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RANDOM ALLERGEN-SPECIFIC IgE EXPRESSION IN ATOPIC FAMILIES: EVIDENCE FOR INHERITED ‘STOCHASTIC BIAS’ IN ADVERSE IMMUNE RESPONSE DEVELOPMENT TO NON-INFECTIOUS ANTIGENS

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Abstract

The complex inherited human atopic diseases are associated with adverse IgE-mediated immune responses, notably allergen-specific IgE that presumably involves the input from one or more genes. However, gene searches have met with limited success, possibly because a causally direct gene input-trait outcome assumption is not valid for these immune responses. To test this assumption, we determined the probability distributions of quantitative IgE responses associated with atopy, and used these to determine the statistical interdependence among first-degree relatives (parent-child and sibling-sibling) from families with history of atopic asthma (total available N = 1099). Each person was screened for asthma history, pulmonary responses by spirometry and atopic immune responses using serum total IgE and skin prick tests (SPT) to 14 allergens. Heritability estimates were made by variance components analysis for quantitative IgE traits. The serum total IgE distribution comprised statistically independent sub-sets when individuals were categorized as either SPT [–] or SPT [+], reflecting contributions from non-pathology associated basal IgE and pathology-associated allergen-specific IgE. However, heritability estimates were significant only for basal IgE, while total allergen-specific IgE production was a random variable independent of inheritance. Genes for specific IgE-mediated responses are not obligately inherited. Rather, gene products that modulate underlying stimulus-response coupling interactions and alter the probabilities influencing adverse immune responses are inherited, but an individual’s specific pathologic outcome is a random variable. These results support a model of ‘stochastic bias’ that ‘skews’ an immune response to non-infectious antigens among people with an inherited predisposition for atopy.

Keywords

Allergen; Atopy; IgE; Complex Disease; SPT

INTRODUCTION

Atopic disorders, such as allergic rhinitis and bronchial asthma, are common complex human diseases. Epidemiological studies have shown that adverse IgE-mediated immune responses are significant risk factors for these conditions (Pearce et al, 1999). And, due to their tendency

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to cluster within families, they have a significant inherited component (Blumenthal & Björkstén, 1999). Results of numerous genome-wide screens and case-control studies have shown multiple chromosomal regions with suggestive associations to these conditions (Hoffjan et al, 2003). But, there is no clear consensus with regard to either the genes involved (Altmüller et al, 2001) or the mode of inheritance for these conditions (Blumenthal & Björkstén, 1999).

Atopy is an adverse humoral immune response among some people to otherwise benign, non-infectious environmental antigens (allergens) involving the production of allergen-specific IgE. An assumption often made is that specific genes responsible for adverse IgE responses are inherited and passed along within families. However, aside from the possibility of multiple genetic contributions and random environmental modifications of their effects, regulation of IgE production involves numerous metabolic pathways (Geha et al, 2003). Different pathways imply different genes responsible for the outcomes.

Thus, a paradox arises of clinically well-defined diseases known to be inherited, but with no clearly discernible pattern for the genes responsible for their expressions. Two different models could account for these disease processes.

One model is that of Obligate Inheritance, in which it is postulated that there are specific inherited errors in the recognition and/or response to specific allergens, or possibly to allergens in general. In this case, there are assumed genetic errors in the regulation of IgE production leading to adverse responses to allergens. However, the particular regulatory pathways involved need not be the same in all families. This would account for common outcomes observed in population-based epidemiological studies, but a lack of consensus with regard to the “gene patterns” contributing to these disorders.

An alternate model is one of Stochastic Bias. In this case, it is assumed that the regulatory pathways are the same for everyone, but during the evolution of a particular response, temporary changes in physiological status (e.g. immunological skewing) may result in ‘errors’ leading to a pathological outcome. As with other metabolically complex processes, the underlying physiological mechanisms, like receptor-ligand interactions or biochemical feedback systems, are often non-linear with regard to stimulus-response coupling (Glass, 2001). On their own, the function of each pathway may be described in deterministic terms, but their complex interactions may lead to apparently random outcomes. In this model, inheritance is a ‘weighting factor’ contributing to these ‘errors,’ as it only promotes an increased likelihood of atopy arising within members of families, but is not an obligate occurrence.

Heritability estimates and gene searches for complex human diseases are based upon quantitative traits (phenotypes), such as serum total IgE, whose expression levels can be compared by standard statistical analyses among related individuals (Falconer & MacKay, 1996). An implicit assumption in these strategies is that the phenotype expression levels are normally distributed, arising from the influence of one or more genes. It is further assumed that a direct causal relationship exists between gene inputs and phenotypic trait expressions, resulting in positive correlations of trait expression values among members of kinships. Any variation from this assumption is most-often attributed to random environmental factors.

In this study, we analyzed the statistical inter-relationships among kinships for quantitative IgE traits known to be associated with atopy. We focused upon first-degree relatives (parent-child and sibling-sibling), as these were the most likely to demonstrate genetic effects with regard to IgE-mediated responses, and would be the least likely to be significantly influenced by random environmental impacts. Our objective was to determine which of two models most likely accounted for atopic immune responses in these kinships: Obligate Inheritance versus Stochastic Bias.

Overall, the results show that pathology-associated IgE outcomes cannot arise from simple causal stimulus-response coupling mechanisms. Rather, adverse physiological responses, like allergen-specific IgE, arise from mechanisms that alter the probabilities of isotype switching to IgE and result in random phenotype expressions among those who are clinically affected. Future studies related to the inheritance of these complex diseases will have to take these stochastic features into account.

METHODS

Study Population

Families that included two children with a physician's diagnosis of asthma were recruited as previously described (CSGA, 1997). This included 666 members of 26 multi-generation families and 433 members of small nuclear families. Each person, or legal guardian, provided written, informed consent according to the guidelines of the Institutional Review Board of the University of Minnesota. Participants were given a routine physical examination, an interviewer-monitored questionnaire, prepared according to American Thoracic Society guidelines, and additional tests described below.

Asthma Symptoms

A positive response for asthma symptoms was recorded for either (a) Questionnaire reports of 2 or 3 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 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.

Bronchial Hyperreactivity (BHR)

Baseline spirometry measures for FEV1 (Forced Expiratory Volume at 1 second) were made according to American Thoracic Society guidelines [1987], followed by provocative methacholine challenges (CSGA, 1997). Bronchial hyperreactivity (BHR) was recorded if there was a decrease of 20% or more in FEV1 after methacholine challenge (≤ 25 mg/ml maximum dosage), or reversibility of 15% or greater after administration of albuterol.

IgE Screens

(1.) Serum Total IgE Tests—Serum total IgE tests were done using the ACCESS Immunoassay System® (Beckman Coulter, Chaska, MN) (Patterson et al, 1994), according to manufacturer's specifications. All IgE results are reported as IU/ml (1 IU = 2.4 ng).

(2.) Skin Prick Tests (SPT)—Skin prick testing was done as previously described (CSGA, 1997) using 14 standardized allergen extracts including ragweed, molds (3), trees (4), grasses (2), dust mites (2), animal danders (2).

(3) Serum Allergen-Specific IgE Tests—Among those with any SPT [+] test result, we randomly selected 250 individuals without regard for their specific SPT screen results, and analyzed their sera for the presence of detectable amounts of five allergen-specific IgE's by "reverse sandwich" ELISA. The five allergens chosen were those that induced the most frequent SPT [+] reactions in this study population (Jackola et al, 2004a), including ragweed (Amb a), dust mites (Der p and Der f), cat (Fel d) and German cockroach (Bla g). These determinations were adaptations of methods described in detail elsewhere (Allauzen et al, 1999). The lowest limit of detection by this method was 0.02 IU/ml (≈ 0.05 ng/ml), and there was a linear response on a log-log scale of 0.05 to 100 ng/ml.

Definitions of Allergen-Specific and Basal IgE Fractions

(1) Allergen-Specific IgE—As previously shown for this study population (Jackola et al, 2004b), among those with any SPT [+] response, a statistically significant estimate for the allergen-specific IgE level can be found from the # SPT [+] results by: $\text{Log}_{10} [\text{Allergen-Specific IgE}] = 0.269 + 0.765 \times \text{Log}_{10} [\# \text{ SPT (+)}]$.

(2) Basal IgE Estimates—Among those who were SPT [–] for all 14 allergens, the allergen non-specific, or basal, IgE value was the same as the total IgE value. Among those with any SPT [+] result, the basal IgE value was given by: $\text{Log}_{10} [\text{Basal IgE}] = \text{Log}_{10} [\text{Total IgE}] - \text{Log}_{10} [\text{Allergen-Specific IgE}]$.

Non-Linear Model Fitting

Non-linear models were used to describe certain phenomena, such as simple exponential probability functions of the form $P(X) = ae^{-\beta X}$. Data for these non-linear equations were fit using a Levenberg-Marquardt algorithm (Bevington & Robinson, 2003) implemented in WinCurveFit® software (Kevin Raner Software, Mt. Waverly, Victoria, AUS). Assessment for ‘goodness of fit’ was made by a coefficient of determination (R^2) based upon minimizing the least squares sums of errors.

Statistics

Comparisons between groups categorized by demographics or clinical status were made by two-sided t-tests (significance level $\alpha = 0.05$) or analysis of variance (ANOVA) using commercial software (Microsoft Excel® and SPSS®).

Variance components analysis was used to partition total phenotypic variance (V_P) into additive genetic (V_A) and non-genetic, environmental components (V_E): $V_P = V_A + V_E$. The environmental component was further partitioned into two components for common family environment, V_{CE} , and common sibling effects, V_{CS} . The narrow-sense heritability (h^2_N) was defined as the ratio of the variance due to additive genetic effects to all sources of phenotypic variance: $h^2_N = V_A / (V_A + V_{CE} + V_{CS})$.

As previously described by Palmer et al (2000), the variance components were determined from calculations of the covariances (COV) about common mean values for quantitative traits for three kinship pairings (spouse-spouse, parent-child and sib-sib). This results in three algebraic equations that can be simultaneously solved for three unknown values, namely:

$$\text{COV}(\text{Spouse} - \text{Spouse}) = V_{CE} \quad (1)$$

$$\text{COV}(\text{Parent} - \text{Child}) = 1/2 V_A + V_{CE} \quad (2)$$

$$\text{COV}(\text{Sibling} - \text{Sibling}) = 1/2 V_A + V_{CE} + V_{CS} \quad (3)$$

Determinations of these covariances were made using commercial statistical software (Microsoft Excel® and SPSS®).

RESULTS

Study Population Demographics

A total of 1099 Caucasians from families with history of atopic asthma residing in Minnesota were screened. As shown in Table 1, the study population comprised 666 individuals from 26 multiplex families, with representatives from 2 to 4 generations per family, and 433 people

from 110 small nuclear families. Overall, there were roughly equal numbers of females and males, with ages spanning roughly seven decades.

As described in Methods, each person was screened for asthma history, evidence of bronchial hyperreactivity (BHR) by spirometry and atopic immune responses by serum total IgE and skin prick test (SPT) using a battery of 14 common aeroallergens. As shown in Table 1, the study population was significantly skewed toward asthma, BHR [+] responses and atopy reflected by SPT [+] results.

The outcomes of BHR [+] and SPT [+] responses were frequently co-expressed with asthma symptoms determined from 2 X 2 contingency tables and Chi-square statistics. For those with asthma symptoms, comparing the numbers of people with and without BHR [+] results and/or SPT [+] results gave: $\chi^2(1) = 26.13$; $p \ll 0.001$. In contrast, comparisons among those without asthma symptoms gave: $\chi^2(1) = 2.24$; not significant @ $p = 0.05$.

Overall, the study population was significantly skewed for asthmatic and atopic disease, and the adverse physiological respiratory (BHR [+]) and atopic immune (SPT [+]) responses were most-often associated with asthma.

Serum Allergen-Specific IgE Concentrations Are Approximated by SPT Results

Evidence for production of atopic pathology-associated allergen-specific IgE is manifest by SPT [+] reactions. We have previously shown that sensitivity to any particular allergen is a random variable, independent of sensitivity to any other allergen (Jackola et al, 2004a). We have also argued that, in this study population, the number of SPT [+] results is a surrogate marker for allergen-specific IgE levels (Jackola et al, 2004b). Here we confirm this assumption.

We randomly selected 250 individuals from the study population who had any SPT [+] result and screened their sera for allergen-specific IgE for 5 allergens that induced the most frequent SPT [+] results in this population. (Individuals were not selected or excluded based upon their SPT responses to these 5 allergens.) The results of this screen are shown in Table 2. Of the 250 people screened, 225 had detectable amounts of specific IgE for one or more of the five allergens tested.

By ANOVA, there were some significant differences in the average concentrations for the 5 allergens as shown in Table 2. However, on a logarithmic scale, the distribution of the sum of the specific IgE's was approximately normally distributed. Also, on a log-log scale, there was a significant positive relationship between total detectable allergen-specific IgE and the number (N) of SPT [+] results for these 5 allergens as assessed by simple linear regression: $\text{Log} [\text{Sum Specific IgE}] = -0.98 + 2.05 \times \text{Log} [N]$; $F(1, 223) = 361.7$; $p \ll 0.001$.

Most importantly, there was also a significant positive association between serum total IgE values and the specific IgE values for the 5 allergens: $\text{Log} [\text{Total IgE}] = 1.80 + 0.59 \times \text{Log} [\text{Sum Specific IgE}]$; $F(1, 220) = 117.7$; $p \ll 0.001$. And there was a positive association between total IgE values and the number (N) of allergen-specific IgE's detected per person for this screen: $\text{Log} [\text{Total IgE}] = 1.46 + 1.08 \times \text{Log} [N]$; $F(1, 220) = 46.7$; $p \ll 0.001$.

Although the number of allergens to which any individual is sensitized is a discrete variable, the quantitative amount of serum allergen-specific IgE is continuous. However, each particular allergen induces a unique clone of lymphoid cells that produce the specific IgE's, and the sensitivities to specific allergens are independent of one another [Jackola et al, 2004a]. Thus, comparing the numbers of allergens to which individuals are sensitized is a surrogate for comparisons of allergen-specific IgE.

Allergen-Specific IgE Is Elevated With Asthma

Because atopic immune responses were frequently detected among those with asthma in this study population, we compared the IgE values for those with and without asthmatic respiratory involvement. Table 3 shows the results for Log [Total IgE] values for four categories of subjects. In this table, a positive respiratory response was defined as having both characteristic asthma symptoms and BHR [+] results. An atopic response was defined as any SPT [+] result. By one-way ANOVA there are statistically significant differences in the average Log [Total IgE] values for these groups.

As expected, those with any SPT [+] results had higher Log [Total IgE] levels (Mean = 2.06; Std. Dev. = 0.61; N = 761) compared to those who were SPT [-] (Mean = 1.33; Std. Dev. = 0.59; N = 338). By two-sided t-test, these mean values were significantly different: $t(1097) = 18.5$; $p \ll 0.001$.

Further, there were also significant differences in total IgE values due to positive asthmatic respiratory involvement. Among those who were non-atopic (SPT [-] Groups A and B in Table 3), comparisons of mean Log [Total IgE] values by two-sided t-test gave: $t(336) = 2.19$; $p = 0.015$. Serum total IgE levels are elevated among those with asthmatic symptoms and adverse respiratory responses. A similar trend was observed for those who were SPT [+] (Groups C and D in Table 3). By two-sided t-test: $t(759) = 7.10$; $p \ll 0.001$.

The differences in total IgE values for those with any SPT [+] result based upon presence or absence of asthmatic respiratory involvement (Groups C and D in Table 3) could be largely attributed to increased allergen-specific IgE production among those with asthma. For those without asthma (Group C), the average number of SPT [+] results = 4.56 (Std. Dev. = 3.05; N = 351). For those with asthma (Group D), average = 5.75 (Std. Dev. = 3.46; N = 410). Comparing these mean values by two-sided t-test gave: $t(759) = 4.96$; $p \ll 0.001$.

A similar comparison could not be made for Groups A and B as they were SPT [-] to the 14-allergen battery used in this study. It was possible that those with asthma were producing allergen-specific IgE to some allergen(s) other than those used in the screen, or the allergen-specific IgE did not induce a positive SPT reaction [our reference on SPT thresholds]. We did not explore this possibility further as it was most likely that the increased serum total IgE levels among those with asthma was due to enhanced production of allergen-specific IgE.

Stochastic Features of Allergen-Specific Responses in Asthma

From the results in the previous section, allergen-specific responses are elevated among those with asthmatic respiratory involvement. To explore this phenomenon further, we graphically compared the number of SPT [+] results for those with and without asthma. Figure 1 shows the proportions of the number of SPT [+] results for those without (open circles) and with (closed circles) asthma symptoms and BHR [+] responses.

The smooth curves in this figure are best-fit estimates assuming a simple exponential model: $P(N) = \alpha e^{-\beta N}$, where $P(N)$ is the probability of having some number, N , of SPT [+] results ($1 \leq N \leq 14$), and α and β are constants. For those without asthma (dashed line): $\alpha = 0.21 (\pm 0.017)$; $\beta = 0.17 (\pm 0.019)$; $R^2 = 0.87$. For those with asthma (solid line): $\alpha = 0.14 (\pm 0.014)$; $\beta = 0.10 (\pm 0.017)$; $R^2 = 0.68$. By Chi-square test, these distributions were significantly different: $\chi^2(13) = 60.1$; $p < 0.001$. While the average number of SPT [+] results is greater in those with asthma compared to those without asthma, the number of allergens to which an individual is sensitized is a random variable, regardless of respiratory disease status.

Heritability Estimates for Basal and Allergen-Specific IgE

We used variance components analysis to make estimates of the heritability of basal and allergen-specific IgE. We focused upon first-degree relatives as these would be most likely to manifest phenotype expressions resulting from inheritance, and would be the least likely to be impacted by significant environmental modifications. As explained in Methods, this involved computing the covariances of quantitative variables for three kinship pairings: spouse-spouse, parent-child and sibling-sibling. These statistics can then be used to estimate three presumably independent contributions to the total phenotypic variance.

Table 4 shows the narrow-sense heritability estimates for the expressions of three different Log [IgE] values shared by kinships: A. Total IgE, B. Basal IgE (see Methods for definition) and C. Allergen-specific IgE (see Methods). IgE values were not adjusted for age, gender or asthmatic respiratory status for these estimates.

In agreement with previous reports, an estimate for serum total IgE levels is in the range of 45–50% (Part A, Table 4). As shown above, total IgE consists of two physiologically distinct components: basal and allergen-specific IgE. As shown in Part B of Table 4, after subtracting the estimated allergen-specific IgE component for those with any SPT [+] result, the heritability estimate for basal IgE is > 95%. This estimate was not adjusted for age, gender or asthmatic respiratory status, and is probably artificially high as a result.

However, basal IgE is not associated with atopic or asthmatic pathology. The allergen-specific IgE component of serum IgE is. As shown in Part C of Table 4, there is no apparent heritability associated with serum allergen-specific IgE. The next section of Results will demonstrate that this component is a random variable arising independently of inheritance.

Stochastic Features of Allergen-Specific IgE in Kinships

Figure 1 shows that the number of allergens towards which individuals are sensitized is a random variable whose probability of occurrence can be modeled by a simple exponential distribution function. These models can also be used to compare the co-occurrence of two random variables simultaneously, such as those for parents and children or pairs of siblings.

Here we assume two independent variables for the number of allergens, X and Y . The probability of occurrence is described by distinct distributions: $P(X) = \alpha e^{-\beta X}$ and $P(Y) = \gamma e^{-\delta Y}$, where the four constants may or may not be equal. We are interested in the intersection of these distributions in order to assess the conditional probability of the co-occurrence of SPT [+] results among kinships.

Let us say that we have two events of interest, A and B . For example, A is the event that a parent is sensitized to some arbitrary number of allergens, and event B is that the atopic child is sensitized to the same or different number of allergens. Each of these has an associated probability of occurrence, $P[A]$ and $P[B]$.

By definition, the *conditional probability* that B occurs given that A occurred first is: $P[B|A] = P[A \cap B]/P[A]$, where $P[A \cap B]$ is the intersection probability of the two events (Feller, 1967). If, however, the two events are independent of one another, then: $P[B|A] = P[B]$. Event B occurs independently of whether event A occurred or not. By comparing the two expressions for the conditional probability, $P[B|A]$, and rearranging gives: $P[A \cap B] = P[A] \times P[B]$. If the two events are independent of one another, then the intersection probability is equal to the product of the two probabilities for events A and B . This relationship can be used to devise a Chi-square test statistic to determine if the two events are independent of one another or not.

By simple enumeration, the intersection of the number of SPT [+] results shared by parents and children, or pairs of siblings, can be found. These are the observed values for the expression $P[A \cap B]$ to be used in the Chi-square evaluation.

The expected probability distribution of co-occurrence will be given by: $P[A] \times P[B] = (\alpha e^{-\beta X}) \times (\gamma e^{-\delta Y})$ for all 14 by 14 possibilities [$1 \leq X \leq 14$; $1 \leq Y \leq 14$]. The expected number of SPT [+] results will be given by this expression multiplied by the total number of observations made. The expected and observed numbers give a 14 by 14 contingency table to be evaluated by Chi-square test with $(14 - 1) \times (14 - 1) = 169$ degrees of freedom (d. f.).

From tabulated values for Chi-square, a critical value at the 95% confidence level for the given d. f. = 200.3. A calculated Chi-square statistic less than this value will indicate that the observed and expected values derived from the same distribution, indicating for our purposes that the distributions of SPT [+] results for relatives are independent of one another.

We first consider the distributions for parents and their children when both members of these kinship pairings are SPT [+] to one or more allergens each. For the SPT [+] parents: $\alpha = 0.20$ (± 0.023); $\beta = 0.16$ (± 0.026); $R^2 = 0.83$. For their SPT [+] children: $\gamma = 0.15$ (± 0.020); $\delta = 0.11$ (± 0.024); $R^2 = 0.69$. Similar to the results in Figure 1, these results yield two different distributions for the number of SPT [+] results for the parent-child kinships.

These results are shown in Figure 2A for the parents (solid circles, solid line) and the children (open circles, dashed line). By Chi-square test there is no significant difference in the observed frequency of co-occurrence for the number of SPT [+] results shared by these kinships and the expected numbers if these distributions are independent of one another @ $p = 0.05$: $\chi^2(169) = 156.0$.

Figure 2B shows the results for pairs of siblings when both members of the sibship are SPT [+]. In this case, for the first sibling (solid circles, solid line): $\alpha = 0.13$ (± 0.015); $\beta = 0.09$ (± 0.019); $R^2 = 0.67$. For the second sibling (open circles, dashed line): $\gamma = 0.12$ (± 0.017); $\delta = 0.07$ (± 0.022); $R^2 = 0.54$. The two distributions nearly overlap one another. But again, by Chi-square there was no difference between the expected and the observed intersection of co-occurrence of SPT [+] results @ $p = 0.05$: $\chi^2(169) = 177.0$.

As argued above, the number of SPT [+] reactions is a surrogate marker for allergen-specific IgE production. Each SPT [+] result indicates the production of IgE by a unique clone of lymphocytes specific for that particular allergen. As shown here, among pairings of first-degree relatives who are SPT [+] to one or more allergens, the number of SPT [+] results, and hence allergen-specific IgE, is independent. Thus, the amount(s) of serum allergen-specific IgE is not a heritable trait like non-pathology associated basal IgE.

DISCUSSION

The objective of this study was to determine which of two alternative models best accounts for expressions of allergen-specific IgE immune system outcomes in families with significant histories of atopic diseases. One model, Obligate Inheritance, postulates that there are specific inherited genetic alterations resulting in abnormal recognition and /or response to allergens that would directly influence the expression levels of quantitative IgE traits. If correct, then there should be significant correlations of IgE trait expression levels among members of kinships, as these presumed genetic alterations would be passed on within families.

The alternate model, Stochastic Bias, proposes that the immune regulatory pathways are the same for everyone, but temporary changes in physiological status may 'skew' these responses resulting in 'errors' in recognition/response leading to a pathological outcome. In this case,

underlying regulatory pathways may temporarily ‘skew’ an individual’s humoral response. Although the likelihood of an atopic immune response may be inherited, the allergen-specific IgE responses are random variables unrelated to outcomes expressed by their relatives. The results favor this Stochastic Bias model.

Atopic, IgE-mediated disorders are common chronic human diseases that presumably arise from inherited errors in immune system recognition and/or response to otherwise benign, non-infectious environmental antigens (allergens). Results of numerous genome screens and case-control studies show that multiple genetic elements contribute to these conditions (Hoffjan et al, 2003). However, regardless of the number of genetic alterations that may be involved, the underlying mechanisms cannot be accounted for by simple Mendelian inheritance that directly or indirectly imply a causal physiological linkage between stimulus (allergen exposure/recognition) and response (humoral immune development). Results presented here and in previous studies argue strongly against this likelihood.

Evidence that the atopic disorders are inherited derives primarily from epidemiological studies: (1) these conditions tend to ‘cluster’ within families, (2) there is a higher concordance of atopic outcomes among identical (monozygotic) twins compared to fraternal (dizygotic) twins and (3) the concordance of atopic responses is higher among first-degree relatives (parent-child or sibling-sibling) than in other pedigree relationships (Blumenthal & Björkstén, 1999). But, epidemiological studies also show that there are large world-wide variations in atopy prevalence, being as low as < 5% of the populations in less-developed countries, to as high as 25–40% of the populations in highly industrialized countries (ISAAC, 1998). Current consensus holds that IgE-mediated atopic conditions are inherited, but that they can be significantly modulated by random environmental contingencies. Unfortunately, these observations do not address the nature of immune system responses to these environmental challenges.

Epidemiological studies, or most studies related to the biology of these conditions, have focused solely upon the adverse immune manifestation of allergen-specific IgE. However, especially in the cases of the common aeroallergens, like ragweed pollen or house dust mites that initiate immune responses within the respiratory tract, specific IgE production is only one manifestation of immune response. In fact, most (all) people will mount a response to aeroallergens involving any or all of the IgA and IgG classes or subclasses (Batard et al 1993a, 1993b; Jackola et al, 2002). It is only among those with an inherited atopic tendency that one also finds a specific IgE response. Yet this is not absolute, as it has been shown in the earliest years of life that production of allergen-specific IgE is common, at least up to the age of two years (Hattevig et al, 1993). A distinction arises in that among children who do not develop atopy this phenomenon is transient, while in children who do develop atopy the phenomenon is persistent. Atopic disorders involve a developmental progression (the so-called “atopic march”).

Two key determinants in whether or not an atopic, allergen-specific IgE will arise involves the likelihood (probability) of an isotype switch to IgE and the relative ‘vigor’ of this response based upon the binding affinity of the antibody for the allergen that induced its production. We have shown that the atopic response is characterized by an extremely high binding affinity by IgE for allergen(s) (Pierson et al, 1998; Pierson-Mullany et al, 2000). We have also found that atopy involves not only a high affinity IgE response, but an attenuated, low binding affinity IgG1 response, whereas a non-atopic response evolves to a high affinity IgG1 response that is equal in ‘vigor’ to the high affinity atopic IgE (Jackola et al, 2002). This study suggested that there is a ‘selective competition’ among antibody isotypes akin to Darwinian evolution on a microscopic scale, in which different “species” (antibody isotypes) “compete” for a common

“resource” (allergen). Both allergen-specific isotypes may co-exist, but the one with the “selective advantage” (highest binding affinity) will dominate.

Further, in the earliest years of life (newborn to 6 years), the developmental “trajectories” of high affinity atopic IgE and high affinity non-atopic IgG1 are comparable (Jackola et al, 2005). Taken together, these results suggest that the difference between an atopic and a non-atopic response to the same allergen(s) is due to (1) the likelihood of switching to a particular isotype, (2) how vigorous this ‘isotype choice’ is and, most importantly, (3) atopic IgE may only be an ‘error’ in isotype switching during an otherwise normal process of antigen recognition and humoral response evolution.

The induction of the characteristic wheal and flare reaction in the percutaneous skin prick test (SPT) is evidence for the production of high binding affinity, allergen-specific IgE (Pierson-Mullany et al, 2002). Figures 1 and 2 of the current report show that the number, N, of SPT [+] results for any person is a random variable. And, this number reflects the induction of unique clones of lymphoid cells that produce these vigorous IgE responses to one or more allergens. We have previously shown that the specific allergens to which people become atopically sensitized are random variables independent of ant sensitivity “patterns” within families (Jackola et al, 2004a).

Also, from our previous experimental work (Jackola et al, 2002), in contrast, among those people who are not atopically sensitized to some of the allergens used in this battery, they are with high probability producing a vigorous IgG1 response that does not involve a clinical manifestation. The allergens used in this screen are widely distributed in this geographic locale, and the results presented here are from families who with high likelihood share similar environmental conditions, including the allergens to which they are exposed. Thus, lack of a clinical response manifestation (SPT [+] result) is not due to lack of exposure to these allergens.

Figure 1 also points to another contingency involved with the likelihood or not of an atopic IgE switch. Among those people who have any SPT [+] result, but who differ with regard to the presence or absence of asthma, the numbers of allergens to which they are sensitized are random variables, approximated by exponential probability distributions. The difference is that those with asthma have a higher probability of producing IgE to more allergens than those without asthma. These results are not unique to this study population. Epidemiological studies have also observed these exponential distributions; as for example Table 2 in Pearce et al (1999), a comprehensive review of the epidemiological literature relevant to atopic disease, and the large United States health survey NHANES III (Figure 1 in Arbes et al, 2005).

By diagnostic criteria, asthma is a chronic inflammation of the airways. This inflammation in large part involves components of the mucosal immune system that are prone to develop germinal center-like foci (so-called mucous associated lymphoid tissue, or MALT), akin for example to Peyer’s Patches in the gut, that can be involved in immunoglobulin production and isotype class switching. These foci are a common feature of chronic inflammatory diseases of the immune system, such as autoimmune disorders (Aloisi & Pujoll-Borrell, 2006). It has been suggested that they are also involved in the atopic disorders, including atopic asthma (reviewed in Gould et al, 2006). References in this review show that only among people with an inherited propensity for atopy will IgE be found in these foci. Non-atopics produce either IgA or IgG in these foci, if any detectable response is present.

Whether or not these foci make significant contributions to atopic disease is controversial. But, they highlight the importance of germinal center (GC) reactions in “decision making” in the evolution of a humoral response to antigens. Current consensus on GC reactions proposes that there are at least three critical checkpoints in formation of an antigen-specific response (Lindhout et al, 1997; Tarlinton & Smith, 2000): (1) Antigen-specific initiation of the GC

reaction, (2) Selection of the best-fitting B cell receptor (BCR) by follicular dendritic cells (FDC's) and (3) Antigen-dependent, T-cell-mediated isotype switching. Factors that promote antigen retention by FDC's and induce the most vigorous (highest affinity) BCR also promote the probability of isotype switching, especially to IgE; antigen retention and the overall "intensity" of the GC reaction make significant contributions to what antibody isotype will predominate.

The importance of the total time in the GC reactions for isotype switching has been given support from *in vitro* studies of isotype switching by initially IgM+ expressing B cells (Tangye et al, 2002; Hasbold et al, 2004). Given the appropriate stimuli and co-factors, these cells will progress through several rounds of the cell division cycle (CDC) prior to switching to IgG. In fact, this group has shown that the total number of CDC's is critical for the initiation of class switching to IgG. An additional number of CDC rounds are also required before an IgE switch is detected. Whether or not this involves a sequential mechanism, like IgM → IgG → IgE, by the same cells is not known. An alternative is that some IgM cells can switch directly to IgE, and by-pass the IgG switch (saltatory switch), which is supported by a murine model (Jung et al, 1994). In either case, these results show that switching to IgE is less common than switching to IgG, and requires more total time for lymphoid cells to make this switch decision.

The *in vitro* studies also highlight another important feature of immune system "decision making"; at the cellular level, metabolic decisions between alternate pathways are stochastic in nature rather than strictly deterministic (Hasbold et al, 2004). Indeed, it has been argued that these stochastic mechanisms are the rule rather than the exception in most immunological information processing at the cellular/molecular level, as these mechanisms promote response plasticity and the fidelity of developing a robust response to highly variable and unpredictable environmental circumstances (Germain, 2001).

Among some of these stochastic networks is the well-known CD28/ICOS/CTLA4 family of co-factors that promote interactions between T cells and antigen-presenting cells (APC) or B cells (Green, 2000). Both CD28 and CTLA4 bind to the same counter receptors on APC's or B cells, B7.1 (CD80) and B7.2 (CD86), but they differ in function. CD28 is an amplifying cofactor, while CTLA4 is an inhibiting co-factor. The Inducible Co-factor of Stimulation (ICOS) apparently modulates the activities of the other two. Taken together, this family describes a classic network involving feedback regulation (Jansson et al, 2005). The relative strength and duration of the countervailing co-factors' interactions will determine the final response, which is not absolute but contingent upon the interplay of these elements. In fact, studied separately, both CTLA4 (Hizawa et al, 2001) and ICOS (Shilling et al, 2005) have been implicated in atopic IgE production.

Regardless of the specific network or metabolic system(s), the overall effect is variability in possible responses, and not deterministic regulation. Genetic alterations in some element(s) of a network, like a feedback loop, may alter the probability of one outcome as opposed to another, but this is not deterministic; it is stochastic. If the evolution of immune responses, such as the development of allergen-specific IgE atopy, was strictly deterministic and under the control of some finite number of genes, then there would be significant correlations in the expression levels of quantitative traits among genetically similar people, such as parent-child or sibling-sibling kinships. Yet, as shown in this study, we can find no evidence for this based upon large numbers of atopically sensitized people from numerous families with history of these diseases. This rules out Obligate Inheritance of allergen-specific IgE-mediated disorders.

The alternative is that a model describing Stochastic Bias is in force. Again, it is most likely that all people share the same metabolic pathways and networks contributing to the development of humoral immune responses to common non-infectious allergens. But,

temporary physiological biases may ‘skew’ the responses among some people and lead to a pathological outcome as an alternative to an otherwise normal process of antigen recognition and immune response development. In this model, inheritance plays a role of one among several factors that contributes to skewing a response.

In this light, specific IgE production is not a cause of atopic disorders, it is a consequence of them. As it is most likely that people from families with history of these diseases will develop an atopic immune response to some number of allergens, what is inherited is the likelihood of atopy but not the specific manifestation. Future gene searches for these complex diseases should focus upon elements that increase the likelihood of these adverse responses, and not the specific responses themselves.

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References

- Allauzen S, Blocki F, Pierson-Mullany L, Jackola D, Dalan D, Larue C, Blumenthal M, Rosenberg A. Expression of IgH chain genes in antibody response to ragweed and purified Amb a 1: evidence for differential regulation of isotype production. *Molec Immunol* 1999;36:587–589. [PubMed: 10499812]
- Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. *Nat Rev Immunol* 2006;6:205–217. [PubMed: 16498451]
- Altmüller J, Palmer LJ, Fischer G, Scherb H, Wjst M. Genomewide scans of complex human diseases: true linkage is hard to find. *Am J Hum Genet* 2001;69:936–950. [PubMed: 11565063]
- Arbes S Jr, Gergen P, Elliott L, Zeldin D. Prevalences of positive skin test responses to 10 common allergens in the US population: Results from the Third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2005;116:377–383. [PubMed: 16083793]
- American Thoracic Society. Standardization of spirometry—1987 updated 1987. *Am J Respir Crit Care Med* 153:1285–1298. [PubMed: 8616555]
- Batard T, Basuyaux B, Lambin P, Bremard-Oury C, Hamilton RG, David B, Peltre G. Isotypic analysis of grass pollen-specific immunoglobulins in human plasma., 1. Specialization of certain classes and subclasses in the immune response. *Int Arch Allergy Immunol* 1993a;100:68–78. [PubMed: 8428167]
- Batard T, Basuyaux B, Laroze A, Lambin P, Bremard-Oury C, Aucouturier P, Hamilton RG, David B, Peltre G. Isotypic analysis of grass-pollen-specific immunoglobulins in human plasma. 2. Quantification of the IgE, IgM, IgA class and the IgG subclass antibodies. *Int Arch Allergy Immunol* 1993b;102:279–287. [PubMed: 8219781]
- Bevington, PR.; Robinson, DK. Data reduction and error analysis for the physical sciences. McGraw Hill; Boston, MA: 2003. p. 161-164.
- Blumenthal, MN.; Björkstén, B., editors. Genetics of allergy and asthma. Marcel Dekker, Inc; New York, NY: 1997.
- CSGA (Collaborative Study on the Genetics of Asthma). A genome-wide search for asthma susceptibility loci in ethnically diverse populations. *Nature Genetics* 1997;15:389–392. [PubMed: 9090385]
- Falconer, DS.; Mackay, TFC. Introduction to quantitative genetics. Addison Wesley Longman, Ltd; Essex, UK: 1996.
- Feller, W. An introduction to probability theory and its applications. I. Wiley; New York, NY: 1967. p. 113-145.
- Geha RS, Jabara HH, Brodeur SR. The regulation of immunoglobulin E class-switch recombination. *Nat Rev Immunol* 2003;3:721–732. [PubMed: 12949496]
- Germain RN. The art of the probable: System control in the adaptive immune system. *Science* 2001;293:240–245. [PubMed: 11452112]
- Glass L. Synchronization and rhythmic processes in physiology. *Nature* 2001;410:277–284. [PubMed: 11258383]

- Gould HJ, Takhar P, Harries HE, Durham SR, Corrigan CJ. Germinal-centre reactions in allergic inflammation. *Trends Immunol* 2006;27:446–452. [PubMed: 16949872]
- Hattevig G, Kjellman B, Björkstén B. Appearance of IgE antibodies to ingested and inhaled allergens during the first 12 years of life in atopic and non-atopic children. *Pediatr Allergy Immunol* 1993;4:182–186. [PubMed: 8298709]
- Hizawa N, Yamaguchi E, Jinushi E, Konno S, Kawakami Y, Nishimura M. Increased total serum IgE in patients with asthma and promoter polymorphisms at CTLA4 and FCER1B. *J Allergy Clin Immunol* 2001;108:74–79. [PubMed: 11447385]
- Hoffjan S, Nicolae D, Ober C. Association studies for asthma and atopic disease: a comprehensive review of the literature. *Resp Res* 2003;4:14–26.
- ISAAC. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). *Eur Resp J* 1998;12:315–335.
- Jackola DR, Pierson-Mullany LK, Liebeler CL, Blumenthal MN, Rosenberg A. Variable binding affinities for allergen suggests a 'selective competition' among immunoglobulins in atopic and non-atopic humans. *Molec Immunol* 2002;39:367–377. [PubMed: 12220894]
- Jackola DR, Liebeler CL, Blumenthal MN, Rosenberg A. Random outcomes of allergen-specific responses in atopic families. *Clin Exp Allergy* 2004a;34:540–547. [PubMed: 15080805]
- Jackola DR, Blumenthal MN, Rosenberg A. Evidence for two independent distributions of serum immunoglobulin E in atopic families: Cognate and Non-Cognate IgE. *Human Immunol* 2004b; 65:20–30. [PubMed: 14700592]
- Jackola DR, Liebeler CL, Lin C-Y, Chiu Y-K, Blumenthal MN, Rosenberg A. Evidence that negative feedback between antibody concentration and affinity regulates humoral response consolidation to a non-infectious antigen in infants. *Molec Immunol* 2005;42:19–30. [PubMed: 15488940]
- Jansson A, Barnes E, Klenerman P, Harlen M, Sorenson P, Davis SJ, Nilsson P. A theoretical framework for quantitative analysis for the molecular basis of costimulation. *J Immunol* 2005;175:1575–1585. [PubMed: 16034096]
- Jung S, Siebenkotten G, Radbruch A. Frequency of immunoglobulin E switching is autonomously determined and independent of prior switching to other classes. *J Exp Med* 1994;179:2023–2026. [PubMed: 8195724]
- Lindhout E, Koopman G, Pals ST, de Groot C. Triple check for antigen specificity of B cells during germinal center reactions. *Immunol Today* 1997;18:573–577. [PubMed: 9425734]
- Patterson W, Werness P, Payne WJ, Matsson P, Leflar C, Melander T, Quast S, Stejskal J, Carlson A, Macera M, Shubert FW. Random and continuous-access immunoassays with chemiluminescent detection by Access® Automated Analyzer. *Clin Chem* 1994;40:2042–2045. [PubMed: 7955376]
- Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? *Thorax* 1999;54:268–272. [PubMed: 10325905]
- Pierson L, Allauzen S, Blumenthal M, Rosenberg A. An automated method for determination of antibody affinity functions with nanogram quantities. *J Immunol Methods* 1998;211:97–109. [PubMed: 9617835]
- Pierson-Mullany LK, Jackola DR, Blumenthal MN, Rosenberg A. Characterization of polyclonal allergen-specific IgE responses by affinity distributions. *Molec Immunol* 2000;37:613–620. [PubMed: 11163397]
- Pierson-Mullany LK, Jackola DR, Blumenthal MN, Rosenberg A. Evidence of an affinity threshold for IgE-allergen binding in the percutaneous skin test reaction. *Clin Exp Allergy* 2002;32:1–10. [PubMed: 12002725]
- Shilling RA, Pinto JM, Decker DC, Schneider DH, Bandukwala HS, Schneider JR, Camoretti-Mercado B, Ober C, Sperling AI. Cutting edge: polymorphisms in the ICOS promoter region are associated with allergic sensitization and Th2 cytokine production. *J Immunol* 2005;175:2061–2065. [PubMed: 16081771]
- Tarlinton DM, Smith KGC. Dissecting affinity maturation: a model explaining selection of antibody-forming cells and memory B cells in the germinal center. *Immunol Today* 2000;21:436–441. [PubMed: 10953095]

Figure 1.

Probability Distributions of Skin Prick Test (SPT) Results. For those with any SPT [+] result, the proportions of SPT [+] results are plotted against N. These proportions were fit to a simple exponential model: $P(N) = \alpha e^{-\beta N}$, where $P(N)$ is the probability of having some number, N, of SPT [+] results ($1 \leq N \leq 14$), and α and β are constants. This figure compares the results for those with asthma symptoms plus BHR [+] results (closed circles, solid line) and those without asthma plus BHR [+] (open circles, dashed line). Values for the constants α and β are given in Results.

Figure 2.

Comparisons of SPT Probability Distributions for Kinships. For kinships (parent-child or sibling-sibling), the probability distributions for the # SPT [+] results are given as in Figure 1. Panel A (top) gives the distribution for Parents (closed circles, solid line) and Children (open circles, dashed line). Panel B (bottom) gives the distributions for Siblings, with first sibling (closed circles, solid line) and second sibling (open circles, dashed line) results nearly overlapping. Details for the constants giving best-fit estimates are in Results.

Table 1
Study Population Demographics

[[[]]]	[[Multiplex Families[]]]	[[Nuclear Families[]]]
Total N (# Families)	666 (26)	433 (110)
Females	332	213
Males	334	219
Average Age [Age Range]	34.5 yrs. [6–81]	28.0 yrs. [6–75]
Atopic Status		
Asthma Symptoms	465 (69.8%)	379 (87.5%)
BHR [+] ¹	278 (41.7%)	291 (67.2%)
Asthma + BHR [+]	231 (34.7%)	279 (64.4%)
Any SPT [+] ²	453 (68.8%)	308 (71.1%)

¹. BHR = bronchial hyperreactivity, defined in methods.

². SPT = (percutaneous) skin prick test.

Table 2

Serum Allergen-Specific IgE Screen Results

[[Allergen]]	[[Average (IU/ml)]]	[[Std. Dev.]]	[[N]]
Dust Mite (Der p)	1.56	3.13	225
Ragweed (Amb a)	3.59	12.30	172
Cat (Fel d)	1.87	42.06	159
Cockroach (Bla g)	0.37	0.08	157
Dust Mite (Der f)	0.86	17.40	128
Total	1.70	6.72	841
	F (4, 836) = 7.34	[[ ]]	[[ ]]
	p-value < 0.001	[[ ]]	[[ ]]

Table 3

Log [Total IgE] By Atopic Status

[[Group]]	[[Respiratory]] ¹	[[Any SPT [+]]]] ²	[[Average]]	[[Std. Dev.]]	[[N]]
A	NEG	NEG	1.282	0.566	238
B	POS	NEG	1.436	0.639	100
C	NEG	POS	1.894	0.591	351
D	POS	POS	2.201	0.596	410
[[ ]]	[[ ]]	[[ ]]	F (3, 1095) = 137.6³ p-value << 0.001		[[ ]]
[[ ]]	[[ ]]	[[ ]]			[[ ]]

¹. Respiratory = Asthma Symptoms + BHR [+].². Any Positive Skin Prick Test (SPT).³. F-ratio for comparison of average values by ANOVA.

Table 4
Heritability Estimates for Basal and Allergen-Specific IgE

A. Log [Total IgE] [[Kinship]]		[[Covariance]]	t (d.f.) ¹	[[p-value]]
Spouse-Spouse		0.0496	1.65 (198)	0.101
Parent-Child		0.0743	4.46 (704)	≪ 0.001
Sibling-Sibling		0.0820	6.45 (948)	≪ 0.001
	Estimated Heritability ²			46.3%
B. Log [Basal IgE] ³				
Kinship		Covariance	t (d.f.) ¹	p-value
Spouse-Spouse		0.0194	0.88 (198)	0.38
Parent-Child		0.0492	3.80 (704)	0.0001
Sibling-Sibling		0.0439	4.38 (948)	≪ 0.001
	Estimated Heritability			> 95%
C. Log [Allergen-Specific IgE] ⁴				
Kinship		Covariance	t (d.f.) ¹	p-value
Spouse-Spouse		0.0113	1.63 (83)	0.11
Parent-Child		0.0057	1.75 (329)	0.18
Sibling-Sibling		0.0096	3.63 (559)	0.003
	Estimated Heritability			0.0%

t-Test value assuming covariance = 0.0.

². Heritability estimates described in Methods.

^{3, 4} Descriptions for Basal and Allergen-Specific IgE values described in Methods.