

Allergen-Specific IgG1 Provides Parsimonious Heritability Estimates for Atopy-Associated Immune Responses to Allergens

Carol L. Liebeler, Saonli Basu, and Duaine R. Jackola

ABSTRACT: Although serum total immunoglobulin E (IgE) is generally elevated in atopic conditions, it is an unreliable trait for dissecting the genetic and environmental components contributing to atopic immune responses, because it can be significantly confounded by demographic factors (age, gender, and race) and clinical status (atopic vs nonatopic). Allergen-specific IgE is a discontinuous trait present only in those with sensitivity to allergens. However, all people will produce allergen-specific immunoglobulin G1 (IgG1), which is elevated among those atopically sensitized to specific allergens. We screened 91 Caucasian nuclear families ($N = 367$) with medical histories of atopic diseases and used variance components analysis to compare heritability estimates for total IgE and IgG1 produced against the common major allergen from house dust mite *Dermatophagoides pteronyssinus* (Der p 1).

An estimate of total IgE heritability was about 48%, although this was significantly confounded by age, gender, and clinical atopic status. In contrast, Der p 1-IgG1 demonstrated a significant inherited component of about 62% that was not influenced by age, gender, or clinical status. For genetic studies of atopic humoral responses, allergen-specific IgG1 may be a more reliable quantitative trait than serum IgE. Moreover, atopy is an inherited deregulation of immune responses to noninfectious antigens, involving antibody isotypes other than IgE. *Human Immunology* 68, 113–121 (2007). © American Society for Histocompatibility and Immunogenetics, 2007. Published by Elsevier Inc.

KEYWORDS: Allergen; atopy; IgE; allergen-specific IgG1; variance components

ABBREVIATIONS

BHR bronchial hyperreactivity
Der p 1 *Dermatophagoides pteronyssinus*
IgE immunoglobulin E

IgG1 immunoglobulin G1
SPT skin prick test

INTRODUCTION

Atopic disorders, such as allergic rhinitis or atopic bronchial asthma, are common, complex human diseases that result from adverse immune system responses to noninfectious environmental antigens (allergens). Although it has been demonstrated that there is an inherited component for these conditions, they are also influenced by numerous environmental factors. As a result, quantita-

tive traits associated with these diseases indicate a great amount of phenotypic variability.

Genetic and environmental components can be dissected by comparisons of trait values among kinships by regression statistics or comparable analyses. Employing these methods assumes that the traits are continuously distributed, that there are detectable, measurable trait levels in all people screened, and that the sources of variation are known or can be reasonably assumed. Among the most frequently used quantitative traits for genetic studies of atopy is serum total immunoglobulin E (IgE), as this is often elevated with atopic disease.

However, the use of total IgE as a quantitative trait for genetic studies of atopy has produced widely conflicting results, with evidence for codominant genetic effects [1],

From The Asthma and Allergy Program, Department of Medicine (C.L.L., D.R.J.); and Division of Biostatistics, School of Public Health (S.B.), University of Minnesota Medical School, Minneapolis, MN, USA.

Address reprint requests to: Dr. Duaine R. Jackola, The Asthma and Allergy Program, University of Minnesota Medical School, Mayo Mail Code 434, 420 Delaware St. S.E., Minneapolis, MN 55455; Tel: (612) 626-4624; Fax: (612) 624-9188; E-mail: jacko001@umn.edu.

Received September 14, 2006; revised November 27, 2006; accepted December 1, 2006.

recessive effects [2–4], pleiotropic effects [5], and other modes of inheritance [6–10]. Further, it has not been clearly established that enhanced total IgE production is a predisposing cause for atopy, as it may only be a consequence that accompanies clinical conditions, so the physiological sources of total IgE are unclear.

We have previously argued that serum total IgE consists of at least two physiologically distinct components [11]. One of these is a basal (noncognate) component apparently unrelated to atopic disease, whereas a second arises from atopic sensitization to allergens, resulting in allergen-specific (cognate) IgE. Yet, this cognate IgE fraction is not a reliable marker for genetic studies because it is a discontinuous trait that is only produced by those with atopic sensitivity to allergens. It is influenced by the number of allergens to which these individuals are sensitized [11], and the number and specific kinds of allergens to which people are sensitized are random variables [12]. Thus, specific IgE is not a useful marker for dissecting the genetic and environmental components related to atopic immune responses using regression-based methods.

Aside from clinical associations, production of allergen-specific IgE is the result of an adaptive, memory-forming humoral immune response to noninfectious environmental antigens (allergens). Save for specific IgE production, it has been demonstrated that it is equally likely for anyone to mount a T-cell dependent humoral response to allergens involving varying amounts of the IgA and IgG isotype subclasses [13–15]. In particular, both specific IgA and immunoglobulin G1 (IgG1) are elevated among those with atopic sensitization to a particular allergen.

IgA production is chiefly confined to the mucosa, making it difficult to evaluate as a quantitative trait for genetic studies. In contrast, IgG1 derives from isotype class switch recombination mechanisms in lymph node germinal centers, with the subsequent generation of long-lived memory B and plasma cells that are available to produce IgG1, which is released into the peripheral circulation [16, 17]. Specific IgE production presumably arises and is produced in a comparable fashion. And, it is possible that elevated specific IgG1 among those with atopic sensitivity to allergens parallels the production of specific IgE, as both are influenced by similar metabolic controls like the cytokine IL-4 [18] or neuroendocrine modulators such as β_2 -adrenergic agonists [19].

To evaluate allergen-specific IgG1 as a quantitative marker for genetic studies of atopy-associated immune responses, we screened small nuclear families with medical history of atopic diseases. We used variance component statistical analyses to evaluate heritability for serum total IgE and dust-mite specific IgG1. We chose dust mite as a model, because dust mites are a source of a common indoor, nonseasonal allergen that is ubiqui-

tously distributed and to which most people are continually exposed. We also included an antigen-specific antibody to tetanus toxoid (TT), TT-IgG1, to demonstrate that inheritance of specific antibody responses is not confined to allergens alone.

The results indicate that specific IgG1 provides more parsimonious estimates of heritability for genetic studies on immune responses resulting from allergen sensitization than total IgE, as it is not significantly confounded by demographic cofactors or clinical atopic status unrelated to dust mite sensitivity. Also, the results suggest that the humoral response associated with atopy is a more generalized immune system deregulation involving antibody isotype production other than IgE alone.

METHODS

Study Population

Caucasian families that included two children with a physician's diagnosis of asthma and who resided in the Minneapolis/St. Paul, Minnesota metropolitan area were recruited through media advertisements as part of studies for the genetics of asthma. Greater than two thirds of this population manifested evidence of allergen sensitivity by standard allergen-specific skin prick test (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 367 people from 91 nuclear families that included both parents, the asthmatic children, and their siblings. One hundred sixty-nine parents (mean age 43.7 years, range 22–75 years) and 198 children (mean age 14.9 years, range 6–42 years) were screened. There were 176 females and 191 males. All participants had been previously inoculated against tetanus toxoid antigen.

Asthma and Pulmonary Function Screens

Each subject was given a nurse-administered, standardized questionnaire, prepared according to American Thoracic Society guidelines, regarding history of asthma and other respiratory diseases as previously described [20]. Baseline spirometry measures for forced expiratory volume at 1 second were made according to American Thoracic Society guidelines, followed by provocative methacholine challenges [20]. Bronchial hyperreactivity (BHR) was recorded if there was a decrease of 20% or more in forced expiratory volume at 1 second after methacholine challenge (≤ 25 mg/ml maximum dosage) or reversibility of 15% or greater after administration of albuterol.

IgE Screens

Serum total IgE tests. Serum total IgE tests were done using the Access Immunoassay System (Beckman

Coulter, Chaska, Minn), according to manufacturer's specifications. All IgE results are reported as IU/ml (1 IU = 2.4 ng).

Skin prick tests. Skin prick testing was done as previously described [20] using 13 standardized allergen extracts for dust mites (including Der p allergen extract) (2), ragweed, molds (3), trees (4), grasses (2), animal danders (2). A positive SPT result was recorded for a wheal area ≥ 9 mm² above negative saline control.

Der p 1 Allergen

The major allergen Der p 1 from house dust mite *Dermatophagoides pteronyssinus* (Der p) was purified by methods previously described [21]. We chose Der p 1 allergen as a model because we have previously demonstrated that the atopic response to any particular allergen is not obligately inherited. Rather, it is a random variable independent of any family history of atopic allergen sensitivity [12]. Der p 1 is a common nonseasonal indoor allergen that, for this study population, induced the highest incidence of atopic sensitization among all of the common indoor allergens that were tested in the SPT screens.

Enzyme-Linked Immunosorbent Assays for Serum Der p 1-IgG1 and TT-IgG1

Measures of Der p 1-specific IgG1 were done using modifications of reverse sandwich enzyme-linked immunosorbent assays previously described [15, 22]. Briefly, a capture antibody was an isotype-specific monoclonal antibody (mouse anti-human and anti-IgG1, Sigma-Aldrich Co., St. Louis, Mo) that eliminated possible cross-reactivity with other isotypes. This was bound to wells of microtest plates overnight at 20°C. Nonspecific binding was blocked using 2% bovine serum albumin (BSA) in phosphate buffered saline (PBS). Serum or serially diluted standards were added for 2 hours at 37°C. For serum test samples, captured IgG1 was evaluated for the presence of Der p 1-specific IgG1 by addition of biotinylated Der p 1 standard (Indoor Biotechnologies, Charlottesville, NC). Calibration of the assay was done using human serum immunoglobulin calibrators (The Binding Site, Birmingham, England). Detection antibody for the calibrators was mouse, anti-human IgG1 (Sigma). Controls for assay validation were from a serum pool with known concentrations of Der p 1-IgG1 maintained by us. A biotinylated detection antibody was added for 1 hour at 37°C. Optimized concentrations of streptavidin-conjugated alkaline phosphatase (Sigma) were added for 1 hour at room temperature. A chemiluminescent substrate (Lumi-Phos 550, Beckman Coulter) was added, followed by reading the test signals with a commercial luminometer (Tropix, Inc., Bedford, Mass). This assay

gave a linear response on a log-log scale over the range of 0.05–100 ng/ml.

For TT-IgG1 assays, we modified the assay described by Sedgwick *et al.* [23] by using a direct-binding enzyme-linked immunosorbent assay. Prior to use, tetanus toxoid (Sigma) was dialyzed (10,000 MW cutoff) against 50 mM borate buffer, pH 8.5, overnight. This preparation was diluted 1:1000 in 50 mM carbonate buffer, pH 9.6, and coated to microtest plates wells overnight. Nonspecific binding sites were blocked with 2% BSA. Serum (1:100 dilution) was added for 1 hour at 37°C. Detection antibody was alkaline phosphatase-conjugated mouse, anti-human IgG1 mAb (Zymed Laboratories, S. San Francisco, Calif). Calculations were done as described by Sedgwick *et al.* [23], which provided a linear response in arbitrary chemiluminescent units.

Statistical Analysis

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, Microsoft Corp., Redmond, Wash and SPSS version 10.0 for Windows, SPSS Inc., Chicago, Ill). Age effects for serum antibody concentrations were assessed by simple linear regression and evaluated by ANOVA.

Variance component analysis was used to partition total phenotypic variance (V_P) into additive genetic (V_A) and nongenetic 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 phenotype variance: $h^2_N = V_A/(V_A + V_{CE} + V_{CS})$.

As previously described by Palmer *et al.* [24], 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 sibling-sibling). This results in three algebraic equations that can be simultaneously solved for three unknown values, namely:

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

$$\text{COV}(\text{parent-child}) = \frac{1}{2}V_A + V_{CE} \quad (2)$$

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

Implementation of the variance components analyses and estimates of the narrow-sense heritability, including estimates for the effects of the cofactors of age and gender, were obtained with SOLAR, version 2.1.4 (Southwest Foundation for Biomedical Research, San Antonio, Tex) as first described by Almasy and Blangero [25].

RESULTS

Serum Antibody Concentrations

Age and gender effects. Three hundred sixty-seven individuals from 91 Caucasian nuclear families with history of atopic asthma were screened. On logarithmic scales, concentrations of serum total IgE, Der p 1-IgG1, and TT-IgG1 were approximately distributed normally in this study population. By simple linear regression, there was a significant age effect for log (total IgE) values: $\log(\text{total IgE}) = 2.24 - 0.013 \times \text{age}$, $F(1, 365) = 29.2$, $p < 0.001$.

In contrast, there were no significant age effects for either log (Der p 1-IgG1) or log (TT-IgG1) values: $\log(\text{Der p 1-IgG1}) = -0.75 + 0.001 \times \text{age}$, $F(1, 365) = 0.009$, $p = 0.93$. And, $\log(\text{TT-IgG1}) = -0.17 - 0.002 \times \text{age}$, $F(1, 365) = 1.92$, $p = 0.17$.

To account for gender effects for log (total IgE) values, subjects were first classified as being either SPT positive (+) or SPT (-), because, as expected, there were significant differences in log (total IgE) values based upon these classifications. For those with any SPT (+) result, the mean value for log (total IgE) = 2.13 (SD = 0.64, $n = 262$). For those who were SPT (-), mean log (total IgE) = 1.28 (SD = 0.60, $n = 105$). By two-sided t -test, these mean values were significantly different: $t(205) = 12.1$, $p < 0.001$.

For females who were SPT (-), mean log (total IgE) = 1.18 (SD = 0.62, $n = 57$). For males who were SPT (-), mean log (total IgE) = 1.41 (SD = 0.55, $n = 48$). These mean values were significantly different: $t(103) = 2.01$, $p = 0.024$.

For females who had any SPT (+) result, mean log (total IgE) = 2.05 (SD = 0.68, $n = 119$). For SPT (+) males, mean log (total IgE) = 2.20 (SD = 0.60, $n = 143$). These mean values were also significantly different: $t(239) = 1.82$, $p = 0.035$.

As demonstrated in "Results," mean log (Der p 1-IgG1) and log (TT-IgG1) values were not affected by

SPT results, save for Der p SPT results on log (Der p 1-IgG1). However, there were no gender effects for these antibody measures: $\log(\text{Der p 1-IgG1})$: mean for females = -0.75 (SD = 0.50, $n = 176$); mean for males = -0.75 (SD = 0.46, $n = 191$), $t(365) = 0.04$, $p = 0.49$; $\log(\text{TT-IgG1})$: mean for females = -0.24 (SD = 0.36, $n = 176$); mean for males = -0.20 (SD = 0.39, $n = 191$), $t(365) = 0.91$, $p = 0.18$.

To be used in genetic studies employing variance components analysis, serum total IgE values must be adjusted for age and sex, as well as for evidence or not of sensitivity to allergens. In contrast, Der p 1-IgG1 and TT-IgG1 values are not appreciably confounded by age or sex.

Effects of clinical status. In addition to age and gender effects, serum antibody concentrations can be influenced by clinical atopic status. For this study, each person was screened for asthma symptoms and BHR by standardized spirometry. They were also screened for atopic sensitivity by percutaneous SPT by using a battery of 14 allergens, including Der p extract.

We first subdivided the study population into four categories based upon results for asthma symptoms and BHR, or any SPT (+) result. Table 1 provides the results for the serum antibody concentrations for these categories. By ANOVA, the mean values for log (Der p 1-IgG1) and log (TT-IgG1) were not significantly different based upon these clinical categorizations. However, log (total IgE) values differed significantly.

As demonstrated in Table 1 for those with any SPT (+) result, log (total IgE) values were different for those with or without asthma symptoms plus BHR (+) results. By two-sided t -test, these mean values were significantly different: $t(119) = 4.50$, $p < 0.001$. A similar trend was observed for those who were SPT (-): $t(97) = 2.32$, $p = 0.011$. Serum total IgE values were influenced by clinical respiratory status, whereas Der p 1-IgG1 and TT-IgG1 levels were not.

TABLE 1 Clinical status and serum antibody concentrations

Asthma/BHR ^a	SPT (+) ^b	<i>n</i>	Total IgE ^c	Der p 1-IgG1 ^c	TT-IgG1 ^c
Neg	Neg	54	1.15 (0.52) ^d	-0.72 (0.48)	-0.25 (0.31)
Neg	Pos	71	1.84 (0.64)	-0.68 (0.49)	-0.23 (0.38)
Pos	Neg	51	1.42 (0.64)	-0.78 (0.46)	-0.19 (0.43)
Pos	Pos	191	2.24 (0.61)	-0.77 (0.47)	-0.22 (0.38)
	$F(3, 363)^e$		58.0	0.77	0.25
	<i>p</i> value		<0.001	0.51	0.86

Abbreviations: BHR = bronchial hyperreactivity; SPT = skin prick test; Neg = negative; Pos = positive.

^a Asthma symptoms and bronchial hyperreactivity.

^b Any SPT (+) result.

^c Values are base 10 logarithms.

^d Mean (SD).

^e F ratio from analysis of variance, by column.

TABLE 2 Atopic status and serum antibody concentrations

SPT (+) ^a	Der p (+) ^b	<i>n</i>	Total IgE ^c	Der p 1-IgG1 ^c	TT-IgG1 ^c
Yes	Yes	122	2.29 (0.59) ^d	-0.64 (0.51)	-0.26 (0.35)
Yes	No	140	2.00 (0.65)	-0.84 (0.43)	-0.19 (0.40)
No	No	105	1.28 (0.60)	-0.75 (0.47)	-0.22 (0.37)
	F (3,363) ^e		78.4	6.16	0.99
	<i>p</i> value		<0.001	0.002	0.37

Abbreviation: SPT = skin prick test.

^a Any SPT (+) result.

^b SPT (+) for Der p allergen.

^c Values are base 10 logarithms.

^d Mean (SD).

^e F ratio from analysis of variance, by column.

We next categorized subjects according to SPT results, and Der p sensitivity in particular. This resulted in three categories as shown in Table 2. By ANOVA, serum log (total IgE) values were significantly different for these categories, but log (TT-IgG1) values were not.

Log (Der p 1-IgG1) values were significantly different. However, unlike total IgE, this was not due to generalized sensitivity to allergens (any SPT (+) result). For those who had any SPT (+) result, mean log (Der p 1-IgG1) = -0.74 (SD = 0.48, *n* = 262). For those who were SPT (-), mean log (Der p 1-IgG1) = -0.75 (SD = 0.47, *n* = 105). By two-sided *t*-test, the mean values were not significantly different: *t* (365) = 0.08, *p* = 0.93.

In contrast, for those who had a positive SPT result for the Der p dust mite extract, mean log (Der p 1-IgG1) = -0.64 (SD = 0.51, *n* = 122). For those who were SPT (-) for the Der p extract, mean log (Der p 1-IgG1) = -0.80 (SD = 0.45, *n* = 245). By two-sided *t*-test, these mean values were significantly different: *t* (365) = 3.17, *p* = 0.0008.

Figure 1 illustrates the distributions of the log (Der p 1-IgG1) values, with the % of cases plotted against log (Der p 1-IgG1) values. There is significant overlap of the values for those who were Der p (+) (solid circles, solid line) compared with those who were Der p (-) (open circles, dashed line). This situation is analogous to what we have observed previously for serum total IgE values for those who are SPT (+) compared with those who are SPT (-) (Figure 1 in reference 11); there is considerable overlap in the expressions of the trait levels although they arise from separate mechanisms.

Serum total IgE levels are influenced by clinical respiratory status and atopic sensitivity to allergens, whereas Der p 1-IgG1 levels are only influenced by specific sensitivity to the dust mite allergen.

Heritability Estimates For Serum Antibody Concentrations

We used pedigree-based variance component analysis as implemented in SOLAR version 2.1.4 [25] to estimate the heritability of immune responses reflected by logarithmic values of serum total IgE, Der p 1-IgG1 and TT-IgG1. Heritability was calculated by comparing the covariance of each of these traits among related individuals with the overall variance of the trait. Each of the traits was adjusted for age and sex.

Table 3 gives the results of these analyses, with the estimated heritability, its standard error of estimation and the probabilities that these estimates were influenced by age and gender. Statistically significant estimates of heritability were found for the three serum antibody con-

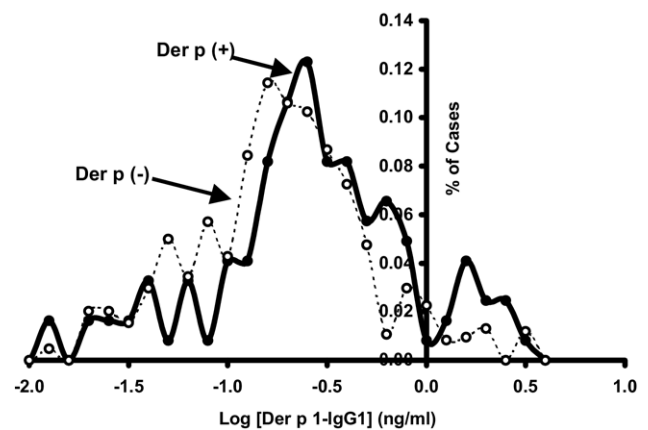


FIGURE 1 Serum Der p 1-IgG1 distributions: Der p (+) versus Der p (-). The percent of cases are plotted against serum Log₁₀ (Der p 1-IgG1) for those who were SPT (+) to the Der p allergen extract (Der p (+) = solid circles, solid line) or those who were SPT (-) to this extract (Der p (-) = open circles, dashed line). For Der p (+), mean = -0.65 (SD = 0.51, *n* = 122). For Der p (-), mean = -0.80 (SD = 0.45, *n* = 245). Der p = *Dermatophagoides pteronyssinus* (dust mite allergen extract); SPT = (percutaneous) skin prick test.

TABLE 3 Heritability estimates for serum antibody concentrations

	Total IgE	Der p 1-IgG1	TT-IgG1
Heritability			
Estimated value	0.478	0.615	0.687
SE of estimate	0.103	0.079	0.080
<i>p</i> value of estimate	8.00×10^{-7}	2.21×10^{-15}	1.22×10^{-14}
Cofactors			
<i>p</i> value for age effect	4.01×10^{-10}	0.66	0.49
<i>p</i> value for gender effect	0.004	0.58	0.71

centrations, reflected by the *p* values of these estimates. Both age and sex did not contribute significantly on Derp1-IgG1 and TT-IgG1 (Table 3) and hence were removed from the final analysis.

The estimated heritability of total IgE was lower than those for Der p 1-IgG1 and TT-IgG1. However, the source of production for IgE cannot be determined with any certainty. As demonstrated above, serum total IgE values were significantly influenced in this study population by sensitivity or not to allergens and clinical respiratory status. Der p 1-IgG1 and TT-IgG1 values were independent of respiratory status, and Der p 1-IgG1 values were only elevated among those with evidence of sensitivity to the Der p allergen. Whereas the IgG1 values are reflective of humoral response capabilities to defined antigens, total IgE could have arisen from one or more sources (*e.g.*, basal IgE, allergen-specific IgE or IgE associated with respiratory status).

We included heritability assessments for the tetanus toxoid antibody, TT-IgG1, to demonstrate that there can be a significant inherited component involving antigen-specific antibodies other than those produced against allergens. Although the heritability estimates for Der p 1- and TT-IgG1 were similar, these arose from independent physiologic mechanisms as there was no association between these serum antibody concentrations as assessed by simple correlation $r = 0.050$, $t(366) = 0.93$, $p = 0.35$. These responses do not arise from interhuman infectious transmission, and only the Der p 1 allergen is known to be associated with atopic immune responses.

Finally, the heritability for Der p 1-IgG1 responses were equally likely to arise from either parent, as parent-child correlations were similar for mothers and fathers: mother-child: $r = 0.52$, $t(96) = 5.98$, $p < 0.001$; father-child: $r = 0.53$, $t(97) = 6.19$, $p < 0.001$.

DISCUSSION

Although the results of this study may not directly relate to genetic factors contributing to atopic predisposition,

two observations merit discussion. First, aside from genetic atopic predisposition, allergen-specific IgG1 provides parsimonious estimates for the genetic factors related to atopy-associated humoral immune responses to noninfectious environmental allergens, as it is not significantly affected by demographic cofactors. Its source is clearly defined, arising from a specific immune response to a defined antigen, whereas the physiologic sources of total IgE are unclear. Second, the genetic components of atopic sensitization to allergens contribute to a more generalized immune system deregulation influencing antibody isotype production other than IgE.

These findings are in contrast to those of other studies in which only IgE measures were used, particularly those in which it was implicitly assumed that atopic sensitization to allergens is an IgE-related phenomenon only. Using total IgE as a quantitative trait for genetic studies of atopy has produced widely conflicting results, particularly with regard to the proportions of the responses putatively attributed to inheritance [1–10]. Although controlled by tight metabolic regulation, the production of IgE involves multiple pathways [26–28], but the details of these interrelationships are not completely understood.

Epidemiologic studies have demonstrated that serum total IgE levels are significantly influenced by demographic factors—like age, gender, and race—and atopic clinical status [29–32], which undoubtedly confound the studies of inheritance. This was also observed in our study population, as demonstrated in the results of Tables 1 and 2. Although the IgG1 values reflect immune response capabilities to defined antigens, it cannot be determined with any certainty what the sources are for IgE production.

The measured serum IgE values could conceivably arise from three—possibly interdependent—sources: basal IgE production unrelated to atopic disease (non-cognate fraction), specific IgE directed against one or more allergens (cognate fraction) [11], or elevated IgE production due to atopic respiratory conditions (ectopic fraction). Attempting to account for one or more of these factors suggests that IgE production is influenced by multiple genes, some of which may not be related to immune system regulatory elements [24, 33, 34].

A comprehensive survey of genetic studies on atopy-related phenotypes demonstrated that no less than 64 genes or chromosomal regions have been implicated in these disorders, of which roughly 50%–60% were immune regulatory genes, including inflammatory and antibody production promoters, whereas the remainder manifested no apparent association with immune system regulation [34]. These complex scenarios argue against any obligatory inherited pathways leading to an atopic outcome.

As we have previously argued [12], the atopic predisposition more likely arises from a stochastic bias model. In this scenario, it is equally likely for anyone to mount an immune response to allergens, but the specific outcome is a random event. The specific outcome is dependent upon the interplay of numerous genetic and environmental elements that, in certain circumstances, may skew a response towards atopy, whereas others experiencing similar conditions remain clinically unaffected. A first step toward unraveling this complexity is to understand the commonality of the atopic and nonatopic humoral responses, as opposed to attempting to clearly delineate the presumed differences.

We have previously demonstrated among randomly selected individuals that those with and without atopic sensitization to a particular allergen can mount an equally vigorous humoral immune response against that allergen [15]. The atopic response was characterized by the production of specific IgE with high equilibrium binding affinity for the allergen and an attenuated, lower affinity specific IgG1 response. In contrast, the nonatopic response was characterized by specific IgG1 with an equilibrium binding affinity for the allergen comparable to that of the atopic IgE response.

We have found that, in the earliest years of life when most atopic humoral responses develop, there is a nonlinear increase in the age-dependent developmental trajectories of humoral responses involving specific IgE among atopic children, and specific IgG1 among nonatopic children are remarkably similar [35]. Interestingly, this study also demonstrated that the atopy-associated specific IgG1 response was deregulated compared with the IgG1 response among nonatopic children, because there was no comparable nonlinear increase with age. Rather, the vigor of the atopic IgG1 response remained relatively unchanged, suggesting again that stochastic features among immune regulators favored a vigorous IgE response at the expense of a vigorous IgG1 response among those children with an atopic predisposition.

From these results, we have surmised that atopy may only be an error in isotype class switching during an otherwise normal process of antigen recognition and humoral response development. If this is so, then as demonstrated in Table 3, about 60% of the factors that contribute to this error are inherited. This does not imply, however, that these factors will be common to all people with the atopic predisposition or that families with similar atopic histories will also share the same genetic complement predisposing to atopy. Again, from the literature survey by Hoffjan *et al.* [34], there are numerous genes that may contribute to an atopic immune response. By probability alone, it is extremely unlikely that all people with atopy or all families with

atopic histories could possibly share the same combinations of these genes.

Recent evidence suggests that single nucleotide polymorphisms (SNPs) in the promoter regions of immune regulatory genes can profoundly impact upon the specific details of an immune response to allergens [36]. Three specific examples include SNPs in the CD14 endotoxin receptor gene that influences both allergen-specific IgE and IgG1 production among those with atopy [37], SNPs in the promoter region for inducible cofactor of stimulation that mediates T cell–B cell interaction during humoral response development [38], and CTLA-4 gene promoter SNPs that also impacts upon T cell–B cell interaction [39, 40].

Given that each of these gene promoter elements can have at least one of three genotypes (*e.g.*, C/C, C/T, or T/T for the CD14 promoter), then there are at least $3 \times 3 \times 3 = 27$ possible combinations taking all three together. If only one of these combinations is the correct one for atopy, then in the total population there is about a 1 of 27—or about 4%—chance of an atopic response, which clearly does not conform to the current atopy incidence rates in industrialized nations of about 25%–40%. Of course, any number of the 27 possible combinations could be proposed to be correct to account for current atopy incidence, but assuming that any of these combinations will be exactly represented in all atopic families is, again, extremely unlikely.

What is more likely, however, is that IgE and IgG1 production may have more immune regulators in common with one another than are disparate, and a more thorough search among these regulators might provide clues to the probability of isotype switching errors. For example, considerable attention has been paid to the genes in the cytokine cluster region on human chromosome 5q31 [41] that includes genes for IL-4, CD14, and the beta-2 adrenergic receptor (β_2 -AR), among others.

By focusing upon this region, it was hoped that the gene alleles that promote enhanced IgE production would become evident and provide clues to the atopic predisposition. However, the cytokine IL-4 is a critical antibody isotype switch factor for both IgG1 and IgE [18], CD14 modulates both allergen-specific IgG1 and IgE responses [37], and β_2 -AR agonists have been demonstrated to enhance the amount of IgG1 and IgE produced per B cell *in vitro* [19]. Any one alone or in combination could account for enhanced production of both specific IgG1 and IgE.

However, the dynamics of humoral response development are not totally accounted for by isotype switching and the amount of the particular isotype that is produced. Somatic hypermutation of the genes encoding for the variable heavy and light chains of antibodies are also involved, which leads to enhanced antibody binding to

antigen during affinity maturation in germinal center reactions [42].

The fully developed humoral response is a function of both the concentration of the particular antibody isotype (IgX) and the binding affinity, K , of this antibody for the antigen that induced its production. The mathematic product of these two variables, that we have called the total antibody binding capacity ($CAP = [IgX] \times K$) is the variable that clearly distinguishes the atopic from the nonatopic humoral response [15]. It is the age-dependent, nonlinear increase in total IgG1 and IgE capacities that are comparable in nonatopic and atopic children [35].

Both the atopic and the nonatopic humoral responses are the result of the interactions among numerous genes that control isotype class switching, the amount of the particular isotype that is produced, and factors involved in affinity maturation. It has been argued that the mechanisms of isotype class switching and affinity maturation within lymph node germinal centers are independently regulated [43]. In effect, the particular antibody repertoire that develops in any given circumstance is a matter of most probable outcomes, akin to Darwinian competitive selection on a microscopic scale [15]. From the results of the present study, allergen-specific IgG1 is a robust and convenient quantitative marker to be used to unravel the genetic complexities of the atopy-associated humoral response to allergens.

ACKNOWLEDGMENTS

This work was supported by NIH Grant 2 RO1-HL049609-11, and the Asthma and Allergy Research Fund, Department of Medicine, University of Minnesota, Malcolm N. Blumenthal, M.D. This is manuscript MSP-003-2006 of the Asthma and Allergy Center, University of Minnesota. We gratefully acknowledge Andreas Rosenberg, Emeritus Professor, University of Minnesota, for a critical reading of the manuscript.

REFERENCES

- Martinez FD, Holberg CJ, Halonen M, Morgan WJ, Wright AL, Taussig LM: Evidence for Mendelian inheritance of serum IgE levels in Hispanic and non-Hispanic white families. *Am J Hum Genet* 55:555, 1994.
- Meyers DA, Beaty TH, Colyer CR, Marsh DG: Genetics of total serum IgE: regressive model approach to segregation analysis. *Genet Epidemiol* 8:351, 1991.
- Dizier MH, Hill M, James A, Faux J, Ryan G, le Souef P, Lathrop M, Musk AW, Demenais F, Cookson W: Detection of a recessive major gene for high IgE levels acting independently of specific response to allergens. *Genet Epidemiol* 12:93, 1995.
- Palmer LJ, Cookson WOCM, James AL, Musk AW, Burton PR: Gibbs sampling-based segregation analysis of asthma-associated quantitative traits in a population-based sample of nuclear families. *Genet Epidemiol* 20:356, 2001.
- Borecki IB, Rao DC, Lalouel JM, McGue M, Gerrard JW: Demonstration of a common major gene with pleiotropic effects in immunoglobulin E levels and allergy. *Genet Epidemiol* 2:327, 1985.
- Postma DS, Bleecker ER, Amelung PJ, Holroyd KJ, Xu J, Panhuysen CIM, Meyers DA, Levitt RC: Genetic susceptibility to asthma-bronchial hyperresponsiveness co-inherited with a major gene for atopy. *New Engl J Med* 333:894, 1995.
- Xu J, Levitt RC, Panhuysen CIM, Postma DS, Taylor EW, Amelung PJ, Holroyd KJ, Bleecker ER, Meyers DA: Evidence for two unlinked loci regulating total serum IgE levels. *Am J Hum Genet* 57:425, 1995.
- Blumenthal MN, Namboodiri K, Mendell N, Gleich G, Elston RC, Yunis E: Genetic transmission of serum IgE levels. *Am J Med Genet* 10:219, 1981.
- Hanson B, McGue M, Roitman-Johnson B, Segal NL, Bouchard TJ Jr, Blumenthal MN: Atopic disease and immunoglobulin E in twins reared apart and together. *Am J Hum Genet* 48:873, 1991.
- Gerrard JW, Rao DC, Morton NE: A genetic study of immunoglobulin E. *Am J Hum Genet* 30:46, 1978.
- 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* 65:20, 2004.
- Jackola DR, Liebeler CL, Blumenthal MN, Rosenberg A: Random outcomes of allergen-specific responses in atopic families. *Clin Exp Allergy* 34:540, 2004.
- 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* 100:68, 1993.
- 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* 102:279, 1993.
- 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* 39:367, 2002.
- Slifka MK, Ahmed R: Long-lived plasma cells: a mechanism for maintaining persistent antibody production. *Curr Opin Immunol* 10:252, 1998.
- McHeyzer-Williams MG, Ahmed R: B cell memory and the long-lived plasma cell. *Curr Opin Immunol* 11:172, 1999.
- Stavnezer J: Molecular processes that regulate class switching. *Curr Topics Micro Immunol* 245:127, 2000.
- Kasprowicz DJ, Kohm AP, Berton MT, Chruscinski AJ,

- Sharpe A, Sanders VM: Stimulation of the B cell receptor, CD86 (B7-2), and the β 2-adrenergic receptor intrinsically modulates the level of IgG1 and IgE produced per B cell. *J Immunol* 165:680, 2000.
20. CSGA (Collaborative Study on the Genetics of Asthma): A genome-wide search for asthma susceptibility loci in ethnically diverse populations. *Nature Genetics* 15:389, 1997.
21. Pierson LK, Allauzen S, Blumenthal MN, Rosenberg A: An automated method for determination of antibody affinity distribution functions with nanogram quantities. *J Immunol Methods* 211:97, 1998.
22. 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* 32:107, 2002.
23. Sedgwick AK, Ballou M, Sparks K, Tilton RC: Rapid quantitative microenzyme-linked immunosorbent assay for tetanus antibodies. *J Clin Microbiol* 18:104, 1983.
24. Palmer LJ, Burton PR, Faux JA, James AL, Musk AW, Cookson WOCM: Independent inheritance of serum immunoglobulin E concentrations and airway responsiveness. *Am J Respir Crit Care Med* 161:1836, 2000.
25. Almasy L, Blangero J: Multipoint quantitative trait linkage analysis in general pedigrees. *Am J Hum Genet* 62:1198, 1998.
26. Sutton BJ, Gould KJ: The human IgE network. *Nature* 366:421, 1993.
27. Oettgen HC: Regulation of the IgE isotype switch; new insights on cytokine signals and the functions of ϵ germ-line transcripts. *Curr Opin Immunol* 12:619, 2000.
28. Geha RS, Jabara HH, Brodeur SR: The regulation of immunoglobulin E class-switch recombination. *Nature Rev Immunol* 3:721, 2003.
29. Barbee RA, Halonen M, Lebowitz M, Burrows B: Distribution of IgE in a community population sample: correlations with age, sex, and allergen skin test reactivity. *J Allergy Clin Immunol* 68:106, 1981.
30. Barbee RA, Halonen M, Kaltenbron W, Lebowitz M, Burrows B: A longitudinal study of serum IgE in a community cohort: correlations with age, sex, smoking, and atopic status. *J Allergy Clin Immunol* 79:919, 1987.
31. Freidhoff LR, Meyers DA, Bias WB, Chase GA, Hussain R, Marsh DG: A genetic-epidemiologic study of human immune responsiveness to allergens in an industrial population: I. Epidemiology of reported allergy and skin-test positivity. *Am J Med Genet* 9:323, 1981.
32. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG: Association of asthma with serum IgE levels and skin-test reactivity to allergens. *New Engl J Med* 320:271, 1989.
33. Xu J, Postma DS, Howard TD, Koppelman GH, Zheng SL, Stine OC, Bleecker ER, Meyers DA: Major genes regulating total serum immunoglobulin E levels in families with asthma. *Am J Hum Genet* 67:1163, 2000.
34. Hoffjan S, Nicolae D, Ober C: Association studies for asthma and atopic disease: a comprehensive review of the literature. *Resp Res* 4:14, 2003.
35. Jackola DR, Liebler 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* 42:19, 2005.
36. Palmer LJ, Cookson OCM: Using single nucleotide polymorphisms as a means to understanding the pathophysiology of asthma. *Resp Res* 2:102, 2001.
37. Jackola DR, Basu S, Liebler CL, Willaert R, Luah SS, Oetting W, King R, Blumenthal MN: CD14 polymorphisms in atopic families: implications for modulated allergen-specific IgE and IgG1 responses. *Intl Archives Allergy Immunol* 139:217, 2006.
38. 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* 175:2061, 2005.
39. Hizawa N, Yamaguchi E, Jinushi E, Konno S, Kawakami Y, Nishimura M: Increased total serum IgE levels in patients with asthma and promoter polymorphisms at CTLA4 and FCER1B. *J Allergy Clin Immunol* 108:74, 2001.
40. Howard TD, Postma DS, Hawkins GA, Koppelman GH, Zheng SL, Wysong AKS, Xu J, Meyers DA, Bleecker ER: Fine mapping of an IgE-controlling gene on chromosome 2q: analysis of CTLA4 and CD28. *J Allergy Clin Immunol* 110:743, 2002.
41. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, Beaty TH: Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 264:1152, 1994.
42. Berek C, Ziegner M: The maturation of the immune response. *Immunol Today* 14:400, 1993.
43. 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* 21:436, 2000.