
Influence of dose and frequency of antigen injection on IgE development in young children: a comparison of fire ant stings and tetanus immunizations

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Background: Previous studies suggest that small antigen doses given frequently are more likely to induce IgE production than are large antigen doses given infrequently.

Objective: To compare the prevalence of antitetanus IgE resulting from the relatively large dose of tetanus toxoid delivered by standard immunizations at 2, 4, 6, and 18 months of age with the previously reported prevalence of anti–fire ant venom IgE resulting from the relatively small dose of venom delivered sporadically by accidental fire ant stings in children younger than 5 years.

Methods: This study uses previously published data on the prevalence of IgE antibodies to imported fire ant venom among children living in an imported fire ant endemic area of Georgia and antitetanus IgE measurements of children recruited between August 1, 2003, and December 30, 2007, as part of the Wayne County Health, Environment, Allergy, and Asthma Longitudinal Study in Michigan, where there are no imported fire ants. The prevalence of anti–fire ant venom IgE antibodies was compared with the prevalence of antitetanus IgE antibodies in these 2 cohorts of children.

Results: The reported prevalence of IgE to fire ant venom among 42 children 2 to 5 years old was 57.1% using a cutoff of 0.1 IU/mL and 35.7% using a cutoff of 0.35 IU/mL. The prevalence of antitetanus IgE in 395 children 2 years old was 52.9% using a cutoff of 0.1 IU/mL and 42.7% using a cutoff of 0.35 IU/mL. The proportion of children with detectable anti–fire ant venom IgE was not statistically significantly different from the proportion of those with antitetanus IgE at either cutoff level ($P = .74$ and $.50$ at 0.1 and 0.35 IU/mL, respectively).

Conclusions: The relatively large dose of tetanus toxoid delivered 4 times during the first 24 months of life produces detectable tetanus specific IgE antibodies as frequently as the smaller doses of venom delivered sporadically by fire ant stings in young children.

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INTRODUCTION

We have previously reported that more than 50% of pre-school children residing in a fire ant endemic area of the United States have IgE antibodies to fire ant venom.¹ This high prevalence of sensitization to imported fire ant venom (IFAV) was surprising. However, children and adults living in areas where fire ants are endemic are stung frequently. One study reported a sting rate of 51% among 137 medical students during 3 weeks of training in Texas.² This rate is similar to a report of stings in 39% of 176 children during the previous month.¹ This high exposure rate could result in IgE

antibody formation in several ways. IFAV could have unique properties that strongly promote IgE production, such as substances with strong adjuvant properties. IgE production to IFAV could also be promoted by intracutaneous delivery of small doses of venom by accidental stings at the relatively frequent intervals previously reported.

Several studies have suggested that IgE production is more likely to occur if an antigen is encountered frequently at low doses rather than infrequently at high doses.^{3,4} This is an important question because there is a growing debate about the value of trying to reduce allergen exposure in early life with the intent of preventing sensitization vs increasing allergen exposure with the intent of inducing tolerance.⁵ The concept of shifting a T_H2 response from IgE to IgG4 with high-dose allergen exposure as a means of reducing allergic disease has also been introduced.⁶

Vaccines are normally thought of as a method of inducing IgG antibodies for protection against infectious diseases by administering sufficiently large amounts of immunogen as infrequently as possible. Vaccines contain well-controlled

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doses of antigens to be delivered by injection on prescribed schedules. Thus, vaccines are examples of relatively high doses of antigen encountered infrequently in early childhood.⁷ Most children receive diphtheria and tetanus toxoids and acellular pertussis (DTaP) vaccines at intervals close to the currently recommended schedule for primary immunization in the United States calling for DTaP injections at 2, 4, 6, and 15 to 18 months of age. The question of how frequently vaccines induce the production of vaccine antigen specific IgE antibodies has received little attention.⁸⁻¹² However, previous investigations have shown that tetanus and pertussis vaccines induce IgE specific antibodies in more than 90% of 4- to 10-year-old children receiving booster immunizations.^{11,13}

Our primary hypothesis was that sporadic exposure to the relatively small amounts of IFAV through natural accidental stings in young children would produce a significantly higher prevalence of detectable IFAV IgE than would be found in similar age children undergoing routine immunizations with tetanus toxoid. Each imported fire ant (*Solenopsis invicta*) sting transfers 10 to 100 ng of protein, and presumably these stings become more common after children begin to crawl and walk between 6 and 24 months of age.^{14,15} In contrast each dose of tetanus toxoid for primary immunization of children contains at least 5 to 10 Lf units (15 to 30 μ g of protein).^{16,17}

As part of a birth cohort study of factors related to the risk of allergic disease, antitetanus IgE was measured in children at 6, 12, and 24 months of age. Our primary analysis compared the previously reported prevalence of IFAV IgE to that of antitetanus IgE determined in our birth cohort. We also analyzed the prevalence of antitetanus IgE at 6, 12, and 24 months of age with stratification by sex or race.

METHODS

This study uses previously published data on the prevalence of IgE antibodies to IFAV¹ among children living in an imported fire ant endemic area of Georgia and data collected as part of the Wayne County Health, Environment, Allergy, and Asthma Longitudinal Study (WHEALS) in Michigan, where there are no imported fire ants. The institutional research board of the Medical College of Georgia approved the study of IgE to fire ant venom. WHEALS was approved by the institutional research boards of the Henry Ford Hospital and the Medical College of Georgia as appropriate.

Serum samples for measurement of anti-IFAV IgE were obtained as residual serum from samples submitted to an immunology laboratory for clinical assay in a fire ant endemic area of Georgia. No information was available concerning the children from whom these samples were taken beyond their age and sex.¹ For the WHEALS cohort, pregnant women were recruited after giving informed consent if they resided in an area of western Detroit and adjacent suburbs defined by contiguous zip codes. Recruitment began on August 1, 2003, and was completed on December 30, 2007. To be eligible, pregnant women in their second or third trimester

had to be at least 21 years of age, live in the defined recruitment area, and attend 1 of 5 selected Henry Ford Hospital clinics for their prenatal care. Women were required to speak English well enough to provide written informed consent. Mothers were asked to identify their race and the race of their child using the categories of the 2000 US Census surveys. All children were expected to receive routine health care, including all immunizations, as recommended by the American Academy of Pediatrics. The recommended schedule includes DTaP immunizations at 2, 4, and 6 months of age with a booster immunization at 15 to 18 months of age. Most children in our cohort received Pediarix vaccine within the Henry Ford Hospital system. We did not obtain the brand names of vaccines given to children from other health care sources, but all brands are considered interchangeable for purposes of immunizing children in the United States.¹⁷ Blood samples were obtained during home visits when the children were approximately 6 and 12 months of age and during a clinical examination at approximately 24 months of age.

Both anti-IFAV and antitetanus IgE antibodies were assayed using the Pharmacia UniCAP assay (Phadia AB, Portage, Michigan). As previously reported, the solid phase for anti-IFAV IgE was prepared from IFAV, which differs from the standard UniCAP reagent, which uses a whole-body extract. For antitetanus IgE, the Pharmacia CAP Rc208 was used, which is a research-only reagent. The ranges of the assays are from 0.1 to 100 kU/L of IgE. Samples with values of 0.1 kU/L or higher were considered positive, although the results for IgE levels of 0.35 kU/L or higher are given for comparison with other studies. Repeat assay of samples consistently demonstrated coefficients of variation among assays of less than 6%.

To test the linearity and analytic sensitivity of a value of 0.1 IU/mL in the antitetanus IgE assay, we selected 4 plasma samples with sufficient volumes and values of 0.56 to 1.20 IU/mL and measured antitetanus IgE after performing 5 serial 2-fold dilutions of each sample. The calculated IgE concentration of each dilution was compared with the actual measured concentration. The measured concentrations of the dilutions decreased as expected to less than 0.1 IU/mL, and the Pearson correlation between calculated and measured values was $r = .992$ ($P < .001$).

Data were described using routine descriptive statistical techniques. The IgE values were log transformed to reduce the influence of outliers on the estimated mean; the means reported are geometric means. Prevalences were compared using χ^2 tests. $P < .05$ was considered statistically significant. All statistical analysis was performed using SAS statistical software, version 9.1 (SAS Institute Inc, Cary, North Carolina).

RESULTS

Our previous report of IgE to IFAV involved a sample of 183 children of whom 42 were 2 to 5 years of age.¹ Of these 42, 22 (52%) were female and 20 (48%) were male. Information on race was not obtained for these children. Information on

antitetanus IgE was obtained for 395 children from the WHEALS cohort. Four hundred four infants had completed a 6-month follow-up in our birth cohort and were potentially eligible; however, after excluding 1 child in each set of twins and those ineligible for other reasons, such as area of residence, 395 children remained. Of these children, 193 (48.9%) were female, 225 (57.0%) were black, 116 (29.4%) were white, and 54 (13.7%) were from other racial groups. Results from fewer children were available at 12 and 24 months of age because they had not yet reached these ages or because they had not yet been evaluated.

As indicated in Table 1, the 52.9% prevalence of detectable antitetanus IgE at 24 months of age using 0.1 kU/L or higher is not statistically significantly different from the 57.1% prevalence of anti-IFAV IgE in children 2 to 5 years of age. There were 14 children who were 24 months old among those studied for anti-IFAV IgE, and 6 (42.9%) of these had detectable anti-IFAV IgE.

Detectable antitetanus IgE increased with age from 15.2% at 6 months to 52.9% at 24 months as indicated in Table 2. If an antitetanus IgE antibody level of 0.35 kU/L or higher is used as the cutoff, the prevalence increases from 6.6% to 42.7% between 6 and 24 months of age, respectively. The prevalence reported at each age reflects all children with valid values at that age regardless of whether they had samples from other ages, resulting in variation in the numbers of children. At 6 months, boys were significantly more likely to have detectable antitetanus IgE than girls, and this difference was nearly significant at 12 months but had disappeared by 24 months of age. The differences in the prevalence of antitetanus IgE did not differ by reported race at any of the 3 ages.

Some children developed relatively high levels of antitetanus IgE as indicated in Table 3. By 24 months of age nearly 23% of children had serum antitetanus IgE concentrations of 1 kU/L or higher, and almost 5% had concentrations of 5 kU/L or higher (data not shown). In the fire ant group from 12 to 36 months of age, 32.2% had IgE concentrations of 1 kU/L or higher.

We next examined the prevalence of antitetanus IgE at 6 months of age in relationship to the number of DTaP immunizations reported for each child. As indicated in Table 4, none of the 31 children who had only received 1 immuniza-

tion had detectable antitetanus IgE, but no significant difference was found in the prevalence of antitetanus IgE levels among children who had received 2 or 3 immunizations ($P = .24$), although the geometric mean antitetanus IgE level was significantly higher in those with 2 compared with those with 3 immunizations ($P < .001$).

DISCUSSION

Our findings do not support the hypothesis that the prevalence of anti-IFAV IgE antibodies would be significantly higher than the prevalence of antitetanus IgE antibodies in young children because of differences in the antigen doses and patterns of exposure.^{3,4} However, the modest number of children studied precludes a definitive conclusion of no difference in prevalence. The amount of tetanus toxoid protein delivered per injection (15,000 to 30,000 ng) is approximately 150 to 3,000 times larger than the total amount of protein delivered by a fire ant sting (10 to 100 ng). The difference in dose between tetanus and IFAV would be reduced if large numbers of fire ant stings were experienced at one time. It is not known whether the nonprotein components of IFAV act as adjuvants for IgE production, but tetanus toxoid is adsorbed onto alum to enhance immunogenicity.¹⁷ Although exposure to both antigens is by injection, tetanus toxoid is delivered intramuscularly, whereas fire ant stings only penetrate into the epidermis.

Allergic reactions to fire ant stings are well known by allergists practicing in endemic areas.¹⁴ However, available information suggests that the risk of allergic reactions to tetanus toxoid immunization is approximately 1:1,000,000.^{18,19}

Few studies have directly examined the relationship between antigen dose and probability of IgE production in humans with neoantigens.⁴ Riedl et al³ used the neoantigen, keyhole limpet hemocyanin, at doses of 0.1, 10, 1,000, and 100,000 μg , with diesel exhaust particles as adjuvant delivered intranasally to study IgE sensitization in 51 atopic adults. The 10- μg dose produced sensitization in 100% of the study participants, whereas the 100,000- μg dose produced sensitization in only 11%, supporting the hypothesis that high doses of an antigen are less likely to produce IgE than are lower doses.

Our findings concerning the production of antitetanus IgE in young children confirm and extend those of others. Dannemann et al⁸ found that approximately 50% of 538 children had developed detectable antitetanus IgE at 24 months of age, and Gruber et al¹⁰ reported a prevalence of 53.6% compared with our finding of 52.9%. These numbers are remarkably consistent considering that the other studies used a different technology, the radioallergosorbent test, to detect antitetanus IgE. The populations they studied were drawn from several European countries, and all participants were likely of European ancestry. Levels of antitetanus IgE are thought to decrease in most, if not all children, until their next DTaP booster as was observed in a study of children boosted at 10 years of age and followed up for 2 years.²⁰

Table 1. Comparison of the Prevalence of Tetanus Toxoid Specific IgE Among Children 2 Years of Age With the Prevalence of Imported Fire Ant Venom (IFAV) Specific IgE Among Children 2 to 5 Years of Age^a

Test result	Antitetanus IgE ≥0.1 kU/L, No. (%)	IFAV IgE ≥0.1 kU/L, No. (%)
Positive	109 (52.9)	24 (57.1)
Negative	97 (47.1)	18 (42.9)
Total	206 (100)	42 (100)

^a $\chi^2 = 0.11$, $P = .74$.

Table 2. Prevalence of Antitetanus IgE Level of 0.1 kU/L or Higher by Sex and Race

Child's age, mo	Children by sex, % (No. positive/total No.) ^a			P value ^b	Children by race, % (No. positive/total No.) ^a		P value ^b
	All children	Male	Female		White	Black	
6	15.2 (60/395)	19.3 (39/202)	10.9 (21/193)	.02	16.4 (19/116)	13.8 (31/225)	.52
12	35.9 (113/315)	40.7 (63/155)	31.1 (50/160)	.08	40.9 (45/110)	33.1 (58/175)	.18
24	52.9 (109/206)	53.4 (55/103)	52.4 (54/103)	.89	49.3 (37/75)	51.0 (51/100)	.83

^a Prevalence of detectable antitetanus IgE antibodies at cutoff values of 0.1 kU/L. The numbers of white and black children do not equal the total number of children because children of other racial groups are included in the category of all children.

^b P values are related to the difference in the prevalence of antituberculosis IgE between male and female children and between white and black children at each age studied using a Wilcoxon rank sum test.

Table 3. Distributions of Antitetanus IgE Concentrations at 6, 12, and 24 Months of Age and Anti-Imported Fire Ant Venom IgE at 12 to 36 Months of Age

IgE concentrations, kU/L	Antitetanus IgE, No. (%)			Anti-fire ant venom IgE in children 12–36 months of age, No. (%)
	6 Months of age	12 Months of age	24 Months of age	
<0.1	335 (84.8)	202 (64.1)	97 (47.1)	18 (58.1)
0.1–0.99	52 (13.2)	71 (22.5)	62 (30.1)	3 (9.7)
≥1.0	8 (2.0)	42 (13.3)	47 (22.8)	10 (32.2)

Table 4. Relationship Between the Number of DTaP Injections Received and the Prevalence and Average Concentrations of Antitetanus IgE Antibodies at Approximately 6 Months of Age

No. of injections before 6 months	No. of children	Prevalence, % (No. positive/total No.) ^a	Geometric means of positive samples	
			Geometric mean, kU/L ^b	Ln IgE mean (SD) ^c
0	20	0 (0/20)		
1	31	0 (0/31)		
2	235	15.7 (37/235) ^d	0.46 ^e	−0.78 (1.08)
3	106	21.7 (23/106) ^d	0.30 ^e	−1.22 (0.63)
4	3	0 (0/3)		
Total	395	15.2 (60/395)	0.39	−0.94 (0.95)

Abbreviation: DTaP, diphtheria and tetanus toxoids and acellular pertussis.

^a Prevalence of detectable antitetanus IgE antibodies at 0.1 kU/L or higher followed by number of positive and total number of samples tested.

^b Geometric mean concentration of antitetanus IgE antibodies for all samples.

^c Natural logarithms of mean (SD) concentration of antitetanus IgE antibodies for all samples tested.

^d $\chi^2 = 1.399$, $P = .24$.

^e t test = 3.904, $P < .001$.

Strengths of our study include the ability to compare the prevalence of specific IgE to 2 different antigens in young children using the same IgE assay system. The use of the

same IgE assay system reduces the risk that a difference in prevalence would be caused by differences in assay sensitivity. Both antigens studied are delivered by injection, although at different depths. The antitetanus IgE portion of the study used samples from a large, geographically based, multiracial, birth cohort, allowing evaluation of antitetanus IgE at 3 ages during early childhood. The assay reagents used for measuring antitetanus IgE are commercially available as research reagents.

The principal weakness of our study is that anti-IFAV and antitetanus IgE were measured in studies differing in design and intensity of data collection. In the future we hope to be able to follow the development of both forms of specific IgE in a single population. We chose to compare specific IgE at 2 years of age because our previous work with anti-IFAV IgE suggested that antigen specific IgE developed rapidly from birth to 2 years of age and increased more slowly after 2 years. The geometric mean values of anti-IFAV IgE also increased with age. The rate of increase in the prevalence of anti-IFAV IgE was modest between 2 and 5 years of age to 6 to 10 years of age, increasing from 57.1% to 64.4%,¹ suggesting that most sensitization occurred during the preschool years. Although the number of children studied at 2 years for anti-IFAV was small, the prevalence of IgE in these 2-year-old children was only slightly lower than the prevalence for the entire age group. Additional weaknesses of our study include the complexity of the antigens and other materials present in both DTaP immunizations and IFAV stings. There may have been variations in the number and timing of each DTaP immunization for participating children that we did not adjust for, and some children did not provide blood at all time points. As with all prospective studies, there are some children who were lost to follow-up or for whom samples were missed, but there is no reason to suspect that loss from the study is related to antitetanus IgE formation. We did not question parents in detail about adverse reactions to immunizations, so we cannot determine whether the presence of antitetanus IgE was associated with more frequent adverse reactions. However, Edelman et al²¹ did not find an association between local reactions after booster immunization in 71 two-year-old children and the presence of detectable anti-pertussis toxin IgE antibodies.

All DTaP vaccines currently used in the United States contain acellular pertussis antigens. The 52.9% prevalence of antitetanus IgE we found is similar to the 53.6% prevalence at 24 months for children who had received tetanus and diphtheria toxoids but not whole-cell pertussis antigens during primary immunizations as reported by Gruber et al.¹⁰ However, when Gruber et al coadministered whole-cell pertussis toxin with tetanus and diphtheria toxoids, the prevalence of antitetanus IgE antibodies was only 12.7% in contrast to the 53.6% prevalence found in children not given whole-cell pertussis. This finding suggests that acellular pertussis vaccines do not shift antibody responses away from IgE production to the same extent that whole-cell pertussis vaccines do as shown by Ausiello et al.²² Whether this has an effect on the risk of later allergic disease in children has not been well studied.

In summary, we found that by 24 months of age 52.9% of unselected children receiving a primary series of 2 or 3 DTaP immunizations formed antitetanus IgE antibodies and that this prevalence was not statistically significantly different from the 57.1% prevalence of anti-IFAV IgE antibodies we previously reported. Knowledge of the high prevalence of IgE to both of these potential allergens is important to physicians called on to evaluate children who have had apparent allergic reactions because positive test results are found in most children who have not had any symptoms of an allergic reaction when exposed to these antigens.^{19,23,24}

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