higher than 0.35 kU/l ($\geq$ CAP class 1). For statistical analysis children were divided into three groups according to total serum IgE levels < 10, 10–100, > 100 kU/l at 24 months of age.

D and T IgE and IgG4 Antibody Assays

D and T toxoid were coupled to CNBr-activated Sepharose (Pharmacia Fine Chemicals; 1 mg protein/g Sepharose; 1 mg corresponds to 1,500 Lf T toxoid and to 1,800 Lf D toxoid). The Sepharose was suspended at 2 mg/ml in PBS containing 10 mM EDTA, 0.2% Tween 20 and 0.05 mg/ml NaN_3 (IgE), PBS with 0.5% sheep serum (v/v), 4.5% bovine serum (v/v), 0.2% Tween 20 (v/v), 0.3% BSA (w/v) and 10 mM EDTA (IgG4). The RAST for IgE and IgG4 was performed by incubation of 50 μ l of serum (IgE undiluted; IgG4 1:40 dilution) with 250 μ l of Sepharose. After incubation overnight washing with PBS-Tween 0.1% (5 times). The second incubation was performed with 500 μ l of medium (0.5% sheep-serum, v/v; 4.5% bovine serum (v/v); 0.2% Tween 20 (v/v); 0.3% BSA (w/v); 10 mM EDTA in PBS (pH 7.4)) plus 50 μ l of sheep-polyclonal anti-IgE or with 500 μ l of medium (0.3% BSA; 0.2% Tween 20; 10 mM EDTA in PBS, pH 7.4) plus 50 μ l of monoclonal anti-IgG4 (both radiolabelled with ^{125}I). After 12 h final washing with PBS-Tween 0.1% (4 times); the percentage of bound ^{125}I was measured in a gamma counter. The results were read from a standard calibration curve for IgE in RAST units (RU) (27, 9, 3, 1, 0.3 RU;

Fig. 1. Distribution of the D and T IgE and IgG4 antibody responses. IgE units (RU; left side of the figure) and IgG4 units (RU; right side of the figure) are shown on the Y-scale. The corresponding percentiles are shown on the X-scale. The percentiles are shown in 10% steps in the figure and in 5% steps in the table under the figure. Each X-scale percentile value has four corresponding Y-scale values (1. D-IgE; 2. T-IgE; 3. D-IgG4; 4. T-IgG4).

For example: 75. percentile: 1. Diph-IgE: 2; 2. Tet-IgE: 2; 3. Diph-IgG4: 60; 4. Tet-IgG4: 43.



1 RU $\equiv$ 1.54 IU/ml). For IgG4 arbitrary RUs (a-RU) were calculated by an IgG standard curve dilution, which was a mixture of samples from 2 grass-pollen-treated patients at dilutions of 1:45, 1:90, 1:180, 1:360, 1:720, 1:1,440.

Statistical Analysis

The statistical analysis was performed using SPSS/PC. Since the data of IgE and IgG4 antibody measurements are positively skewed, they were log-transformed. Correlations among the different types of antibodies were studied by computing partial correlations adjusting for the time period (in days) after the last immunization. D and T antibody levels in different subgroups were compared by the Mann-Whitney U test using the Bonferroni correction for multiple comparisons. To determine whether subgroup differences in the IgE responses to T and D antigen are related to the time period between the last immunization and IgE antibody measurement, these individually variable periods were grouped into one four equal time intervals.

Results

No major systemic or large local clinical reactions were reported after immunization with D and T toxoids.

T-specific serum IgE antibodies were detected in about 50% of the samples, D-specific IgE antibodies were detectable in 55% of the samples, while T-specific IgG4 antibodies could be found in about 80% and D-specific IgG4 antibodies in 85% of the samples, as shown in figure 1.

The time intervals between the last D and T immunization and the D- and T-specific IgE antibody analysis were variable ranging from 1 week to 21 months. The D and T antibody response was investigated with regard to the time interval since the last D and T immunization. The intervals

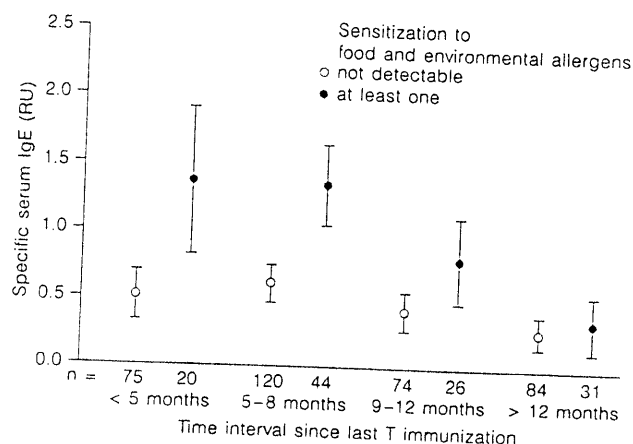


Fig. 2. T-specific serum IgE antibody concentrations in 2-year-old children with and without atopic sensitization in relation to the time interval since the last immunization. For each group, mean IgE values and 95% confidence intervals are presented.

of <5 months, 5–8 months 9–12 months and >12 months show differences between the group with and without atopic sensitization. With a tendency for higher mean D and T IgE antibody responses in the group of children with atopic sensitization, children with shorter intervals (≤ 4 months, 5–8 months) to the last D and T immunization and atopic sensitization tended to have higher mean D and T antibody levels than children with longer time intervals (9–12 months and >12 months). The results are presented in figure 2.

There was no correlation between D and T IgE serum levels and atopic family history, gender, social status or number of immunizations.

T IgE and IgG4 antibody levels were positively correlated (adjusted for the time period after the last immunization). Positive correlations were also found between D IgE and IgG4 antibody levels, T IgE and D IgE antibody levels and T IgG4 and D IgG4 antibody levels. D IgE and T IgE antibody levels were also correlated with the total IgE level. Children who did not receive any T or D immunization in the first 2 years of life did not show a detectable specific antibody response to D or T toxoid. Results are shown in table 3.

Associations between immunological parameters of atopy and the T and D IgE and IgG4 antibody response are shown in table 4. Children with elevated cord blood IgE (>0.9 kU/l) and higher total IgE (10–100 and >100 kU/l) at 24 month as well as specific sensitization at 24 months showed significantly higher D and T IgE antibody levels than children with lower cord blood IgE or total IgE values. Children with higher total IgE levels (10–100 kU/l) in the D IgG4 assay group and higher total IgE levels (10–100 and >100 kU/l) in the T IgG4 assay group tended to have higher D and T IgG4 antibody responses.

In contrast to the different T and D IgE responses depending on the different levels of immunological parameters of atopy, the median T or D IgE antibody levels did not differ significantly between children with and without atopic manifestations (table 4).

Discussion

Our results clearly show that 24-month-old children with and without early manifestation of atopic symptoms are capable of producing IgE antibodies in response to T and D immunizations. About 50% of the infants in this study developed detectable specific T and D antibodies: comparable findings were reported previously [14]. Children with immunological indicators of atopy such as specific sensitizations already at the age of 2 show significantly higher toxoid-specific IgE responses.

Other studies investigated the specific IgE response before and after booster immunizations and the data of a longitudinal follow-up with a temporary course in mostly older individuals with wide age ranges [10, 12, 15]. In contrast to other study designs, we present comparable data of children of the same age and complete records of vaccinations and a number of different immunological data from birth until 24 months of age.

Table 3. Partial correlation coefficients of D and T IgE with D and T IgG4 after controlling for time period after last immunization

Antibodies	Partial correlation of D and T IgE and IgG4				
	n	ND	CC	95% CI	p value
D IgE with D IgG4	263	19	0.51	0.42–0.59	0.0001
T IgE with T IgG4	486	60	0.43	0.36–0.50	0.0001
D IgE with T IgE	300	94	0.85	0.82–0.88	0.0001
D IgG4 with T IgG4	421	21	0.73	0.67–0.77	0.0001
D IgE with total IgE	302	7	0.65	0.58–0.71	0.0001
T IgE with total IgE	484	11	0.64	0.59–0.69	0.0001

ND = Non detectable; CC = correlation coefficient; 95% CI = 95% confidence interval.

The analysis of toxoid-specific serum IgE antibody levels in relation of different time intervals between the last immunization and the serological analysis indicates that IgE antibodies tend to decrease after a period of 8 months after the last immunization. However, since IgE antibodies may still be found after an interval of more than 12 months, this IgE response is not short-lived. We cannot draw definite conclusions about the duration of the specific IgE antibody responses from our study since only cross-sectional data obtained at the age of 2 are available.

As is known from other studies, the development and expression of IgG4 are age dependent and vary widely among children of different age groups [16–18]. This might be an explanation for our results indicating that 15% of the children of our study population have undetectable IgG4 antibodies to both toxoids.

Toxoid-specific IgE and IgG4 antibody responses are found to be associated. Correlations between specific IgE and IgG4 responses are in agreement with the concept that these immunoglobulins are regulated in a similar way via IL-4 and TH2 cells although the mechanism of this regulation is not completely understood yet [14, 19, 20].

It has been speculated that immunizing agents and their adjuvants may influence the natural course of atopic disease and may induce the development of specific IgE antibodies to atopic allergens. Especially, the role of pertussis infections as well as pertussis immunizations as a stimulating factor for IgE antibody responses in both atopic and non-atopic individuals has been addressed in several studies [8, 15, 17]. Correlations between total serum IgE and pertussis IgE antibodies have been described, particularly in atopic children [15]. An IgE-antibody-inducing effect of DPT vaccine was observed by Matuhasi et al. [5], who described elevated levels of T-specific IgE antibodies in individuals

Table 4. Differences in D and T IgE and IgG4 antibody responses according to different total serum IgE subgroups, sensitization status, atopic eczema and wheezing at 2 years of age

Subgroup	D IgE					T IgE				
	n	mean	SD	median	p value ¹	n	mean	SD	median	p value ¹
Cord blood IgE										
<0.9 kU/l	250	2.30	7.19	0.22	0.007	389	1.72	5.72	0.07	0.0001
≥0.9 kU/l	45	4.71	6.71	1.89		80	4.03	6.63	1.91	
Total serum IgE ¹										
<10 kU/l	107	0.13	0.36	0.00	0.0001	174	0.15	0.43	0.00	0.0001
10–100 kU/l	159	2.00	3.11	0.83		249	1.64	2.66	0.77	
>100 kU/l	36	12.32	16.24	6.87	0.0001	61	8.52	13.06	4.33	0.0001
Specific sensitization										
None	219	1.81	5.27	0.11	0.0001	353	1.30	3.27	0.03	0.0001
≥1 positive CAP	77	4.86	10.44	1.32		121	3.99	9.48	1.11	
Atopic eczema										
No symptoms	121	2.04	4.30	0.33	0.6	176	1.74	4.23	0.29	0.2
Probable	126	2.70	6.70	0.21		219	1.78	4.04	0.07	
Obvious	58	3.66	11.24	0.28	0.6	94	3.33	10.24	0.18	0.3
Wheezing										
None	274	2.47	6.94	0.25	0.39	426	2.07	5.98	0.18	0.57
Probable	10	5.55	8.81	0.59		19	2.50	4.34	0.79	
≥2 episodes	20	3.36	7.83	0.53	0.6	40	2.01	5.27	0.00	0.39
Subgroup	D IgG4					T IgG4				
	n	mean	SD	median	p value ¹	n	mean	SD	median	p value ¹
Total serum IgE ²										
<10 kU/l	154	42.1	184.6	6.91	0.0001	179	98.4	637.5	3.43	0.0001
10–100 kU/l	210	179.6	769.0	22.31		262	198.8	884.1	10.71	
>100 kU/l	55	271.4	907.4	22.73	0.38	64	259.4	1048.6	17.22	0.08

¹ Mann-Whitney U test for the respective groups using Bonferroni correction for multiple comparisons.

² At 2 years of age.

vaccinated with DPT vaccine [10]. Haus and Weinberg [17] suggest that atopic symptoms in a group of 1-year-old children were caused by DPT immunization during the first year of life. Follow-up studies will be needed in order to determine whether or not immunizations with toxoids and the degree of the specific IgE responses to them are playing any role in enhancing or suppressing IgE-mediated immune responses to atopic allergens or modulating the clinical course of atopic diseases, as suggested by some investigators [21, 22].

Compared to other studies which observed hypersensitivity reactions or local side effects after immunization with D, T or pertussis toxoids, no severe side effects were reported in our study. This confirms the observation that the incidence of local as well as serious systemic adverse reactions has remained low [1, 2].

In summary, our data indicate that immunizations with D and T toxoids result in the development of specific IgE antibodies in about 50% of children. This antibody response is not limited to the subgroup of children with early clinical manifestations of atopic disease.

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