

## Robustness Into Advanced Age of Atopy-Specific Mechanisms in Atopy-Prone Families

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We evaluated atopy-associated parameters in 1099 people (aged 6–84 years) from families with history for atopy. All were tested for serum total immunoglobulin E (IgE) and allergen sensitivity by skin prick test. Specific IgE tests were done in randomly selected families. There was a decline with age in serum total IgE values, and relative atopy “incidence rates” were slightly lower among those older than 60 years. However, there was no change with age in sensitivity or severity of atopy. Among those sensitized to ragweed (*Ambrosia artemisiifolia*), there was no age-associated change in IgE levels specific to Amb a 1, a major allergen extracted from ragweed, and no change in the binding affinity of IgE for the Amb a 1 allergen. Among families with atopic histories, the underlying atopic mechanisms are particularly robust, and the atopic propensity remains into advanced age. In addition, established atopic responses may be focused in an immune system compartment either independent of or minimally influenced by T-cell activity.

ATOPY is a relatively common, adverse humoral immune system response to common environmental agents (allergens) involving the production of allergen-specific immunoglobulin E (IgE). Epidemiological investigations of allergen sensitivity in a community-based population (1–4) and an industrial setting (5,6) show that the propensity for and the relative incidence of atopic disorders tend to change with age. Serum total IgE values decline with age in the general population, and there are significantly fewer cases of atopy among elderly subjects (60 years and older) compared with younger subjects when evaluated for allergen sensitivity by standardized skin prick tests (SPT) using batteries of allergens common to the particular geographical locale. Additionally, there was a significant concordance of atopic parameters among family members in the community-based study (7). However, indicators of the propensity for atopy or tests for allergen sensitivity do not address the specific mechanistic aspects of these disorders.

We have been interested in the mechanistic basis of atopic disorders, with specific focus on the chemical binding affinity between purified allergens and allergen-specific IgE. The reaction between allergen and receptor ( $F_{c\epsilon}RI$ )-bound IgE on mast cells or basophils initiates the signaling cascade leading to the release of mediators, such as histamine, associated with allergic symptoms following provocative challenges. We have found that the allergen-specific IgE produced by atopic humans is of extremely high binding affinity (8), and that there is a direct relationship between the IgE-allergen binding and SPT reactivity (9). Independent investigations have shown that the strength of IgE-allergen binding is positively correlated with the release of histamine by basophils in vitro (10). Additionally, among atopic adults it would appear that the immunoglobulins

produced against allergens are directed toward only a few immunodominant epitopes (11), agreeing with models of time-dependent immune response maturation (12).

We have also been interested in the genetic basis of atopic disorders, particularly asthma, and have studied families with positive atopic histories. These families include both multigeneration families (three or four generations each) and smaller, nuclear families (asthmatic child, his or her siblings, and both parents). Among all these individuals, there is a wide range of age covering eight decades (preteens to octogenarians). Although there is a pronounced selection bias in this study population, invalidating it for epidemiological considerations, the effects of age on atopy-associated parameters in these atopy-prone families is a valid consideration.

It is well established that fundamental declines occur with age in immune system responses, and a consensus view holds that most changes occur either among T lymphocytes or functions mediated by T cells (13). For example, we have shown that the frequency of CD4+ T cells capable of producing interleukin-2 (IL-2) significantly declines with donor age (14). Humoral immune responses also change with age, although the underlying mechanistic basis is controversial.

Current thought posits that age-associated changes in B-cell function are primarily due to changes in the T-cell compartment and a resultant dysregulation on B-cell function (15). Altered function is primarily associated with exposure to “new” antigenic challenges, as for example the less efficient therapeutic value of inoculation regimes among the elderly population. In Le Maoult et al.’s view, humoral immune alteration is more a function of dysregulation than of immune humoral deficiency. Although both T cells and B cells are involved with the onset of atopy, the

effector mechanisms are principally humorally driven and focused on the production and regulation of IgE. To what extent this regulation is T cell dependent or T cell independent is not well established, and it is also not known to what extent age influences this regulation.

In this study, we evaluated the effects of age on the underlying mechanistic aspects of atopy, using short ragweed (*Ambrosia artemisiifolia*) as a model allergen in families with positive atopic histories. The results suggest that the physiological mechanisms of atopy are particularly robust in atopy-prone families, and that, once established, humoral immune regulation of atopy may be either independent of or minimally affected by the T-lymphocyte compartment.

## METHODS

### Donor Selection and Ascertainment

Families that included at least one child with a physician's diagnosis of asthma were recruited. To qualify, an asthmatic child had to have both characteristic asthma symptoms and positive evaluation of bronchial hyperreactivity (see descriptions that follow). 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 1099 family members ranging in age from 6 to 84 years (553 male and 546 female subjects). As detailed in the Results section, 666 of the donors came from 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 they have been included in ongoing studies concerning the genetics of asthma and other atopic disorders. An additional 433 came from nuclear families that included both parents, the asthmatic child, and his or her 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 symptomology and other respiratory diseases, as well as history of allergic disease. Each participant was also tested for atopic status and respiratory function (tests are described later).

### Assessment of Atopic Status

**Skin testing and skin test index.**—Each participant was tested for sensitivity to a battery of 14 allergens common to the Upper Midwest. The SPT was administered with Duo-Tip Test Applicators (Lincoln Diagnostics, Inc., Decatur, IL) and used standardized extracts (from Bayer Corp., Elkhart, IN) from ragweed (1), molds (3), trees (4), grasses (2), dust mites (2), and animal danders (2). Results were scored for wheal area (square millimeters) by planimetry, with a positive result  $\geq 9 \text{ mm}^2$  above saline control. A positive control was also included by using histamine base (6 mg/ml final concentration derived from diluting hista-

mine dihydrochloride at 10 mg/ml initial concentration; histamine from Greer Laboratories, Lenoir, NC).

To gauge relative degrees of atopic severity in the groups of families, we devised a skin test index (STI) to compare results among all donors with any positive skin test reaction (14 allergens tested). A total of 761 individuals (69.2%) had one or more positive results. For each allergen tested, histograms of responses (wheal area in square millimeters) were approximately normally distributed on a  $\log_{10}$  scale (not shown).

For each allergen, the response ( $f$ ) for each person was normalized by the relation  $f_i = (X_i - \text{MIN})/(\text{MAX} - \text{MIN})$ , where  $X_i$  was the wheal area for the  $i$ th allergen tested, and MAX and MIN were the maximum and minimum wheal areas for the  $i$ th allergen from the pool of all positive results for that allergen. For each individual, the STI was determined from the sum of all normalized positive reacts for that individual:

$$\text{STI} = \sum_i f_i.$$

A histogram of the  $\log_{10}$  (STI) results was approximately normally distributed.

**Serum IgE tests.**—All serum IgE tests were done by using the Access Immunoassay System developed by Sanofi Diagnostics Pasteur, Inc. (Division of Beckman Coulter, Chaska, MN) (16). Serum was separated and stored frozen ( $-80^\circ\text{C}$ ) until assayed. Total IgE determinations were done by using commercial kits according to the manufacturers' specifications. Allergen-specific IgE determinations were done by using the purified allergen Amb a 1, a major allergen extracted from short ragweed, *A. artemisiifolia*, a common seasonal allergen (17). (Among allergen-sensitized donors in this study group,  $\approx 50\%$  had positive skin test reactions to the extract of short ragweed.) The methods are described in detail elsewhere (8). Results for both total and allergen-specific IgEs are reported as international units per milliliter (1 IU/ml = 2.4 ng/ml).

For allergen-antibody binding affinity and capacity, we have devised a method to study the polyclonal binding reactions between purified allergens (Amb a 1) and allergen-specific IgE (8). In all cases tested, we found two or three allergen-specific reactions from sensitized individuals' sera independent of the allergen-specific IgE concentration. Our method determines the number of binding reactions involved ( $n$ ), the binding affinities of the reactions ( $K$ ), and the relative proportion of each reaction to the total of all reactions involved ( $p$ ).

### Definition of Asthma

For purposes of this study, asthma was defined as having both characteristic symptoms of asthma and a positive result for bronchial hyperreactivity (BHR).

**Asthma symptomatic.**—A positive result for *asthma symptomatic* was given for (a) questionnaire reports of two or three symptoms of coughing, wheezing, or shortness of breath associated with chest tightness, in the absence of

a cold or flu, or (b) self-reported asthma with confirming physician's diagnosis. Only symptoms occurring within the previous 12 months were considered. Self-reports were accepted from adults older than 18 years. Reports for minor children were accepted from a parent or an adult familiar with the child's medical history.

**BHR.**—Baseline spirometry measures for forced expiratory volume in 1 second ( $FEV_1$ ) were made according to American Thoracic Society guidelines (18), followed by provocative methacholine challenges at 5-minute intervals. Methacholine (doubling concentrations from 0.15 to 10.0, then 25 mg/ml) was administered with a DeVilbiss 646 dosimeter (Laboratory for Applied Immunology, Inc., Fairfax, VA) (0.6-second delay; 0.6-second duration). Methacholine challenges were not administered to those with baseline  $FEV_1$  values of 70% or less based on expected values adjusted for age, gender, and body mass index. For these cases, airway reversibility was assessed by administering albuterol with a handheld nebulizer. For this study, a positive result for BHR was recorded if there was a decrease of 20% or more in  $FEV_1$  after methacholine challenge ( $\leq 25$  mg/ml maximum dosage), or reversibility of 15% or more after administration of albuterol.

### Statistical Analyses

We made basic comparisons between the major family groups (multigeneration and nuclear) in order to determine if there were similar "rates" and "degrees of severity" for atopy within the groups. These and other comparisons described in the Results section were made by an analysis of variance (ANOVA) and *t* tests. For evaluating age-associated changes in atopy-associated parameters, we used simple linear regression with age as the independent variable. All parameters were evaluated after  $\log_{10}$  transformation, which precluded statistical weighting adjustments for age and gender. Significance of the regression parameters was assessed by ANOVA.

## RESULTS

### Comparisons Between the Multigeneration and Nuclear Families With Atopic Histories

A total of 1099 members of families with histories of asthma were evaluated (553 male and 546 female subjects). This total comprised 666 members from 27 multigeneration families (representatives from three or four generations) and 433 members from 112 nuclear families (asthmatic child, both parents, and the asthmatic child's siblings). The mean donor age among the multigeneration families (34.5 years) was significantly older than that of the smaller, nuclear families (28.0 years) by *t* test ( $t = 6.18$ ;  $df = 1014$ ;  $p \ll .0001$ ). However, the age ranges for the two groups of families were comparable (Table 1).

Basic comparisons between the family groups for general atopic status are also given in Table 1. On the basis of STI results, there was a higher proportion of atopics in the nuclear families (79.5%) than there was in the multigeneration families (62.6%). This was a result of the smaller average family size among the nuclear families (relatively

Table 1. Comparisons Among Families For General Atopic Status

| Characteristic   | Multigeneration | Nuclear     | Combined    |
|------------------|-----------------|-------------|-------------|
| No. of families  | 27              | 112         |             |
| <i>n</i>         | 666             | 433         | 1099        |
| Mean family size | 24.7            | 3.9         |             |
| Age range (y)    | 6–84            | 6–79        |             |
| Average age (SD) | 34.5 (18.6)     | 28.0 (16.0) |             |
| SPT(–)           |                 |             |             |
| Log (IgE)*       |                 |             |             |
| Mean             | 1.32            | 1.41        | 1.34        |
| SE               | 0.035           | 0.033       | 0.022       |
| <i>n</i> (%)     | 249 (37.9%)     | 89 (20.6%)  | 338 (30.8%) |
| SPT(+)           |                 |             |             |
| Log (IgE)        |                 |             |             |
| Mean             | 2.06            | 2.08        | 2.07        |
| SE               | 0.03            | 0.03        | 0.02        |
| <i>n</i> (%)     | 417 (62.6%)     | 344 (79.5%) | 761 (69.2%) |
| Log (STI)        |                 |             |             |
| Mean             | 1.57            | 1.66        | 1.61        |
| SE               | 0.041           | 0.028       | 0.026       |
| <i>n</i>         | 417             | 344         | 761         |

Notes: Multigeneration and nuclear family definitions are given in the Results section. SPT(–) = skin prick test negative (14 allergens possible); SPT(+) = skin prick test positive (1 or more allergens); STI = skin test index (defined in Methods).

\*Serum total IgE.

more atopics per average number of family members). As expected,  $\log_{10}$  (total IgE) values were higher among those with positive skin test results than among those with negative results. There was also a positive correlation between  $\log_{10}$  (Total IgE) and  $\log_{10}$  (STI) ( $r = .43$ ;  $p \ll .001$ ). However, among those with positive SPT results, SPT(+) in Table 1, there were no significant differences in mean  $\log_{10}$  (Total IgE) or  $\log_{10}$  (STI) values between the multigeneration and nuclear families by *t* test ( $p = .05$ ).

Because of the selection bias for asthma, there was a significantly higher overall proportion of atopic donors (69.2%) than might be expected if the donors had been randomly selected from the general population ( $\approx 20$ –30%). However, there were no major differences between the multigeneration and nuclear families with regard to atopic status or severity. Thus, we pooled all results for the assessments to be described.

### Age and Parameters Associated With Atopic Propensity

To compare the results of our atopy and asthma-biased study population with those of epidemiological investigations of the population at large, we subdivided our population into five age categories (0–15, 16–30, 31–45, 46–60, and >60 years). Subjects were further categorized according to gender and any SPT results—SPT(+) or SPT(–) for tests to 14 allergens.

Overall trends for atopic propensity, reflected by  $\log_{10}$  serum total IgE values, are given in Figure 1A. Overall trends for atopic incidence, reflected by % SPT(+) results, are given in Figure 1B. Detailed results for  $\log_{10}$  serum total IgE, SPTs, and mean wheal areas for positive control histamine skin test are given in Table 2.

As shown in Figure 1A, there is a slight decline with age in mean  $\log$  total IgE values. As expected, mean values were

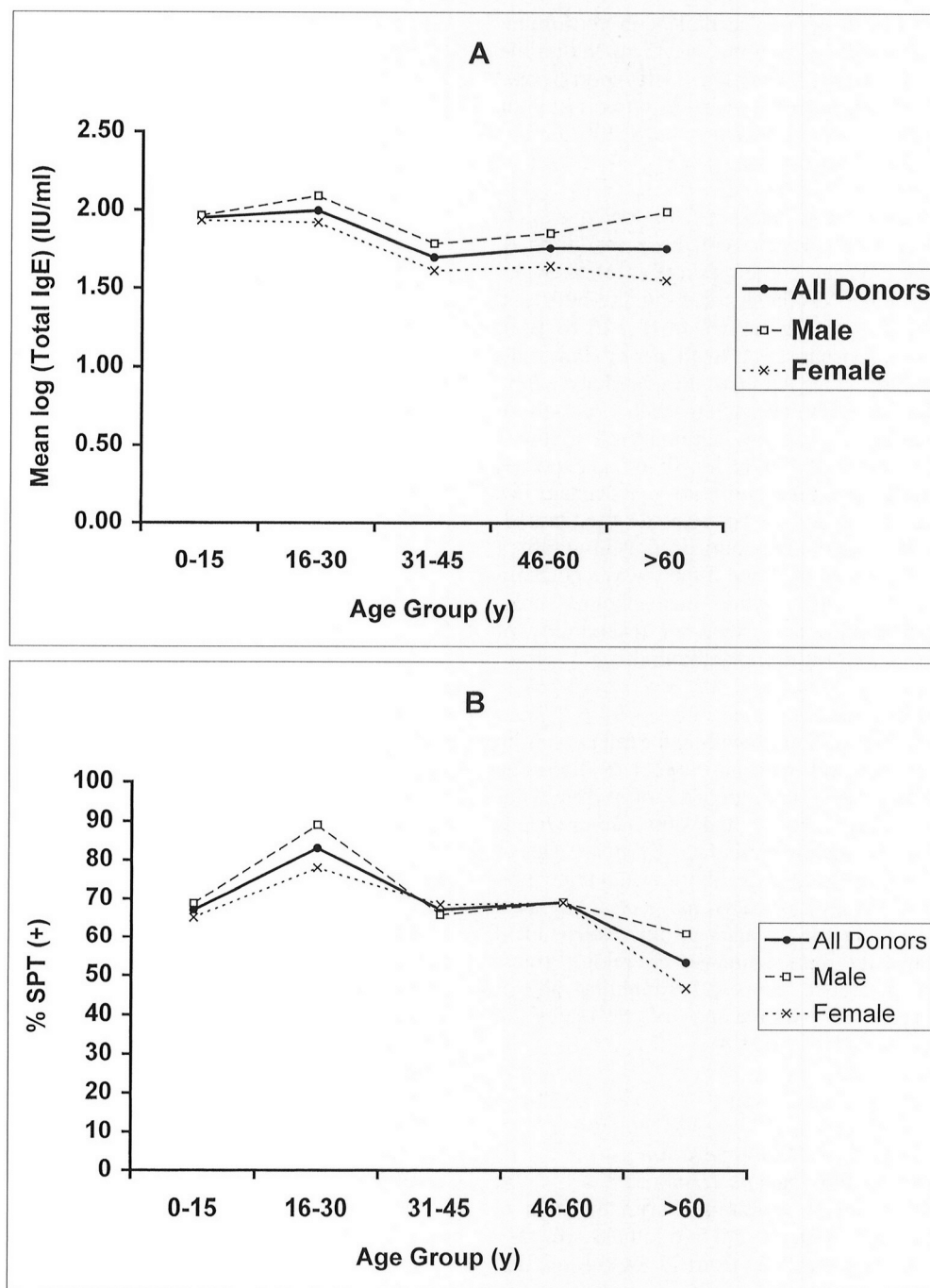


Figure 1. Atopic propensity vs donor age: **A**, mean  $\log_{10}$  serum total IgE values (IU/ml) by age group; **B**, percentage of skin prick test positive [% SPT(+)] results by age group.

higher among those with any SPT(+) result than for those who were SPT(-) for all allergens (Table 2). The most significant decline with age is apparent in female subjects who were SPT(+), in agreement with previous studies. It has been shown that smoking history can have an impact on serum IgE levels (4). In our population, there was a slight effect of smoking on IgE levels, although this was not taken into account for these most basic comparisons.

There was a statistically significant decline with age in serum total IgE values. All donors:  $\log_{10} (\text{Total IgE}) = 2.0091 - 0.0055 \times \text{Age}$ ;  $F(1, 1097) = 2.24$  by ANOVA,  $p \ll .0001$ . This result is equivalent to  $(\text{Total IgE}) \approx 100 \times 10^{-0.0055 \times \text{Age}}$ . For example, over a 30-year span, serum total IgE values would decline by approximately 30%.

However, this decline was only apparent in those with SPT(+) results, whereas there was no apparent change in

IgE values among those who were SPT(-). All SPT(+) donors:  $\log_{10}(\text{Total IgE}) = 2.260 - 0.0065 \times \text{Age}$ ;  $F(1, 759) = 25.77$ ,  $p \ll .001$ . All SPT(-) Donors:  $\log_{10}(\text{Total IgE}) = 1.305 + 0.000665 \times \text{Age}$ ;  $F(1, 336) = 0.16$ ,  $p = .69$ .

On the basis of comparisons to overall percentages of SPT(+) responses, the results by age group are also comparable with previous investigations, with a slightly elevated "relative rate" among younger subjects in the second and third decades (16-30 years), and a slightly diminished "rate" among those older than 60 years (Figure 1B; Table 2). In addition, and in agreement with others (6), there is no apparent age effect for mean histamine skin test response, although this is in contrast to results of the community-based study that found a decline in this parameter with age (1).

Aside from the pronounced selection bias in our study population, the parameters that indicate a propensity for serum IgE or presence of SPT(+) atopy show comparable age-associated effects as those seen in epidemiological investigations.

#### Age and Parameters Associated With General Atopic Mechanisms in Atopy-Prone Families

We next focused on the general mechanistic aspects of atopic responses reflected in the characteristics of the SPT(+) responses. For all donors with any SPT(+) results, regardless of age, there was a positive correlation between  $\log_{10}(\text{Total IgE})$  and  $\log_{10}(\text{Average SPT wheal Area})$  ( $r = .428$ ;  $p \ll .0001$ ). The average wheal area was calculated for each SPT(+) individual by summing all wheal areas for positive results and dividing by the number of positive results. There were no apparent age-associated effects for the relative presence or severity of the atopic responses. A simple regression of the number of SPT(+) results versus age gave the following: number of SPT(+) =  $4.99 + 0.0069 \times \text{Age}$ ;  $F(1, 759) = 0.96$ ,  $p = .33$ . The slope of this line was not statistically different from zero.

Similarly, a regression of  $\log_{10}(\text{mean SPT wheal area})$  versus age gave  $\log_{10}(\text{Wheal Area}) = 0.95 + 0.0003 \times \text{Age}$ ;  $F(1, 759) = 0.18$ ,  $p = .67$ . Again, the slope of this line was not significantly different from zero.

Thus, although it would appear that there are some age-associated declines in the propensity for atopy (results in the previous section), there does not appear to be an age-associated effect on the general mechanistic aspects of atopy among atopy-prone families.

#### Age and Parameters Associated With Ragweed Sensitivity

Because of its ubiquitous distribution in this geographic locale, we used ragweed allergen as a model for exploring underlying atopic mechanisms. Among those with evidence for allergen sensitivity in this study population,  $\approx 50\%$  (552/1099) demonstrated evidence for sensitivity to short ragweed (*A. artemisiifolia*) by SPT to ragweed extract. For those with SPT(+) results for ragweed extract, there was a slight increase in average wheal area with age:  $\log_{10}(\text{Amb a wheal area}) = 1.457 + 0.0029 \times \text{Age}$ ;  $F(1, 550) = 6.38$ ,  $p = .012$ .

Table 2. Age and Predisposition to Atopy

|                       | Age Group (y) |       |       |       |      | All    |
|-----------------------|---------------|-------|-------|-------|------|--------|
| Characteristic        | 0-15          | 16-30 | 31-45 | 46-60 | >60  | Donors |
| Log (total IgE)       |               |       |       |       |      |        |
| Males                 |               |       |       |       |      |        |
| SPT(+) Mean           | 2.26          | 2.20  | 2.00  | 2.04  | 2.22 | 2.14   |
| SD                    | 0.67          | 0.52  | 0.54  | 0.55  | 0.67 | 0.59   |
| n                     | 110           | 82    | 116   | 58    | 25   | 391    |
| SPT(-) Mean           | 1.32          | 1.18  | 1.38  | 1.44  | 1.64 | 1.38   |
| SD                    | 0.66          | 1.00  | 0.46  | 0.55  | 0.51 | 0.59   |
| n                     | 50            | 10    | 60    | 26    | 16   | 162    |
| Females               |               |       |       |       |      |        |
| SPT(+) Mean           | 2.26          | 2.05  | 1.81  | 1.85  | 1.84 | 1.98   |
| SD                    | 0.57          | 0.55  | 0.68  | 0.66  | 0.58 | 0.63   |
| n                     | 88            | 85    | 128   | 47    | 22   | 370    |
| SPT(-) Mean           | 1.32          | 1.46  | 1.19  | 1.18  | 1.30 | 1.28   |
| SD                    | 0.58          | 0.77  | 0.58  | 0.46  | 0.52 | 0.59   |
| n                     | 47            | 24    | 59    | 21    | 25   | 176    |
| % SPT(+)              |               |       |       |       |      |        |
| Total                 | 67.1          | 83.1  | 67.2  | 69.1  | 53.4 | 69.2   |
| Males                 | 68.8          | 89.1  | 65.9  | 69.1  | 61.0 | 70.7   |
| Females               | 65.2          | 78.0  | 68.5  | 69.0  | 46.8 | 67.8   |
| Log (histamine wheal) |               |       |       |       |      |        |
| Males                 |               |       |       |       |      |        |
| Mean                  | 0.72          | 0.64  | 0.68  | 0.71  | 0.64 | 0.68   |
| SD                    | 0.31          | 0.20  | 0.23  | 0.26  | 0.19 | 0.26   |
| Females               |               |       |       |       |      |        |
| Mean                  | 0.68          | 0.64  | 0.68  | 0.67  | 0.63 | 0.67   |
| SD                    | 0.31          | 0.24  | 0.24  | 0.23  | 0.17 | 0.25   |

Notes: SPT(+) = skin prick test positive (1 or more allergens); SPT(-) = skin prick test negative (14 allergens possible); log (histamine wheal), area measured in square millimeters.

To study more specific details of the responses to ragweed allergen, we randomly selected five of the multi-generation families. These families comprised 165 total members ranging in age from 6 to 73 years (82 male and 83 female subjects). Of these people, 73 (44%) had SPT(+) results to ragweed extract. An additional 27 members who were SPT(-) to ragweed extract had measurable amounts of serum Amb a 1-specific IgE. Among the 73 people from these families with SPT(+) results to ragweed extract,  $\log_{10}(\text{Amb a wheal area}) = 1.579 + 0.0047 \times \text{Age}$ ;  $F(1, 72) = 3.03$ ,  $p = .086$ . Although not statistically significant, the regression parameters are in reasonable agreement with the results for all ragweed SPT(+) donors in the entire study population.

We used purified Amb a 1, the major allergen from short ragweed, for the specific analysis of atopic mechanisms. For the 73 people with SPT(+) results to ragweed extract, there was a strong positive correlation between concentrations of serum Amb a 1-specific IgE and wheal area for SPT ragweed extract results:  $r = .68$ ,  $p \ll .0001$ . There was, however, no significant change with age for Amb a 1-specific IgE:  $\log_{10}(\text{Amb a 1-IgE}) = 0.189 - 0.0039 \times \text{Age}$ ;  $F(1, 72) = 0.35$ ,  $p = .56$ . (Although ragweed is a seasonal aeroallergen, the exposure to which can influence serum specific IgE values, there were too few people in this study subset to be able to adequately account for this variable.)

Using purified allergens, such as Amb a 1, we are able to determine binding affinity parameters in heterogeneous, polyclonal systems by using an "affinity distribution"

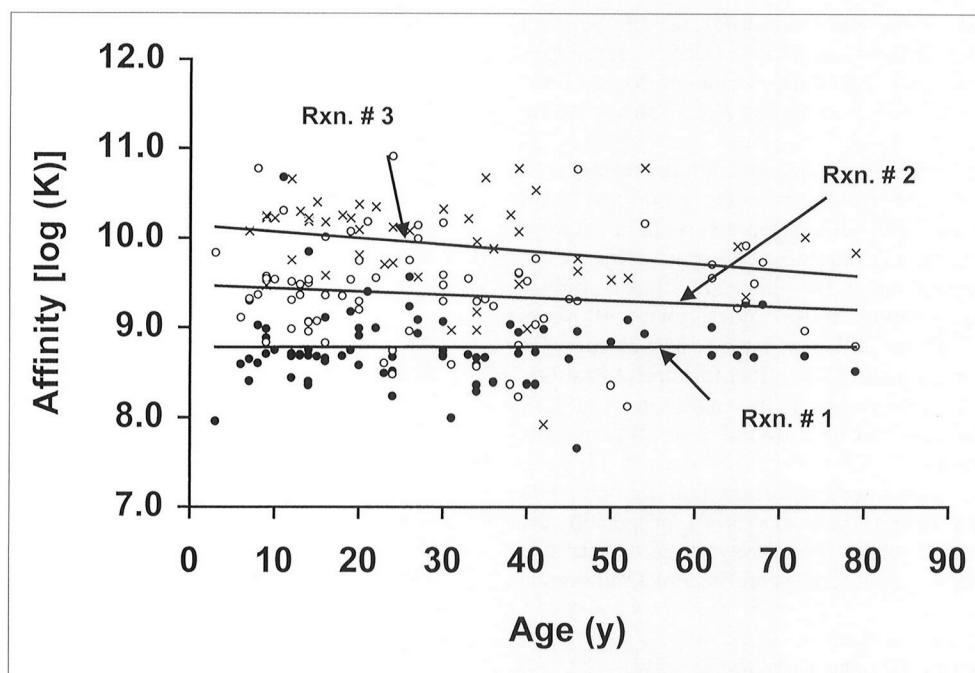


Figure 2. Allergen-specific IgE binding affinity vs donor age: the  $\log_{10}$  of the most probable binding affinity constants [ $K$ ; units = L/M] vs age for the specific reactions between the major ragweed allergen Amb a 1 and serum Amb a 1-specific IgE (additional details are given by Pierson and colleagues) (8). Results for linear regressions of  $\log [K]$  vs age are given in the Results section. Reaction 1 (Rxn. #1, ●); had the lowest affinity values, reaction 2 (Rxn. #2, ○) had intermediate affinity values, and reaction 3 (Rxn. #3, ×) had the highest affinity values for all donors tested.

method (8,9,11). This method determines the number of reactions that occurred in complex systems ( $n$ ), the mean binding affinity (reaction association) constant ( $K$ ), and the relative proportionate amount of each reaction constituent ( $p$ ). For the 73 people tested, all demonstrated at least two reactions between IgEs and (presumed) epitopes on the Amb a 1 allergen, and 49 (67%) demonstrated three specific reactions.

Perhaps the most telling indicator of the robustness of this atopic reaction mechanism is that there was no change with age in the binding affinity constants. Figure 2 shows the  $\log_{10}(K)$  values against donor age for the three detectable reactions in this study group. For each of the reactions in this figure (labeled Rxn. #1, Rxn. #2, and Rxn. #3), the slopes of the lines derived from simple linear regression do not vary significantly from zero.

Rxn. #1 (low affinity):

$$\log_{10}(K1) = 8.777 + 0.00014 \times \text{Age};$$

$$F(1, 72) = 0.003, \quad p = .96.$$

Rxn. #2 (intermediate affinity):

$$\log_{10}(K2) = 9.475 - 0.00345 \times \text{Age};$$

$$F(1, 72) = 0.900, \quad p = .34.$$

Rxn. #3 (high affinity):

$$\log_{10}(K3) = 10.147 - 0.00719 \times \text{Age};$$

$$F(1, 48) = 2.680, \quad p = .11.$$

## DISCUSSION

From a clinical perspective, supported by epidemiological investigations (1–6), there would appear to be a decline with age in both the incidence and severity of atopic diseases, particularly among the elderly population who are 60 years and older. Atopic incidence declines (based on standard assessments such as the SPT), symptoms severity declines, and there would appear to be a general humoral alteration of the propensity for atopy reflected by age-associated declines in serum total IgE values. These changes parallel the well-established declines that occur in overall immune status and functional responses observed in elderly humans and other mammals (13). However, atopic disorders are complex diseases that involve interactions among several physiological systems, including the immune system. It is not well known in which of these several physiological compartments the primary alterations may reside, or if the observed changes reflect a general phenomenon of senescent physiological decline.

In agreement with a community-based population study (1–4) and a population study in an industrial setting (5,6), the parameters that indicate a propensity for serum IgE or presence of SPT(+) atopy show comparable age-associated effects as those seen in the epidemiological investigations. There is an apparent age-associated decline in serum total IgE values (Figure 1A; Table 2). The mean  $\log_{10}$  values for each age group are comparable with those reported by others (Table 1 in ref. 2). In addition, the relative rates of

allergen sensitivity (SPT results) are comparable (Figure 1B; Table 2), with a slight increase in the years 16–30 and a marked decrease among those older than 60 years. However, in contrast, the underlying specific mechanistic parameters associated with atopy (allergen-specific IgE levels and binding affinities) do not change significantly with age.

As part of our ongoing studies related to the genetics of asthma and other atopic diseases (19), we have attempted to more clearly define the basic mechanistic features of the atopic humoral immune response. Our focus has been on developing ultrasensitive, chemiluminescence-based detection methods (20) to analyze the extremely low concentrations (nanomolar or less) of allergen-specific immunoglobulins in human serum, and devising methods to analyze the complex binding reactions between allergens and allergen-specific immunoglobulins (8).

These analyses have demonstrated that the clinically oriented phenotypes in both genetic and epidemiological investigations may not be entirely reliable. For example, the standard SPT for detecting sensitivity to allergens may miss significant numbers of people with underlying atopic disorders as a result of "false negative" results (9). Additionally, our most recent evidence shows that the difference between atopic and nonatopic humoral responses may not be as great as that inferred from studies that rely upon clinical evidence for disease (21).

We have also studied age-associated declines in general immune functional responses. For example, we have shown that the frequency of CD4<sup>+</sup> T cells capable of producing IL-2 significantly declines with age in elderly humans (14). In agreement with others' assessments, this decline was centered in a so-called memory compartment (CD45R0<sup>+</sup>) of T cells. The relative proportions of "naïve" (CD45RA<sup>+</sup>) and memory (CD45R0<sup>+</sup>) CD4 and CD8 T cells change with age, with most changes occurring during the first two to three decades of life (22). Interestingly, however, the relative proportions of these CD4–CD8 subsets remains relatively constant during the later generations of life, at least based on assessments that use peripheral blood lymphocytes, suggesting that age-associated changes occur as a result of physiological changes in the cohorts of cells themselves, rather than being influenced by external or environmental elements.

Indeed, it is this feature of immune functional decline that is at the focus of much current research in immunogerontology; fundamental alterations occur in the physiology of immune system cells, primarily the CD3<sup>+</sup> T-cell component (both CD4<sup>+</sup> and CD8<sup>+</sup> cells). One hypothesis proposes a "functional mosaic model" (23,24). According to this model, some, but not all, T cells become functionally incompetent with age, whereas other cells remain as functionally robust in the elderly population as comparable cells from the young. An alternative model, although not mutually exclusive, proposes that there is a dynamic restructuring with age in the T-cell complement that involves alterations in the ways in which T cells are able to regulate and coordinate immune responses (25). At the center of these hypotheses are the long-term consequences of T-cell status and function, although they are difficult to

investigate as the life spans of lymphocytes have proven difficult to define and measure (26).

In any event, a consensus opinion holds that the fundamental alterations that occur with age in the immune systems of mammals are due to changes in either T lymphocytes or functions that are mediated by T cells (13). These changes are also reflected in the T-cell-mediated humoral responses of B lymphocytes. Although age-associated alterations of B cells' functions are not as clear cut as those of the T-cell compartment, the general consensus holds that specific changes occur upon exposure to "new" antigen challenges in aged mammals, including production of lower affinity, less-specific immunoglobulins (15). These changes, all T-cell dependent, result in significant changes with age in antibody selection repertoire, diminished capacity for affinity maturation, and less efficient antibody function (15,27). However, what of long-term humoral responses, or the continued humoral responses to common environmental challenges such as allergens?

Aside from its reputed protective functions against parasitic infestations, the "reason" for the evolution of and the specific immune function of IgE is not well known (28). However, it is known that IgE regulation involves both T-cell-dependent (e.g., IL-4, CD40–CD40L) and T-cell-independent (e.g., Fc $\epsilon$ R/CD23) components. The initiating events leading to T-cell-dependent production of IgE, as well as other immunoglobulins, occurs primarily in the germinal centers of secondary lymph nodes (29) and derives from the direct, physical interactions between T and B cells. One consequence of these interactions is the maturation of high affinity antibody-producing memory B cells.

A fundamental feature of these memory cells is membrane-bound, high affinity, and antigen-specific immunoglobulin (mIg) that is the principal component of the B-cell receptor (BCR) (30). Upon subsequent exposure to the antigen that induced the production of the mIg, the B cell becomes activated, leading to the production and secretion of soluble immunoglobulin (sIg). The higher the affinity of the mIg, the more sIg that will be produced. A higher-affinity mIg is mechanistically tied to a slower dissociation rate between antigen and mIg and a longer "residence time" for BCR ligation and B-cell activation (31).

However, in the absence of antigen exposure, or possibly the loss of or functional inadequacy of a secondary signal provided by T cells, these memory B cells may die by means of apoptosis. Production of a new "generation" of memory cells is possible, though, upon reintroduction of antigen concomitant with the requisite T-cell help. In this scenario, it can be envisioned how fundamental changes could occur in the humoral repertoires of aged humans after exposures to "periodic" or "infrequently encountered" antigens based on the known alterations in T-cell help. (Such a scenario appears to be the case with the known therapeutic inefficiency of inoculation regimes in elderly humans.)

It is now becoming evident, however, that the majority of immunoglobulins found in the serum are not the result of this memory B-cell compartment. (The immunoglobulins produced by the memory compartment are primarily involved with antigen site-specific responses, such as in the

gut, skin, and respiratory system.) Rather, most serum immunoglobulins derive from the production by relatively long-lived, antigen-specific plasma cells found in the bone marrow, or the mucosa in the cases of the IgA subclasses (32). This "long-term compartment" derives from maturation of cells in the memory B-cell compartment and the subsequent relocation to the bone marrow or mucosa.

It has been observed both in aging humans and rodent models that antigen-specific humoral responses from this long-term compartment, derived from antigen exposure in early life, do not fundamentally change with age (15). Additionally, recent experimental evidence in a rodent model has demonstrated a distinct compartment of these antibody-forming cells (AFCs) in the bone marrow that is functionally distinct from the B-cell memory compartment (33). The key distinction between these compartments was the lack of "markers" for somatic hypermutation (SH) associated with affinity maturation among the bone marrow AFCs. This indicates that these cells have attained their highest functional status, and no longer require mediation of T-cell help required for SH and other alterations during immunoglobulin repertoire selection and development (34).

A key feature that we have observed for the IgE-mediated atopic humoral response is that the allergen-specific IgE produced is of extremely high binding affinity (8,9,11). Most of these observations were made from adults (typically 18–40 years of age) with established sensitivities to aeroallergens such as ragweed or house dust mites. The high-affinity IgE is related to a strong, positive SPT reaction, which appears to be due to the strength of binding between allergen and membrane-bound IgE involved with histamine release (10). We have also observed a subset of clinically atopic humans who had detectable amounts of serum allergen-specific IgE but who were SPT(–) (9). The IgE produced among these subjects was characterized by lower binding affinity for allergen than that produced by SPT(+) subjects. It is worth noting that the majority of these subjects were children younger than 15 years of age, suggesting, possibly, that their atopic humoral responses had not yet "matured" to the level observed in the majority of adult atopic cases.

In the present investigation we extended these analyses to a large population consisting of atopy-prone families. In agreement with epidemiological investigations, there was a "downward trend" with age in atopic propensity, which was reflected by diminishing levels of serum total IgE, and atopic incidence, which was reflected by a lower percentage of SPT(+) subjects in the oldest age category (60 years and older; see Figure 1 and Table 2). However, there were no apparent age-related declines in the parameters associated with the specific mechanisms of atopy.

Among general atopic mechanisms, the average number of SPT(+) results and the average wheal areas for SPT(+) reactions did not change with age. These results suggest that the ability to respond to and the degree of response to allergens do not change as a function of age, at least among people in atopy-prone families, who have similar lifestyle patterns and probabilities of exposure to common allergens.

More importantly, however, the specific mechanisms associated with atopy did not significantly change with age.

Serum allergen-specific immunoglobulins (Amb a 1-IgE) did not change and the Amb a 1-IgE binding affinity for allergen did not vary with age (Figure 2). These results suggest that, in atopy-prone families, the underlying mechanisms of the atopic humoral response are particularly robust.

In addition, on the basis of what has been observed regarding a long-term compartment of antibody-forming plasma cells (32,33), these results suggest that the high-affinity IgE-producing cells among atopic humans reside in a T-cell-independent compartment. We can provide no direct evidence for a (possibly) bone marrow located compartment of these cells. Yet, this would seem to be a plausible explanation. In contrast, among the human population at large, the diminished rates or propensities for atopy with age could result from alterations in the generation of a B-cell memory compartment, resident in such anatomic locales as the gut, skin, or respiratory system. These compartments would be compromised by diminished T-cell activity with age, and they could account for the diminished atopic phenomena observed in the at-large population.

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