

Allergen Skin Test Reaction Patterns in Children (≤ 10 Years Old) from Atopic Families Suggest Age-Dependent Changes in Allergen-IgE Binding in Early Life

Duaine R. Jackola Lisa Pierson-Mullany Malcolm N. Blumenthal
Andreas Rosenberg

Asthma and Allergy Center, University of Minnesota Medical School, Minneapolis, Minn., USA

Key Words

Allergen · Antibody affinity · Atopy · Pediatric allergy · Skin prick test

Abstract

Background: Genetic studies of atopy rely upon evidence of abnormal IgE production, usually elevated total IgE or skin prick test (SPT) reactions. However, these measures may change with subject age. **Methods:** We screened 1,099 members of atopic families (aged 6–87 years) by serum total IgE and SPT for 14 allergens. For those SPT negative, we screened for Amb a 1- and Der p 1-specific IgE. Der p 1 IgE-Der p 1 allergen binding affinities were done on randomly selected subjects. **Results:** There were significantly fewer atopics ≤ 10 years old (69.1%) compared to those > 10 years old (75.8%) based upon any SPT-positive result. Children ≤ 10 years had fewer SPT-positive reactions and smaller SPT wheal reaction areas. Yet, mean total IgE values were comparable to those of the older group. Screens for specific IgE showed no differences in proportions of atopics (≤ 10 years old = 83.1% and > 10 years old = 82.3%). Among those SPT-positive for house dust mite extract, there was a positive correlation between Der p 1 binding affinity and the wheal area of the house dust mite extract. There

was a positive correlation between the number of SPT-positive reactions and total IgE for both age groups. However, there was only a significant relationship between SPT-positive wheal area and total IgE for those > 10 years old and no apparent relationship between wheal area and total IgE for those ≤ 10 years old. **Conclusion:** These results suggest that atopy-specific physiological mechanisms, primarily those involving allergen-IgE binding, change during the earliest years of life.

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Introduction

Evidence that atopic disorders are inherited derives from observations that clinical atopic outcomes tend to ‘cluster’ within families [1]. However, the results of several genome-wide screens for the gene or genes involved in atopic diseases among atopy-prone families, based upon these clinical outcomes, have only shown nonsignificant, suggestive linkages to multiple chromosomal areas, but no consensus among any of the reports [2–6]. What these studies confirm, though, is that there may be multiple genes involved or, possibly, that the clinical phenotypes employed have an unusually large degree of inherent errors of classification.

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Correspondence to: Assist. Prof. Duaine R. Jackola
Asthma and Allergy Center, University of Minnesota Medical School
Mayo Mail Code 434, 420 Delaware St. S.E.
Minneapolis, MN 55455 (USA)
Tel. +1 612 626 4624, Fax +1 612 624 9188, E-Mail jacko001@umn.edu

For example, in epidemiological studies it has been shown that the severity of atopic disease and the levels of common markers, such as serum total IgE, decline with age in the adult population [7]. This suggests a possible reversal or loss of basic atopic mechanisms. However, we have recently shown that, although some markers do decline with age, the basic underlying mechanism of IgE-based humoral response remains essentially unchanged, even into the advanced years (>60 years) [8]. In the adult population with established atopic sensitization this indicates that, although the clinical picture might change with age, the basic mechanisms of allergen interactions with IgE antibodies are particularly robust. What might this suggest at the other end of the age spectrum for children who are or will become atopic?

Most cases of atopic allergy and asthma develop in the earliest years of life during a critical 'window of sensitization' period [9]. Major contributing factors to this 'atopic march' are genetic susceptibility, general immaturity of the developing immune system, initial exposure to environmental allergens and other host and environmental factors (maternal sensitization, diet, air pollutants and others) [10, 11]. Yet, the underlying biology of these complex pathological processes remains elusive.

The critical question for genetic studies is at what age can it be reasonably assured that the clinical markers used in adults are valid for classifying children? Several reports have suggested a link between asthma and the atopy-associated measures of serum total IgE values or skin prick test (SPT) reactions [12, 13]. Yet, it remains controversial as to how strong these associations are, and studies involving children and adults are not in concordance [14].

We have investigated the underlying biology of atopy, focusing primarily upon the biochemistry of the binding reactions between immunoglobulins and purified allergens, since allergen-antibody interactions are a critical step in the evolution of atopic outcomes. Using ultrasensitive chemiluminescence-based detection methods [15], we have devised techniques to study the complex reactions between allergens and IgE [16]. These methods demonstrate that there are clear differences among those who have detectable amounts of serum allergen-specific IgE but who vary with regard to standard SPT sensitization tests; those with SPT-positive results have higher allergen-IgE binding affinity values compared to those who are SPT negative [17]. Low IgE binding affinity failed to elicit an SPT reaction, a clear potential source of classification errors.

For this report, we investigated the relationships of SPT reactivity, IgE levels and IgE affinity markers for atopy in children 10 years of age and younger. Comparing these results with those of atopics >10 years old, it appears that there are attenuated affinity and IgE response capacity among the younger individuals who are already allergen sensitized. These results point towards the possibility of errors in phenotype determinations for genetic studies, and underscore the necessity of quantitative determinations of specific IgE response capacity.

Materials and Methods

Atopic Families

Families that included at least 1 child with a physician's diagnosis of asthma were recruited. (Based upon standard atopy screens to be described, >2/3 of this population was also atopic.) To qualify, an asthmatic child had to have both characteristic asthma symptoms and positive evaluation of bronchial hyperreactivity, as previously described [3]. Each person, or legal guardian, provided written, informed consent according to the guidelines of the Institutional Review Board of the University of Minnesota. We evaluated a total of 1,099 family members, of which 666 came from 26 families in which representatives of three or four generations were ascertained. These families have been followed in clinics of the University of Minnesota Medical School for several decades, and have been included in ongoing studies concerning the genetics of asthma and other atopic disorders. An additional 433 came from 112 nuclear families that included both parents, the asthmatic children and their siblings. These families were recruited through media advertisements, also as part of studies for the genetics of asthma.

Participants were given a routine physical examination and an interviewer-monitored questionnaire, prepared according to American Thoracic Society guidelines, regarding asthma symptoms and other respiratory diseases, and allergic histories and symptoms. Baseline spirometry measures for FEV₁ were made according to American Thoracic Society guidelines [18], followed by provocative methacholine challenges as previously described [3]. Each was also tested for sensitivity to a battery of 14 allergens common to the Upper Midwest by standard SPTs.

SPT for Allergen Sensitivities

Skin prick testing was administered with Duo-Tip Test Applicators (Lincoln Diagnostics, Decatur, Ill., USA) using standardized allergen extracts (extracts from Bayer, Elkhart, Ind., USA). Positive histamine and negative saline controls were also administered. Wheal areas (mm²) were measured with a planimeter. A positive response was recorded for any wheal area ≥ 9 mm² above the negative saline control. This method is consistent with current diagnostic procedures for evaluating allergen sensitization. Table 1 lists the allergen extracts tested including the allergen source, and the systematic and scientific names [19].

Serum Immunoglobulin Assays

Whole blood was obtained by sterile venipuncture. Serum was separated and stored frozen (-80°C) until assayed.

Total IgE. Quantitative serum total IgE determinations were made using the ACCESS Immunoassay System® developed by Sano-fi Diagnostics Pasteur (Division of Beckman Coulter, Chaska, Minn., USA). Access® is an automated solid-phase immunoassay system that utilizes chemiluminescence-based detection [20]. Calibration was based upon commercial standards (Beckman Coulter).

Amb a 1- and Der p 1-Specific IgE. In this study population, >50% of those with any SPT-positive result demonstrated sensitivity to ragweed or house dust mite allergens. Quantitative assays for allergen-specific IgE were done using the purified allergens Amb a 1, a major allergen extracted from short ragweed (*Ambrosia artemisiifolia*), and Der p 1, a major allergen extracted from house dust mite (*Dermatophagoides pteronyssinus*). Serum concentrations of these allergen-specific IgEs were measured by an adaptation of the Access-specific IgE immunoassay [15, 16]. The lowest limit of detection using this method is 0.02 IU/ml (1 IU = 2.4 ng). (Note: the Access method for determinations of allergen-specific IgE is currently not commercially available in the US.)

Distribution of Der p 1 Binding Affinity. Our basic experimental method provides information regarding allergen-antibody binding reactions in vitro. Specific details are described elsewhere [16, 17, 21]. The information derived from this method includes the number of reactions involved between an allergen-specific immunoglobulin and the allergen (n), the equilibrium binding affinity constant for each reaction (K_A) and the relative proportion of each reaction to the sum of all reactions involved [$p(K)$]. The data were analyzed using Mathematica® software (Wolfram Research, Champaign, Ill., USA). (A pamphlet detailing the methods used for determining affinity distribution functions is available, at no cost, to interested investigators.)

Statistics

Basic comparisons of age and gender groups were done using analysis of variance (ANOVA) and t tests. Comparisons among quantitative variables were made by simple linear regression of $\log_{10}$ -transformed values. Comparisons of proportions were made by Z tests [22].

Results

Basic Screens for Atopy by Age and Gender

The study population comprised 1,099 members of families with positive atopic history, of whom 666 came from 26 multi-generation families (representatives of 3 or 4 generations) and 433 from 112 nuclear families. The members of the study cohort ranged in age from 6 to 87 years (546 females and 553 males). For basic comparisons, all subjects were screened for their 'atopic status' by standard SPT to 14 common allergens and serum total IgE concentrations. As previously shown, there were no major differences between the multi-generation and nuclear families with regard to prevalence of atopy [8].

We divided the population into decadal groups (e.g. ≤ 10 , 11–20, 21–30 and >60 years). Figure 1 shows the number of SPT results and the average SPT wheal area by age group.

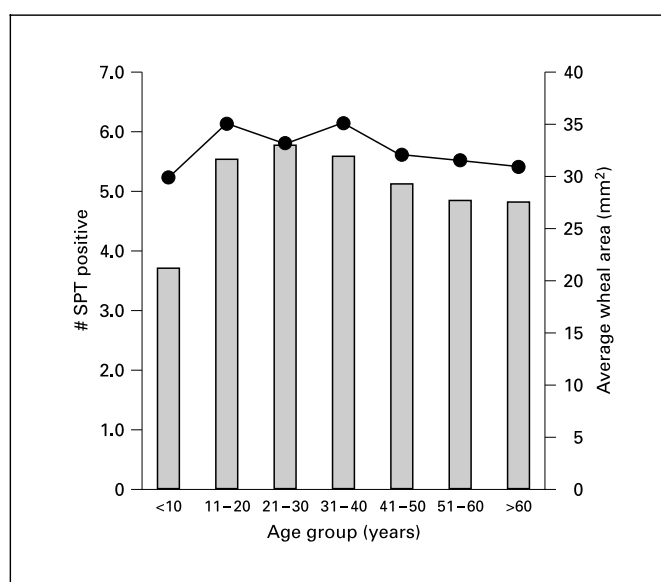


Fig. 1. Average SPT results by age. The average SPT-positive results (■) and average SPT wheal area (●; mm²) are given for each age group.

Table 1. List of allergen extracts tested

Indoor Allergens		
1 Dust mite	Der p	<i>Dermatophagoides pteronyssinus</i>
2 Dust mite	Der f	<i>Dermatophagoides farinae</i>
3 Cat	Fel d	<i>Felis domesticus</i>
4 Dog	Can f	<i>Canis familiaris</i>
5 German cockroach	Bla g	<i>Blattella germanica</i>
6 American cockroach	Per a	<i>Periplaneta americana</i>
Molds		
7 Mold	Cla h	<i>Clad sporium herbarum</i>
8 Mold	Alt a	<i>Alternaria tenuis (alternata)</i>
9 Mold	Asp f	<i>Aspergillus fumigatus</i>
Seasonal allergens		
10 Rye grass	Lol p	<i>Lolium perenne</i>
11 Short ragweed	Amb a	<i>Ambrosia artemisiifolia</i>
12 Birch	Bet v	<i>Betula verrucosa</i>
13 Mugwort	Art v	<i>Artemisia vulgaris</i>
14 Oak	Que a	<i>Quercus alba</i>

By ANOVA, there were statistically significant differences in the number of SPT-positive reactions by age: $F(6, 754) = 4.31$; $p = 0.0003$. The average number of SPT-positive reactions for those ≤ 10 years old was 3.70, while the average for the remainder of the study population was 5.29. This was not due to gender differences as the mean number of SPT-positive results for both males and fe-

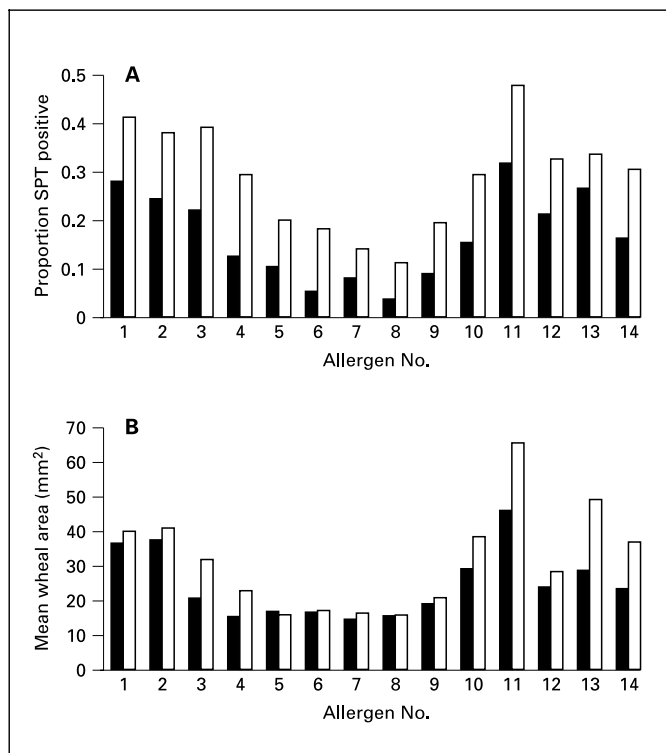


Fig. 2. Proportionate SPT results by age. For each test allergen (table 1), the proportionate number of SPT-positive results (**A**) and mean wheal area in mm² (**B**) were determined for those ≤10 years old (■) and those >10 years old (□).

males ≤10 years old was 3.70, while the mean value for females >10 years was 5.39 and the mean value for males >10 years was 5.19.

In contrast, there were no apparent differences for average SPT wheal areas with age by ANOVA: $F(6, 754) = 1.07$; $p = 0.38$. However, comparing the average wheal areas for those ≤10 years (29.7 ± 2.21 ; $n = 86$) with the average wheal areas for those >10 years (33.5 ± 0.78 ; $n = 676$) by *t* test, a significant difference was noted: $t(760) = 1.63$; $p = 0.05$. This result also was not due to gender differences.

To rule out the possibility that these variations might have been due to differential sensitivities to specific allergens, we calculated the proportion of SPT-positive responses to each of the 14 allergens listed in table 1. Figure 2A shows the proportion of SPT-positive responses for each allergen, comparing the results for those ≤10 years old with those >10 years. Except for variations in proportionate responses by age, there are no discernible 'patterns' to indicate any greater or lesser sensitivity to any particular allergen. Similarly, in figure 2B there are no

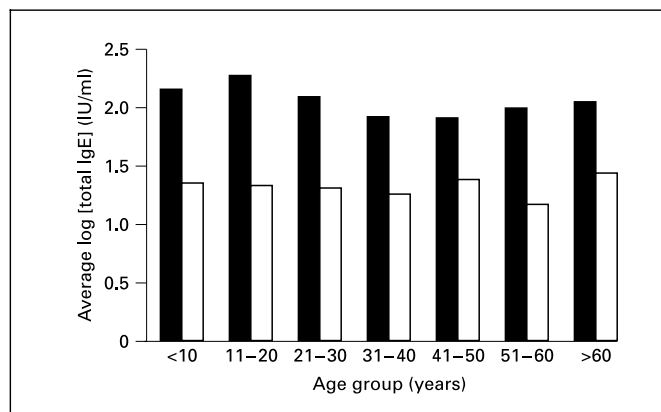


Fig. 3. Comparisons of serum total IgE levels by age. For each age group, the mean log₁₀ [total IgE] levels (IU/ml) were determined for those with any SPT-positive (■) or SPT-negative result (□; 14 test allergens).

discernible 'patterns' for the degree of response based upon SPT wheal areas. Thus, in this study population, variations in responses to allergens were due to age, at least for those ≤10 years but not due to variable sensitivities to particular allergens.

Serum total IgE values are a general indicator for 'atopic propensity' [23]. Figure 3 shows the mean log₁₀ [total IgE] values for each of the age groups according to whether they had SPT-positive or SPT-negative results. ANOVA revealed a significant difference among those with any SPT-positive results: $F(6, 754) = 7.90$; $p < 0.0001$. Although statistically significant differences were observed for mean log₁₀ [total IgE] values among the age groups, this was not due to the mean values for those ≤10 years old (mean log₁₀ [total IgE] = 2.15) compared to the remainder of the population (mean log₁₀ [total IgE] = 2.04). Rather, it was due to slightly elevated values among those in the group 11–20 years old (2.27) and slightly lower values among those aged 31–40 (1.91) and 41–50 years (1.91).

Among those with SPT-negative results to all test allergens, there were no significant differences in mean log₁₀ [total IgE] values by age: $F(6, 331) = 0.81$; $p = 0.56$. As before, for both those with SPT-positive and SPT-negative results, there were no differences attributable to gender in these atopy-prone families.

In contrast to the total IgE values, the results of the basic SPT atopy screens among 1,099 members of atopy-prone families point to significantly diminished responses to common allergens among those ≤10 years old compared to the remainder of the study population.

Table 2. Atopy groups for Der p 1-positive responses

Atopy group	n	Dust mite SPT	Elevated total IgE (>95 IU/ml)	Mean Der p 1 IgE, IU/ml	Mean log capacity
A	32	–	yes	1.07	–1.373
B	32	–	no	10.22	–1.060
C	25	+	yes	11.26	0.828
D	25	+	no	5.83	0.376

ANOVA results: atopy group comparisons by Der p 1 concentration: $F(3, 110) = 1.69$ (not significant, $p = 0.05$); atopy group comparisons by binding capacity: $F(3, 110) = 6.40$ ($p < 0.001$); comparisons of binding capacities by atopy group: groups A vs. C, A vs. D, B vs. C and B vs. D: $p < 0.05$, respectively (comparisons of group means by t tests).

Refined Screens for Atopy

To eliminate the potentially confounding variable of smoking on IgE production, for the remaining analyses we excluded the results from people with a smoking history of 1 or more pack-years ($n = 269$). Among the remaining 830 subjects, based upon any SPT-positive result or elevated total IgE (>95 IU/ml) [23], the percentage of atopics among those ≤ 10 years old ($n = 136$ in total) was 69.1%. In contrast, for those >10 years ($n = 694$ in total), the percentage of atopics was 75.8%. Comparing these proportions by Z test: $Z = 1.601$, a significant difference was found ($p = 0.05$).

For the 14 allergens used for SPT screens, among those with any SPT-positive result $>50\%$ were sensitized to house dust mite (Der p) and/or ragweed (Amb a) allergens. We screened the sera of subjects with SPT-negative results using the major allergens Der p 1 and Amb a 1. Then, defining atopy as any SPT-positive result or evidence of serum allergen-specific IgE, the percentage of atopics among those ≤ 10 years ($n = 136$ in total) was 83.1%. Among those >10 years old ($n = 694$ in total), the percentage was 82.7%. There was no difference in these proportions by Z test: $Z = 0.283$; no significant difference was found ($p = 0.05$). Thus, although not apparent by standard atopy screens, the relative incidence of atopic allergen sensitization in these atopy-prone families was consistent across all age groups.

Effect of Allergen-IgE Binding Affinity on SPT Results

We have previously shown that the allergen-IgE binding affinities vary among those with detectable serum allergen-specific IgE. On average, those with SPT-positive

results have higher reaction binding affinities compared to those with SPT-negative results [17]. Independent investigators have shown that binding affinity is positively correlated with histamine release by basophils in vitro [24].

To determine the effect of allergen-IgE binding affinity and the basic atopy screens in this study population, we randomly selected samples, without regard to subject age, based upon results for SPT reactions to dust mite (Der p) extract and total IgE values. Table 2 gives the SPT-positive/SPT-negative results of four groups with elevated (>95 IU/ml) or normal total IgE values. (All those selected with SPT-negative results had detectable Der p 1 IgE.) Also listed are the mean values for 'binding capacity'.

As previously shown [16, 17], interactions between IgE and purified allergens are complex, heterogeneous reactions. The total binding capacity is defined as: $CAP \equiv [IgE] \times \sum_{i=1}^n K_i \times p[K]_i$, where $[IgE]$ is the specific IgE concentration, n is the number of specific reactions, K_i is the equilibrium binding constant for the i th reaction and $p[K]_i$ is the relative proportionate amount of each reaction. With $[IgE]$ expressed in units of M/L and K expressed in units of L/M, the variable CAP is dimensionless. In the case of the common indoor allergen Der p 1, each of the specific reactions corresponds to IgE produced against a specific immunogenic epitope [21].

By ANOVA, there was no significant difference among the four groups (table 2) based upon mean Der p 1 IgE values. In contrast, based upon mean binding capacities, there were significant differences. Pairwise group comparisons by t tests showed that those with SPT-positive results had higher binding capacities, and hence higher binding affinities, than those who were SPT negative. Thus, the probability of an SPT-positive response was primarily due to the affinity of IgE for allergen rather than the concentration of specific IgE. Further, there was a positive correlation between $\log_{10}$ (capacity) and $\log_{10}$ (wheal area) for those with Der p SPT-positive results ($r = 0.58$; $p = 0.0003$). A similar result was found comparing Amb a 1 capacity and Amb a SPT-positive wheal area ($r = 0.64$; $p < 0.0001$).

Age and Strength of Atopic Response

Due to the limited number of subjects with SPT reactions to dust mite or ragweed among the ≤ 10 -year-old group, we could not directly compare binding affinities and SPT results as a function of age. However, we could make indirect assessments based upon the number of SPT-positive results, SPT wheal areas and total IgE values, since at least some complement must be allergen-specific IgE.

We first assessed the number of SPT-positive results versus $\log_{10}$ [total IgE] by simple linear regression: for those ≤ 10 years old, the number of SPT-positive responses = $0.58 + 1.45 \times \log_{10}$ [total IgE]; $F(1, 84) = 12.66$; $p < 0.0001$, and for those > 10 years old: the number of SPT-positive responses = $0.66 + 2.31 \times \log_{10}$ [total IgE]; $F(1, 673) = 141.8$; $p < 0.0001$.

The slopes of the regression lines were significantly different from zero, indicating that the total IgE complement was associated with increased likelihood of allergen sensitivity in both age groups. Interestingly, comparing the two regression coefficients by *t* test showed that the slope for those > 10 years was significantly greater than for those ≤ 10 years: $t(757) = 4.14$; $p < 0.0001$.

We next compared the average $\log_{10}$ (wheal area) for each SPT-positive subject with his/her $\log_{10}$ [total IgE] value: for those ≤ 10 years old: $\log_{10}$ (wheal area) = $1.26 + 0.06 \times \log_{10}$ [total IgE]; $F(1, 84) = 2.15$; $p = 0.15$. In this case, the slope of the regression line was not significantly different from zero. However, this was in contrast to those > 10 years old: $\log_{10}$ (wheal area) = $1.23 + 0.11 \times \log_{10}$ [total IgE]; $F(1, 666) = 57.9$; $p < 0.0001$.

Although indirect, these results are in agreement with the results of the previous section: increased IgE binding capacity (affinity $\times$ IgE concentration) corresponds to a greater IgE-mediated response (SPT wheal area). Thus, it would appear that the specific IgE contained in the total IgE complement of those > 10 years old is functionally robust in terms of affinity reflected by the strong association between IgE values and SPT-positive wheal reaction areas.

In contrast, while there is evidence for allergen-specific IgE production in those ≤ 10 years, reflected by both SPT-positive results and evidence of serum allergen-specific IgE, the lack of association between IgE and wheal area suggests that this group's IgE has not yet functionally 'matured' to the level of older members of these atopy-prone families.

Discussion

Atopy is a form of immune dysregulation, defined as an aberrant humoral immune response to common environmental antigens (allergens) resulting in the production of allergen-specific IgE. It is thought that most cases of atopic disease are initiated in early life during a critical 'window of sensitization' period [9–11]. Most studies of atopy rely upon evidence of abnormal IgE production: elevated serum total IgE values, any SPT-positive reac-

tion or presence of detectable serum allergen-specific IgE. There are two major concerns regarding the reliability of these assessments and their relationships to atopic disease, particularly for genetic investigations.

First, the distribution of values of these quantitative measures may change as a function of age, especially in the earliest years of life. Second, the presence (or apparent absence) of a particular marker may only reflect a 'physiological potential' (or apparent lack) for a particular response, but the actual response elicited requires some 'physiological force' (e.g. IgE binding affinity for allergen).

In a prospective study, Sasai et al. [25] showed that minute amounts of serum allergen-specific IgE present at < 6 months, with values below the generally accepted, albeit arbitrary, value of 0.35 IU/ml, was a good predictor of atopic sensitization by age 6 years.

In another prospective study of children during the first 12 years of life, Hattevig et al. [26] showed that, as expected, those with atopy tended to have higher values of serum total IgE than children without atopy. However, in the first 2 years of life, both groups of children had detectable amounts of allergen-specific IgE. The difference was that the production of the specific IgE was only transient in the group 'not at risk' whereas it was persistent in the 'at risk' group. In both studies [25, 26] it was shown that significant developmental changes occurred in the earliest years of life in the immune systems of children, at least with regard to the production of the type and amount of IgE. However, neither these nor related studies have suggested an underlying physiological mechanism associated with these differences that might account for differential immunoglobulin production.

The primary humoral response to antigen involves the production of large amounts of relatively nonspecific IgM. Upon subsequent contact with the antigen, engagement of the metabolic pathways involved in isotype class switch recombination results in the production of a different immunoglobulin class or subclass (IgA, IgG or IgE) [27]. Additionally, the secondary immunoglobulin produced is more specific for the particular antigen challenge. Gene rearrangements of the variable heavy and light chains of the antigen-combining region via somatic hypermutation results in 'affinity maturation' so that the antibody binds more efficiently to the antigen that induced its production [27]. Thus, both class switch recombination and somatic hypermutation are simultaneously required for a particular secondary response [28].

We have found among SPT-positive atopic adults that the binding affinity of the specific IgE for allergens is

extremely high (typical values in the range 10^8 to $10^{11} M^{-1}$) [16, 17, 21]. Also, once established, the affinity of the IgE for allergen is particularly robust and does not significantly change even into the later years of life (>60 years) [8]. However, in some cases differential responses regarding affinity have been suggested.

We have compared the IgE-allergen binding affinities among adults with detectable allergen-specific IgE but who varied with regard to skin test reactivity (SPT negative vs. SPT positive). On average, those with SPT-positive results tended to produce more allergen-specific IgE than those who were SPT negative, although there was considerable overlap between these groups. In contrast, the IgE-allergen binding affinity among SPT-positive subjects was significantly higher than in those who were SPT negative (at least 10- to 100-fold higher) [17]. A plausible physiological mechanistic explanation for these differences has been provided by independent investigators who have shown that IgE-allergen binding affinity is positively correlated with the amount of histamine released by basophils in vitro [24].

In the present study, we screened 1,099 members of atopy-prone families for their 'atopic status' using standard methods (total IgE and SPT) and refined methods (allergen-specific IgE; Der p 1 and Amb a 1). On average, when grouped by age, there were no significant differences to be found for total IgE values (fig. 3). In contrast, there were significant differences by age, especially in the group ≤ 10 years old, with regard to the probability of an atopic response, reflected either by the number of SPT-positive reactions or the average wheal area of SPT-positive responses (fig. 1). These differences could not be accounted for by possible variations with age in response(s) to any particular allergen or sets of allergens (fig. 2).

It may have been that the group ≤ 10 years old simply had a lower proportion of atopic individuals in this study population, although this was unlikely as ascertainment criteria were established on asthmatic children. Defining atopy as any SPT-positive result or elevated total IgE (>95 IU/ml) showed that only 69.1% of the group ≤ 10 years old was atopic by these criteria compared to 75.8% in the group > 10 years old. However, an SPT-negative result does not preclude the existence of an underlying atopic mechanism [17].

We screened the sera of those with SPT-negative results for allergen-specific IgE (Der p 1 from house dust mite and Amb a 1 from ragweed). Then defining atopy as any SPT-positive result or evidence of serum allergen-specific IgE showed that the proportions of atopy in the study

population were comparable across the age groups (83.1% for those ≤ 10 years old and 82.3% for those > 10 years old). The differences in the number of SPT-positive results or average wheal areas shown in figure 1 could not be accounted for by a lower proportion of atopic individuals in the youngest age group.

We randomly selected subjects from the study population, without regard to age, depending on their results for SPT reactions to the house dust mite (Der p) allergen extract and serum total IgE values. As shown in table 2, we compared the results of four groups: SPT-negative/high IgE, SPT-negative/normal IgE, SPT-positive/high IgE and SPT-positive/normal IgE. All of these subjects had detectable amounts of allergen-specific IgE when screened using the purified allergen Der p 1.

Based upon serum concentrations of Der p 1-specific IgE, there were no differences among the four groups by ANOVA. In contrast, there were significant group differences in their IgE-allergen binding affinities (incorporated in the parameter of binding capacity). Those with SPT-positive results had, on average, higher binding capacities (affinity $\times$ IgE concentration) than those who were SPT-negative, in agreement with our previous results [17].

Most importantly, among those with SPT-positive results to the Der p extract, there was a strong positive correlation between the SPT wheal area and the Der p 1 binding capacity. (A similar result was found using the Amb a extract wheal areas and Amb a 1-specific binding capacities.) Thus, the total binding capacity (affinity $\times$ antibody concentration) was mechanistically tied to both the likelihood of an SPT reaction and the strength of the reaction (wheal area).

Due to the limited number of subjects with SPT-positive results for Der p or Amb a extracts in the ≤ 10 -year-old group, we could not adequately compare the SPT reaction wheal areas and binding capacity results as a function of age. However, because some fraction of the total IgE complement must consist of allergen-specific IgE, we could make indirect comparisons among those with any SPT-positive result.

By simple linear regression, there was a strong positive association between $\log_{10}$ [total IgE] values and the SPT-positive results for both age groups: ≤ 10 years old and > 10 years old. Thus, by inference, the specific IgE present in the total IgE complement was correlated with the likelihood of the sensitization to some set of allergens for these individuals regardless of age.

In contrast, comparisons using simple linear regression of the $\log_{10}$ [total IgE] values with $\log_{10}$ (wheal area) did not give comparable results for these age groups. For those

>10 years old, there was a strong positive association, suggesting a mechanism akin to the results of table 2: increased IgE binding capacity is correlated with an increased wheal reaction area. However, there was no apparent association between total IgE and wheal area for those ≤ 10 years old. In this case it may be inferred that, although allergen-specific IgE is present it has not reached a state of 'functional maturity', most likely due to mechanisms involved in antibody 'affinity maturation'.

In summary, in a large study population comprising members of atopy-prone families ranging in age from 6–87 years, there are significant age differences in the atopic responses, particularly among those ≤ 10 years old. The number of SPT-positive reactions and the average SPT wheal reaction areas are diminished compared to the remainder of the study population, even though there is evidence of comparable proportions of atopy across all age groups based upon detectable amounts of allergen-specific IgE in the sera. The key atopy-associated physiological mechanism that best accounts for these observations is that among those >10 years old, their allergen-specific IgE has reached an end-stage of functional maturity with regard to the affinity of the IgE for allergen. In contrast, among those ≤ 10 years old, it would appear that the specific IgE has not reached an end-stage of functional

maturity, and that coincident with previously observed changes in allergen-specific IgE concentrations with age in the earliest years of life there is also a corresponding mechanism associated with the 'affinity maturation' of these immunoglobulins.

Prospects for Genetic Studies. The clinical markers of atopy can have variable outcomes, particularly as a function of age. Further, standard markers of atopy are only associated with evidence of abnormal IgE production (SPT results, serum total or specific IgE). Thus, these markers only reflect a 'response potential'. The underlying biology of these complex diseases also involves a physiological 'driving force' (e.g. binding affinity). The overall 'atopic capacity' is a function of both the potential and the driving force that elicit the response. Future genome-wide screens for atopic disease should take both elements into account.

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