

Evidence for Two Independent Distributions of Serum Immunoglobulin E in Atopic Families: Cognate and Non-Cognate IgE

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ABSTRACT: Genetic studies of IgE-mediated atopic disease have produced conflicting results, due largely to variable phenotype definitions. Total IgE concentrations and 14 allergen skin prick tests (SPT) were determined in 1099 members of families with history of atopy. Log_{10} [Total IgE] values were normally distributed in both atopic (SPT [+]) and non-atopic (SPT [–]) groups. The mean Log_{10} [Total IgE] value was higher in the atopic group, although the standard deviations of the distributions were the same. The mean Log_{10} [Total IgE] value of the non-atopic distribution was subtracted from the individual Log_{10} [Total IgE] values of the atopic group giving an allergen-specific fraction. There was a strong positive correlation between the specific IgE fraction and the number (#) SPT [+] results, defined as Cognate IgE.

ABBREVIATIONS

IgE immunoglobulin E

Among the atopics, subtracting the Cognate IgE value from total IgE yielded Non-Cognate IgE. The Cognate and Non-Cognate IgE distributions were statistically uncorrelated. Evidence is presented for two serum IgE fractions that are statistically and physiologically independent of one another in atopic families; a Cognate IgE fraction associated with atopic sensitization and a Non-Cognate IgE fraction unrelated to atopic disease. Elevated serum IgE is a consequence, not a predisposing cause, of allergen sensitization. *Human Immunology* 65, 20–30 (2004). © American Society for Histocompatibility and Immunogenetics, 2004. Published by Elsevier Inc.

KEYWORDS: allergy; atopy; IgE

SPT skin prick test (percutaneous)

INTRODUCTION

A frequently used model for studies of the genetics of disease susceptibility in humans is atopy, defined as an aberrant immune response to common environmental antigens (allergens) involving the production of allergen-specific IgE. Quantitative variables associated with atopy, such as allergen skin test reactions or serum total IgE, offer an unambiguous way in which to define the phenotype linked to the pathophysiology of disease. IgE production is subject to strict genetic regulation [1], and it has been assumed that resolving the regulatory mechanisms involved will lead to an understanding of the inheritance of atopic disease.

It is known that total IgE values differ considerably between those with and without established sensitivity to allergens [2]. Those with allergen sensitivities have, on average, higher total IgE values than those who do not. However, there is a great deal of overlap of IgE values between those with known sensitivity to allergens and those without any evidence of sensitization. Thus the question arises of whether an inherited predisposition to elevated IgE production also predisposes to atopic sensitization to allergens, or is enhanced IgE production among atopic people merely a consequence of the predisposition to allergen sensitization? This unresolved issue has complicated the search for a well-defined atopic genotype and the exploration of genetic markers, such as HLA, associated with the putative genotype.

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Among those with allergen sensitivities, at least some fraction of the total serum IgE complement is allergen-specific IgE that will induce the characteristic wheal and flare reaction after introduction of small quantities of allergen during the percutaneous skin prick test (SPT). The interaction between receptor ($F_{c\epsilon}RI$) bound specific IgE and the allergen that induced its production will initiate a signaling cascade in basophils or mast cells leading to the release of histamine and other mediators [3].

It would appear that there are two fractions of IgE present in serum: one fraction is associated with inducing atopic responses, such as the SPT [+] result; and another fraction is ostensibly unrelated to atopic disease. We determined the statistical distributions of these IgE fractions using serum samples and SPT results from members of families with positive atopic histories. Based upon established theorems for random variables, we demonstrate that these distributions are independent of one another in a probabilistic sense. And, from the results associated with the SPT reaction, we argue that these fractions are physiologically independent: one is a Cognate IgE fraction associated with the SPT [+] results, while a second is a Non-Cognate IgE fraction unrelated to atopic sensitization. Consequently, the use of serum total IgE values alone introduces an error in defining the genotype derived from this “uncorrected” phenotype.

This also focuses attention upon the general question of separating the production of normal, basal levels of any biologically essential compound from those values associated with a pathophysiologic state. It is important to clearly understand the production of these basal levels when attempting to understand the genetic predisposition for a disease, such as atopy, associated with abnormal production of these compounds.

MATERIALS AND METHODS

Study Population

Families that included at least one child with a physician's diagnosis of asthma were recruited as previously described [4]. Although selected for asthma, greater than two of three of this population had evidence of allergen sensitivity by standard SPT. 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 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 fami-

lies that included both parents, the asthmatic child and their siblings. These families were recruited through media advertisements, also as part of studies for the genetics of asthma.

Each was tested for sensitivity to a battery of 14 allergens common to the Upper Midwest section of the United States by standardized percutaneous skin prick tests (see below) and serum samples were obtained for total IgE determinations.

Allergy Tests

Serum total IgE tests. Whole blood was collected by sterile venipuncture, serum was separated, and stored frozen (-80°C) until assayed. Serum total IgE tests were done using the ACCESS Immunoassay System developed by Sanofi Diagnostics Pasteur, Inc. (Division of Beckman Coulter, Chaska, MN, USA) [5]. Determinations were made according to manufacturer's specifications. Results were expressed as IU/ml ($1\text{ IU} = 2.42\text{ ng}$).

Skin prick tests. Skin prick testing was administered with Duo-Tip Test Applicators (Lincoln Diagnostics Inc., Decatur, IL, USA) using 14 standardized allergen extracts (Bayer Corporation, Elkhart, IN, USA). Positive histamine and negative saline controls were also administered. Wheal areas (in mm^2) were measured with a planimeter. A positive response was recorded for any wheal area $\geq 9\text{ mm}^2$ above the negative saline control. Tests included standardized extracts from ragweed, molds (3), trees (4), grasses (2), dust mites (2), and animal danders (2).

Statistical Models

Frequency distributions of serum Log_{10} [Total IgE] values were made for those with any SPT [+] result and those who were SPT [-] to 14 test allergens. After correcting for the fraction of total IgE that was not associated with SPT [+] results, comparison of this specific IgE fraction was made to the number of SPT [+] results by simple linear regression (SLR) of Log_{10} -transformed variables. This result gave a “cognate” IgE fraction, and the remainder of the serum total IgE was described as “non-cognate.” A test for the independence between the cognate and non-cognate IgE fractions was made by SLR and Chi-square analysis [6].

As described in the Appendix, a non-linear model was derived to characterize the probability distribution for the cognate IgE fraction. This model was evaluated using the Mathematica software program NonLinearRegress (Wolfram Research, Inc. Champaign, IL, USA), which utilizes a Marquardt minimization algorithm [7].

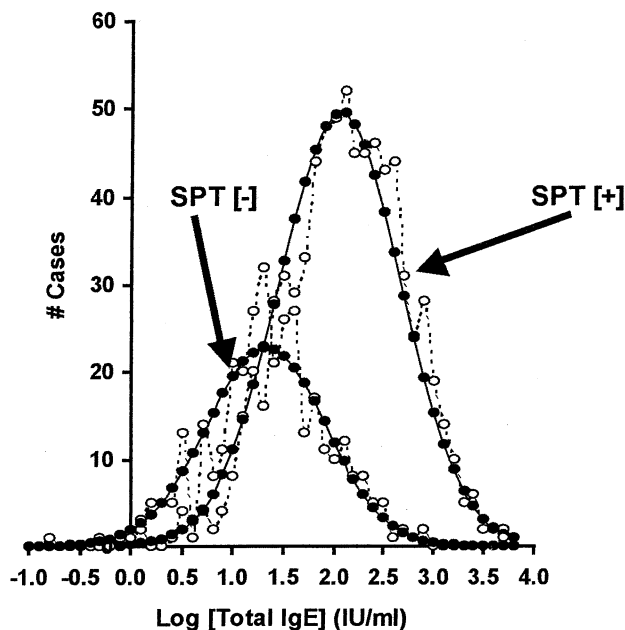


FIGURE 1 Serum total IgE distributions: SPT [+] vs. SPT [-]. The “# cases” are plotted against serum Log_{10} [Total IgE] for those with any SPT [+] result or those who were SPT [-] to a battery of 14 allergens. The dashed lines, either SPT [+] or SPT [-], are the observed number of cases. The solid lines are expected values assuming lognormal distributions. SPT [+] mean = 2.059 (SD = 0.613; $n = 761$); SPT [-] mean = 1.327 (SD = 0.592; $n = 338$). Abbreviations: SPT = skin prick test; SD = standard deviation.

RESULTS

IgE Distributions: SPT[+] Versus SPT[-]

We determined serum total IgE concentration values and the results of SPT reactions to 14 allergens in 1099 people from families with histories of atopic disorders. The study population ranged in age from 6 to 84 years old (553 males and 546 females). Because we were interested in IgE distributions only, we made no corrections for age, gender, or smoking history.

Figure 1 illustrates the distributions of total IgE values for those who were SPT[-] to all 14 allergens and those who had one or more SPT[+] reactions. On the Log_{10} -scale, the mean IgE value for those who were SPT[-] ($n = 338$) was 1.327 (≈ 21 IU/ml). By comparison, the mean IgE value for those who were SPT[+] ($n = 761$) was 2.059 (≈ 115 IU/ml). By t -test, these were significantly different ($p \ll 0.0001$). These values are comparable to those previously reported [2]. It will be noted that the relative “spread” of the two distributions are similar; the standard deviations of the log-normal distributions are virtually identical: SPT [-] standard deviation (SD) = 0.592; SPT [+] SD = 0.613.

On a Log_{10} -scale, both sets of values approximate log-normal distributions depicted by the smooth contin-

uous lines in Figure 1. By Chi-square analysis there was no significant difference between the theoretical distributions and the observed frequency distributions. For those who were SPT [+]: $X^2(50) = 28.1$; no significant difference at $p = 0.012$. Similarly, for those who were SPT [-]: $X^2(50) = 38.1$; no significant difference at $p \ll 0.001$.

As expected, the mean total IgE values for those SPT[+] were, on average, higher than those who were SPT [-]. However, as previously observed by others [2], there was considerable overlap in total IgE values between those who were SPT[+] and SPT[-].

Identifying the Cognate IgE Fraction in Atopics

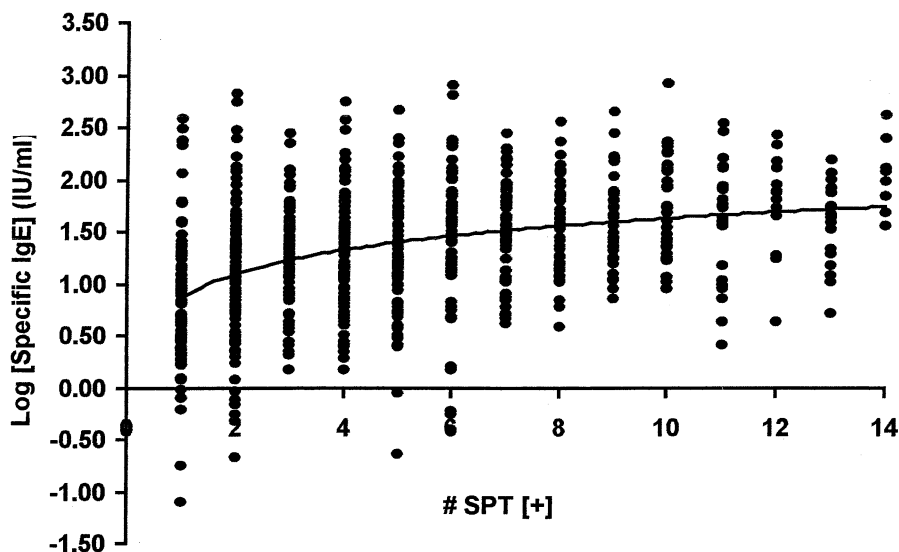
Experimentally, we had two independent determinations: serum Total IgE and evidence for specific IgE based upon the SPT to 14 allergens. Due to the unavailability of suitable standards, quantitative determinations of serum allergen-specific IgE are not reliable. However, among those with any SPT [+] result, there was a positive correlation between serum total IgE values and # SPT [+] results. By SLR: Log_{10} [Total IgE] = $1.68 + 0.072 \times \{\# \text{ SPT } [+]\}$; $F(1, 759) = 140.3$; $p \ll 0.001$. Thus, some fraction of the total IgE complement was due to sensitization to allergens and the production of allergen-specific IgE, with higher total IgE values among those with more SPT [+] results.

We postulated that the total IgE distribution for those with any SPT [+] result was a composite (convolution in the probabilistic sense) of two ostensibly independent distributions [8, 9]. One was a fraction related to the SPT [+] reactions (allergen-specific IgE) and one that was unrelated to atopic sensitization (non-specific IgE). We further proposed that the distribution of non-specific IgE values among those with any SPT [+] result was similar to the distribution of total IgE values for those who were SPT [-] (Figure 1).

To eliminate this non-specific fraction, we subtracted the mean Log_{10} [Total IgE] value of those who were SPT [-] (mean = 1.327) from the individual Log_{10} [Total IgE] values of those with any SPT [+] result. This left a fraction that was ostensibly specific IgE associated with the SPT [+] reaction(s). Figure 2 illustrates the Log_{10} [Specific IgE] values plotted against the #SPT [+] results.

By SLR, the best empirical fit of the data resulted from: Log_{10} [Specific IgE] = $0.269 + 0.765 \times \text{Log}_{10}$ [# SPT (+)]; $F(1, 759) = 154.3$; $p \ll 0.001$. This relation was used to determine the distribution of the cognate IgE fraction among atopic subjects that was correlated to their SPT [+] results.

FIGURE 2 Log [Specific IgE] versus number (#) SPT [+] results. For those with any SPT [+] result, a Log [Specific IgE] value was obtained by subtracting the mean value of 1.327 from their Log [Total IgE] values. As explained in Results section of this report, the solid line was from the best empirical fit of the data by SLR. Abbreviations: SPT = skin prick test; SD = standard deviation; SLR = simple linear regression.



Distributions of Cognate and Non-Cognate IgE in Atopic Patients

For each of the atopic patients in the study population (any SPT [+] result), the cognate IgE value was determined from the SLR results given above. Figure 3A illustrates the frequency distribution of Log_{10} [Cognate IgE] values for these atopic patients (dashed line). The solid line in Figure 3A depicts the expected distribution for cognate IgE values. This distribution was derived in the following manner.

From the frequency histogram of observed patients in Figure 3A, the proportionate value was determined by dividing the # cases for each Log_{10} [Cognate IgE] result by the total # cases ($n = 761$). This gave an empirical distribution for the probability of having some Log_{10} [Cognate IgE] value. A theoretical model of this distribution of probability values is derived in the Appendix based upon the empirical results for the probability of # SPT [+] results in the study population and the relationship between # SPT [+] and the specific IgE results. The best empirical model for the probability of having some value (Y) of Log_{10} [Cognate IgE] was given by:

$$P(Y) = A - BY^C \text{Exp}[-DY^C] \quad (0.27 \leq Y \leq 1.15), \quad (1)$$

where A , B , C , and D are constants with the following derived values: $A = 0.140 (\pm 0.0013)$; $B = 0.0021 (\pm 0.0034)$; $C = 0.733 (\pm 0.245)$; $D = -3.660 (\pm 1.622)$; total $R^2 = 0.96$.

Using the result from Equation 1, the total number of expected cases for each value of Log_{10} [Cognate IgE] was determined by multiplying the total number of cases ($n = 761$) by the probability value $P(Y)$. This result is depicted as the solid line in Figure 3A. By Chi-square

analysis, there was no difference between the expected and observed values: $\chi^2 (13) = 13.97$; $p = 0.005$.

For each atopic patient, the derived Log_{10} [Cognate IgE] value was subtracted from the Log_{10} [Total IgE] value. This gave the Log_{10} [Non-Cognate IgE] result for each atopic person. The frequency distribution of these values is illustrated in Figure 3B (dashed line). The mean value for Log_{10} [Non-Cognate IgE] = 1.325 (SD = 0.559; $n = 761$). As in Figure 1, this distribution was best approximated by a normal distribution. The expected distribution for Log_{10} [Non-Cognate IgE] values is given by the solid line in Figure 3B. By Chi-Square, there was no significant difference between the observed and expected values: $\chi^2 (50) = 39.3$; $p < 0.001$.

Independence of the Cognate and Non-Cognate IgE Fractions

The distribution of Log_{10} [Non-Cognate IgE] values among atopic patients (Figure 3B) was comparable to the distribution of Log_{10} [Total IgE] values among non-atopic subjects (Figure 1). By t -test, assuming equal variances, there was no difference in mean values for these distributions: $t(1097) = 0.074$; $p = 0.47$. Thus, the derived values among atopic patients were comparable to the measured values among non-atopic patients in this study population. The non-cognate fraction represents a basal serum IgE distribution ostensibly unrelated to allergen sensitization.

We compared the Log_{10} [Non-Cognate IgE] and Log_{10} [Cognate IgE] for each of the atopic patients. A scatter plot of this comparison is illustrated in Figure 4. By SLR, there was no apparent relationship between these two IgE fractions in the atopic patients: Log_{10} [Cognate IgE] = $0.735 - 0.0001 \times \text{Log}_{10}$ [Non-Cognate IgE]; $F(1, 759) = 3.8 \times 10^{-5}$; $p = 0.995$. The

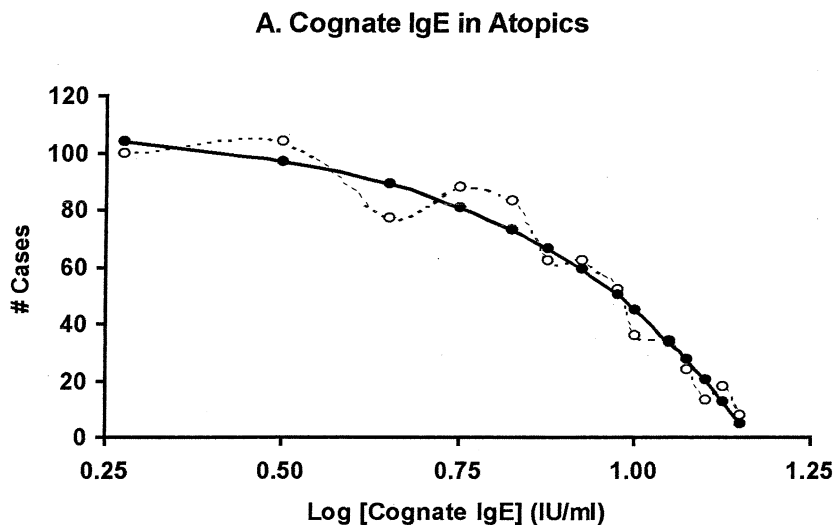
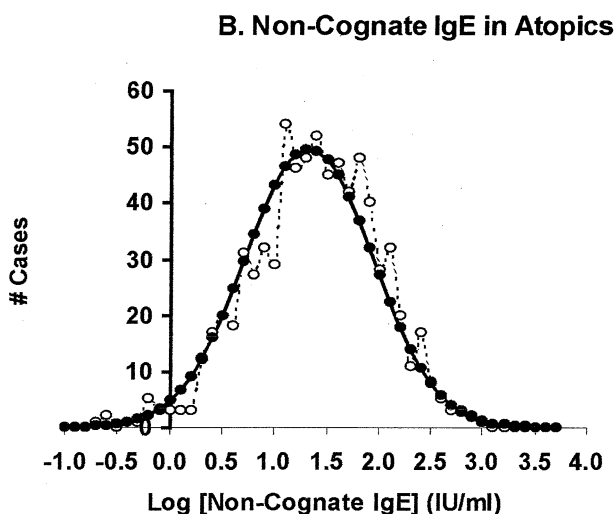


FIGURE 3 Distributions of cognate and non-cognate IgE for SPT [+] patients. (A) As explained in the Results section of this report, the allergen-specific cognate IgE distribution was derived from the relationship of specific IgE and number (#) SPT [+] results in Figure 2. The dashed line gives the observed cognate IgE values and the solid line gives the expected distribution of values derived from Equation 1 in the Results and Appendix. (B) Non-cognate IgE values were derived by subtracting the Log [Cognate IgE] value from the Log [Total IgE] value for those with any SPT [+] result. The dashed line indicates the observed number of cases. The solid line depicts the expected number of cases assuming a log-normal distribution with mean value = 1.325 (SD = 0.559; $n = 761$). Abbreviations: SPT = skin prick test; SD = standard deviation.



regression coefficient was not significantly different from zero, suggesting that the two distributions were independent.

As a further test for independence, we performed a Chi-square analysis [6]. We arbitrarily divided each of the Log_{10} scales into equally spaced intervals. This gave a contingency table with R rows and C columns ($R = 8$, $C = 5$). Entries into each "cell" represented the number of cases with a particular Cognate and Non-Cognate IgE value. The intervals were chosen so that there were no entries of zero in any of the $R \times C$ cells. By Chi-square analysis with $(R-1) \times (C-1)$ degrees of freedom there was no significant difference between the observed and expected values at $p = 0.001$ (X^2 [28] = 21.4).

For summary, the mean values, variances and standard deviations for the cognate and non-cognate values are given in Table 1. Among those with any SPT [+] result,

the sum of mean values for the Log_{10} cognate and non-cognate fractions was: $0.735 + 1.325 = 2.060$. Although these means were from derived values, they were virtually identical to the overall mean total IgE value for the atopic group of 2.059 (Figure 1 and Table 1). Also, the sum of the variances = 0.376 was equal to the variance for total IgE values of 0.376 among SPT [+] subjects (Table 1). Thus, using the derived values for cognate and non-cognate IgE values among the atopic patients, we were able to recover the observed distribution of measured values for serum total IgE in this group (Figure 1).

Study Population Demographics and IgE Distributions

Epidemiologic investigations have demonstrated that there can be alterations in the distributions of serum IgE

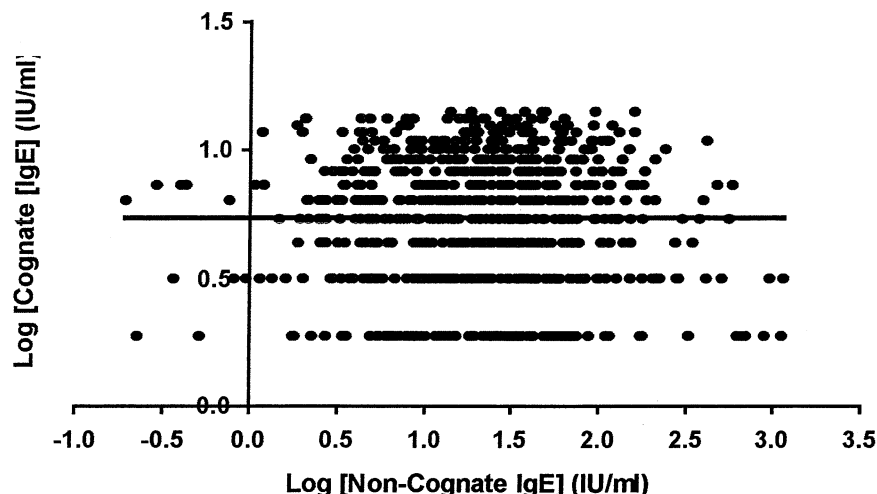


FIGURE 4 Cognate IgE versus non-cognate IgE. For those with any SPT [+] result, the Log [Cognate IgE] value was plotted against their Log [Non-Cognate IgE] value. The solid line is the result of SLR. Abbreviations: SPT = skin prick test; SD = standard deviation; SLR = simple linear regression.

values due to subject age, gender, smoking, or family history of atopy [10–12]. These reports were based upon large samples obtained randomly from the at-large population. To determine if there were similar effects in our study population of atopy-prone families, we also accounted for these demographic variables.

Age and gender effects. We have recently reported that, within this population of atopy-prone families, there are some slight alterations in the IgE distributions due to patient age [13]. Among those with any SPT [+] result, this was due to a lower mean # SPT [+] reactions for children 10 years of age and younger (mean = 3.70; $n = 86$) compared to those older than 10 years (mean = 5.29; $n = 676$). There were no gender effects for the # SPT [+] reactions.

The lower number of SPT [+] responses was not due to lowered production of IgE among the younger patients, as serum total IgE concentrations were relatively constant across all age groups and gender groups in atopy-prone families [13]. Rather, it was due to an apparent lowered IgE affinity for allergen that induces the wheal and flare reactions characteristic of the SPT response. We have previously reported that those with detectable amounts of serum allergen-specific IgE but

who are SPT [–] have lowered IgE binding affinity for allergen compared to those who are SPT [+] [14].

For those 10 years of age and younger ($n = 86$), the mean SPT [+] number of 3.70 translates to about 5.05 IU/ml of specific IgE. For those older than 10 years ($n = 676$), the mean SPT [+] number of 5.29 translates to 6.64 IU/ml of specific IgE. The age effect is negligible in this population of atopy-prone families. Further, as we have also demonstrated, once established, the IgE affinity, and hence the SPT [+] reaction, is particularly robust into the advanced years of life [15].

Smoking. We compared results for those older than 20 years of age. Smokers were those who had a history of 1 or more pack-years (pack-year = 1 package of cigarettes per day per year).

Log [Total IgE] values among those who were SPT [–] to all allergens were as follows: smokers, mean = 1.34, variance = 0.34, $n = 92$; non-smokers, mean = 1.27, variance = 0.32, $n = 227$. Comparison of the mean values by t -test, assuming equal variances, gave: $t(317) = 0.93$, $p = 0.18$. Smoking had no effect upon IgE production for the non-atopic patients.

Among those with any SPT [+] result, the statistics for # SPT [+] were as follows: smokers, mean = 5.94,

TABLE 1 Summary of IgE statistics

Status & measure	Mean	Variance	Standard deviation	Number
Atopic (any SPT [+])				
Log [Cognate IgE]	0.735	0.064	0.252	761
Log [Non-Cognate IgE]	1.325	0.312	0.559	761
Log [Total IgE]	2.059	0.376	0.613	761
Non-atopic (all SPT [–])				
Log [Total IgE]	1.327	0.350	0.592	338

Abbreviation: SPT = skin prick test.

variance = 15.54, $n = 183$; non-smokers, mean = 5.31, variance = 12.77, $n = 342$. There were no effects for gender or total number of pack-years of smoking. Comparison of the mean values by t -test, assuming unequal variances, gave: $t(342) = 1.80$; $p = 0.036$. Although statistically significant, the effect of smoking was negligible as the mean SPT [+] value for smokers translated to 7.26 IU/ml, whereas that for non-smokers translated to 6.66 IU/ml.

Family history of atopy. Because IgE production, either allergen-specific or non-specific, is an ostensibly inherited trait, we made comparisons by correlation analysis among kinships: parent-child and sib-sib.

Among all parent-child pairs, when both had any SPT [+] result (330 pairs), there were no significant correlations for either Log [Total IgE] values or # SPT [+] results. There was, however, a significant correlation for # SPT [+] results if both the parent and the child had asthma: $r = 0.19$; $F(1,127) = 4.70$, $p = 0.032$. It would appear that this weak concordance of parent-child values is due to some mechanism not directly associated with IgE production.

Among all sib-sib pairs, regardless of atopic status, there were significant correlations for the atopic parameters. For Log [Total IgE] values: $r = 0.20$; $F(1,583) = 22.95$, $p \ll 0.001$. For # SPT [+] results: $r = 0.27$, $F(1,583) = 45.52$, $p \ll 0.001$. A similar trend was found when both siblings were SPT [+]. For Log [Total IgE]: $r = 0.17$, $F(1,349) = 10.03$, $p = 0.002$. For # SPT [+]: $r = 0.28$, $F(1,349) = 29.43$, $p \ll 0.001$. However, as with the parent-child correlations, these results were significant only when both siblings were asthmatic: # SPT [+] results (both sibs asthmatic): $r = 0.41$, $F(1,153) = 31.32$, $p \ll 0.001$.

There were no apparent effects of age, gender, smoking or family history of atopy on non-cognate IgE production. For cognate IgE, there were only minimal effects for those 10 years of age and younger that was not due to the ability to produce IgE [13] and smoking. The clearest effect on cognate IgE values resulted from parent-child or sib-sib correlations, but only if both of the members of the kinship were also asthmatic.

DISCUSSION

Genetic studies of IgE production have produced widely conflicting results, with evidence for major recessive genes, codominance, polygenetic effects or genetic heterogeneity (see references in [16]). These disparities appear to be due largely to variable definitions of pertinent phenotypes. A statistical path analysis based upon total IgE values in randomly selected nuclear families suggested inheritance of a gene for allergen sensitivity that

was independent of 'basal' IgE production [17]. Yet, no accounting was made for characteristic allergen sensitivity test results.

Other studies, using statistical segregation analyses, have analyzed IgE values in humans with and without specific allergen sensitivity. Bivariate segregation analysis accounting for atopic symptoms suggested a common major gene with pleiotropic effects [18]. A regressive model using a semiquantitative allergy index as a covariate found evidence for a recessive gene for total IgE levels [19]. And another regressive model suggested evidence of a major recessive gene for high IgE values that acted independently of specific responses to allergen(s) [16]. Yet, these analyses did not account for the statistical characteristics of the underlying probability distributions for IgE that may or may not have been associated with specific allergen sensitivity.

Molecular studies of the inheritance of atopy have focused upon the genetic regulatory elements for IgE production. These studies have also relied mainly upon a phenotype derived from serum total IgE values. Several studies have implicated the "cytokine cluster" on human chromosome 5 [20,21]. These cytokines are involved with isotype class switch recombination (CSR), although none of the cytokines implicated are uniquely involved with IgE production. For example, interleukin-4 (IL-4) has been reported to be an obligate requirement for switching to IgE [22], yet IL-4 is also involved with switching to the IgG subclasses [23, 24].

Other studies have implicated polymorphisms in interleukin gene promoter regions [25], or mechanisms involved with suppression of IgE production via the BCL-6 repressor [26] or the negative regulator Id2 [27]. Yet, it is possible that these mechanisms could be influenced by strong environmental factors that "override" the regulatory controls without involving an inherited predisposition to enhanced IgE production. Or, the possibility exists that immunologic components are involved that increase the likelihood of IgE production although they may not be directly linked to IgE CSR. An example of this has been shown with certain identified polymorphisms of the CD14 endotoxin receptor that enhance the production of IgE, but interestingly only among those with established sensitivities to allergens [28, 29].

These latter reports point to a dilemma in searches for atopy-associated genes. Although it is known that IgE levels are, on average, elevated among those with established sensitivities to allergens compared to those without atopic sensitivity [2], it is not entirely clear that the IgE values reported are necessarily functionally associated with atopy.

A positive association between polymorphisms in the IL-13 gene (a part of the chromosome 5 "cytokine cluster") and serum total IgE levels was found in children,

but only among those who were SPT [−] to a battery of allergens [30]. Weaker or no associations were found for IgE values among those who had any SPT [+] result. In contrast, other investigators found stronger suggestive linkages to chromosome 14q markers, in an area putatively associated with IgE CSR, and a generalized sensitivity to allergens compared to serum total IgE or sensitivity to individual allergens [31].

Although it seems clear that IgE production is under strict genetic regulation due to the minute amounts produced relative to other immunoglobulin classes [1], it is not at all certain that revealing the regulators of IgE production will be causally tied to the atopic predisposition. Enhanced IgE production may either predispose to atopy, or elevated IgE may only be a consequence of sensitivity to allergens.

It is known that IgE has a protective function against parasites, that areas of the world with endemic parasitic infestations are associated with elevated total IgE values but, paradoxically, there is no corresponding increase in the incidence of atopic disorders compared to the rates observed in industrialized nations [32]. Perhaps the dilemma is partly a matter of scale and functionality. From our results in Figure 1, and results reported by others [2], a likely average basal serum total IgE value among those with no evidence of atopic sensitivity to allergens is about 20 IU/ml ($\approx$ 50 ng/ml). From our results in Figure 3A and Table 1, a likely average value for allergen-specific IgE for those with any SPT [+] result is about 5.5 IU/ml ($\approx$ 12 to 13 ng/ml). Compared to the estimated basal IgE value (non-cognate), the allergen-specific IgE (cognate) is close to 25% of basal levels, which would be clearly discernible in a scatter plot comparing those with and without sensitivity to allergen (Figure 1).

In contrast, the estimated basal level of serum IgG is about 10 mg/ml. This represents all IgG fractions that may or may not have functional specificity for antigens. If an antigen-specific IgG is produced, say staphylococcus-specific IgG, it would have to be produced in amounts approaching 2.5 mg/ml to reflect 25% of basal IgG values. Most antigen-specific IgGs are probably in the nanogram/milliliter to microgram/milliliter range, and would not be discernible against the overwhelming basal ('background') IgG distribution.

For thorough genetic investigations, it appears incumbent that IgE fractions should be clearly identified or separated, either biochemically or by estimating the statistical characteristics of their underlying probability distributions. A biochemical separation of all allergen-specific IgEs would be a daunting task as it could never be known with certainty to which allergens any individual or group of individuals would be sensitized. Further, quantitative measures of serum allergen-specific are un-

reliable due to the unavailability of suitable standards. However, from a statistical and probabilistic view, the methods are readily implemented based upon a few simple assumptions.

We have previously reported that there is a significant difference in the total binding capacity of specific IgE when comparing those with evidence of serum allergen-specific IgE but whose SPT reactions differ. On average, those with SPT [+] results have significantly higher binding affinity IgE than those who were SPT [−] [14]. Additionally, the binding affinity is particularly robust among those with SPT [+] results, as the value of this parameter does not change with age even into the later years of life (> 80 years) [15]. Thus, the SPT [+] result is a reliable indicator for the presence of allergen-specific IgE and is physiologically associated with a clinical marker of atopy. While there may be those with detectable amounts of allergen-specific IgE but who are SPT [−], this fraction cannot be reliably associated to the atopic diathesis and may be 'background noise.'

From a statistical-probabilistic perspective, it would be ideal to test all of the people in the study population for all possible allergens. This is obviously not ethical or prudent. But, based upon reliable clinical information for the geographic locale, it can be determined with reasonable certainty what allergens are most likely to be involved with sensitization for the greatest number of atopic cases [4]. Thus, the results given here are a best approximation for this particular study population relative to the most probable allergen exposures in this region.

Based upon these assumptions, the critical question becomes are the two distributions of IgE independent of one another? From our results in the scatter plot illustrated in Figure 4 and the statistical analyses, both by regression (correlation) and Chi-square analysis, there is no evidence to indicate that the Cognate and Non-Cognate IgE fractions are associated with one another. Thus, in both a physiological and a statistical-probabilistic sense, the two fractions are independent.

As illustrated in Table 1 and Figures 1 and 3B, the Non-Cognate IgE distributions are equivalent for those with and without atopic sensitization (SPT [+] vs. SPT [−]). If there is a gene(s) for basal IgE production, it is shared by all in the study population and is unrelated to the propensity for atopic sensitivity. However, as illustrated in Figure 1, serum Total IgE values are higher among those with any SPT [+] result that must be due to the production of allergen-specific IgE in addition to basal IgE. Thus, elevated IgE production among atopic individuals is largely due to the production of allergen-specific IgE alone.

Finally, epidemiologists have reported that IgE distributions can be influenced by the demographic factors

of age, gender, smoking, and family history of atopy [10–12]. These and similar studies were based upon observations of patients selected randomly from the at-large population, with roughly 25% to 40% of these populations demonstrating evidence of atopic sensitivity to allergen(s). While accounting for these demographic features as cofactors for IgE production indicates statistically significant results, in most cases the absolute magnitude of IgE concentration differences are not too great. Further, no accounting was attempted for the possibility of physiologically distinct IgE distributions (cognate/allergen-specific IgE versus non-cognate/non-specific IgE).

Our study population consisted of families with known history of atopic disorders (asthma and allergy) of which two of three had evidence of sensitization to one or more allergens. We found no evidence for the demographic factors influencing basal, non-cognate IgE production. There was only a minimal effect of smoking upon cognate IgE production, reflected by a slightly higher mean number of SPT [+] reactions among smokers compared to non-smokers, and evidence for cognate IgE production (any SPT [+] response) was slightly lower among children aged 10 years or younger. However, age was not a factor for the ability to produce IgE [13]. Rather, it was more likely that many of these children had not yet developed specific IgE with sufficiently high binding affinity to elicit an SPT [+] response to some allergens. IgE binding affinity is a variable that is independent of IgE concentration [14].

With regard to family history, we found weak, although statistically significant, correlations for atopic parameters (Log [Total IgE] or #SPT [+] responses) for parent-child and sib-sib pairings. However, these were most clearly due to the influence of the presence of asthma, as the strongest correlations were found only among those kinship pairings with both having asthma. This suggests that IgE production is influenced by genetic or environmental factors that cannot be accounted for by simple evaluation of IgE distributions alone, as for example the influence of the endotoxin receptor (CD14) upon allergen-specific IgE production [28, 29].

In summary, we have statistically and physiologically identified two independent distributions of serum IgE among members of atopy-prone families. One distribution represents a basal serum IgE that is unrelated to atopic sensitization to allergens (Non-Cognate IgE) and a second that is positively associated with sensitivity to allergens (Cognate IgE). The gene or genes regulating basal Non-Cognate IgE are shared by all. Independent mechanisms probably determine the likelihood of becoming sensitized to allergens, as there is no evidence from these results to suggest that enhanced production of IgE is associated with the propensity for sensitization.

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APPENDIX

Derivation of Equation 1

The equation displayed in the Results section of this study is the best empirical fit of the observed probability distribution for Cognate IgE values related to the SPT [+] results in this study population. This equation is:

$$P(Y) = A - BY^C \text{Exp}[-DY^C] \quad (0.27 \leq Y \leq 1.15), \quad (1)$$

where A, B, C and D are constants determined by a non-linear regression algorithm. This equation was derived from theorems of probability for linear combinations of two random variables [8, 9].

The distribution of Cognate IgE values was derived from the distribution of SPT [+] results. For this study population, it was observed that the probability of having some number (X) of SPT [+] results was:

$$P(X) = \alpha e^{-\beta X} \quad (1 \leq X \leq 14), \quad (2)$$

where α and β are constants.

It was also observed that the value (Y) of Cognate IgE was related to the # SPT [+] results by an equation of the form:

$$\text{Ln}[Y] = \gamma + \delta \text{Ln}[X], \quad (3)$$

where Ln is the natural logarithm (used here for mathematical convenience) and γ and δ are derived constants. A solution for the variable X in Equation 3 is:

$$X = (e^{-\gamma/\delta})Y^{1/\delta}, \quad (4)$$

which is equivalent to:

$$X = \epsilon Y^C. \quad (5)$$

It is known that the total probabilities for two inter-related random variables must be equal:

$$P(Y)|dY| = P(X)|dX|, \quad (6)$$

which upon rearrangement gives:

$$P(Y) = P(X)/\{|dY|/|dX|\}. \quad (7)$$

(Absolute values are used as negative probability values are undefined.)

From Equations 2 and 5:

$$P(X) = \alpha \text{Exp}[-\beta \epsilon Y^C], \quad (8)$$

which is equivalent to:

$$P(X) = \alpha \text{Exp}[-DY^C]. \quad (9)$$

where $D = \beta\epsilon$.

From Equation 3:

$$dY/dX = \delta/X = \delta/\epsilon Y^C. \quad (10)$$

Substituting the results of Equations 9 and 10 into Equation 7 gives:

$$P(Y) = BY^C \text{Exp}[-DY^C], \quad (11)$$

where $B = \alpha\epsilon/\delta$.

With the exception of an additional arbitrary constant, A, Equation 11 is equivalent to Equation 1 above and the displayed equation depicted in the Results section. The constant A was added as a stabilization factor for fitting the data using a non-linear algorithm, as these algorithms yield results that oscillate at the 'end points' of the data.

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