Genetic Control of Basal Serum Immunoglobulin E Level and Its Effect on Specific Reaginic Sensitivity*
(regulator gene/Ir genes/HL-A antigens/allergens/atopy)

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Communicated by Victor A. McKusick, May 28, 1974

ABSTRACT Studies of the distribution of total serum immunoglobulin E levels in nonallergic and allergic populations defined a cut-off point between low and high immunoglobulin E at 95 U/ml, based on Mendelian recessive inheritance of high immunoglobulin E level. Subsequent investigations of the distribution of total serum immunoglobulin E levels in 28 allergic families confirmed the recessive hypothesis. The results of quantitative skin tests in eight families, performed with between five and eight highly purified grass and ragweed pollen allergens per family, demonstrate that the immunoglobulin E-regulating gene exerts a profound effect on specific immunoglobulin E-mediated sensitivity, often masking the effect of HL-A associated immune response genes.

Atopic allergy in man is a complex disease associated with the presence of immunoglobulin E (IgE) reaginic antibodies (1). Since 1972, many authors have reported a familial predisposition toward atopic manifestations in general, as well as to each of separate major symptom types—allergic rhinitis, asthma, and eczema (reviewed in refs. 2-4). A further variable is the relative degree to which different allergic subjects develop IgE-mediated sensitivities to different allergen complexes (e.g., pollens) or, more specifically, to highly purified allergens that may be isolated from these complexes (4-8). From these and other considerations, one would anticipate that the genetics of IgE-mediated allergy would be extremely complicated (2-4, 8).

As a first approach to this problem, we have considered two broad classes of immunogenetic control over IgE-mediated allergy: (i) regulation of the biosynthesis and/or metabolism of all molecules of the IgE class, and (ii) the effects of specific immune response (Ir) genes. Family and twin studies of total serum IgE levels in man, by Hamburger and associates (9), strongly suggested that the basal level of IgE is under a fairly simple genetic control, the mechanism of which was not elucidated. Further genetic experiments in animals showed that certain Ir genes, controlling primarily (if not exclusively) T-cell function, are closely linked to the animal's major histocompatibility type (10, 11). The effects of these genes can only be observed under highly limiting conditions of immunization, a situation which pertains in human allergy to inhaled pollen antigens (4, 5, 8).

Abbreviations: IgE, immunoglobulin E; Ir, immune response (gene); Creg, crossreacting group (of HL-A antigens).

We have recently demonstrated a highly significant association between IgE and IgG antibody production to Ra5, a ragweed pollen antigen [molecular weight = 5000 (12)], and the possession of a major histocompatibility antigen of the HL-A7 crossreacting group (Creg) (7, 5, 13). In studies with more complex allergens, further genetic factors seemed to be masking associations analogous to those observed for Ra5 as well as genetic linkage in families between a specific allergic sensitivity and a specific HL-A haplotype (8). The present communication will show that one important factor that modulates the development of specific IgE antibody response is an autosomal gene that regulates basal serum IgE concentration.

MATERIALS AND METHODS

Unrelated Subjects. The distribution frequency of total serum IgE levels was ascertained on samples taken from 205 unrelated adults (121 men, 84 women) who were highly allergic to ragweed and/or grass pollens and who had not received immunotherapy for at least 2 years. Most samples (70%) were taken when the boosting effect of seasonal allergenic stimulation was minimized11. None of the remainder was taken during the probable period of maximal IgE level11. The distribution frequency of IgE levels in nonallergic subjects was derived from data on 106 unrelated adults, who were completely devoid of symptoms. Intradermal skin tests on 46 unselected nonallergic subjects, using very high concentrations (100 μg/ml) of both dialyzed ragweed and grass pollen extracts, gave negative, trace, or one-plus (0.5-cm wheel) reactions.

Families. The genetics of total IgE levels was studied in 26 Maryland families and 2 other families (1 Pennsylvania Amish and 1 Wisconsin) having a total of 108 children (56)

11 The minimal (basal) IgE levels occurred between mid-February and mid-May for individuals highly sensitive to grass and ragweed pollens. The total serum IgE levels in 17 untreated allergic subjects followed over 2 years (unpublished) showed a year-to-year variation of 16% (±14%). Seasonal allergic exposure produced a mean rise of 85% (±51%) above the basal level for the year, maximal levels occurring between mid-September and mid-October. On the other hand, ongoing ragweed immunotherapy (plus contributing seasonal exposure) caused a mean rise of 120% (±93%) in 20 allergic subjects during the first year, but a depression after several years of therapy. Serum levels of nonallergic subjects varied by no more than 10% throughout the year.
boys, 52 girls). All but two children were at least 7 years of age; the two younger children were included because they had unequivocally high IgE levels (>900 U/ml). The mean adult IgE level is reached before 7 years of age (14). At least one child in each Maryland family was allergic to grass and/or ragweed pollens. Maryland serum samples were drawn between March and early May, except for Families B7, B9, and E3 (Fig. 2), which were bled during late November and December. The Amish (C4) and Wisconsin (C5) families were bled during August. Allergy histories were taken from almost all subjects and confirmed by skin testing with crude and purified grass and ragweed allergens in most cases. Histories of immunotherapy, which can modify IgE levels, are given in the footnote to Fig. 2.

Quantitative Intradermal Skin Testing. Eight immunologically distinct allergens, isolated from grass and ragweed pollens, were used (Fig. 3). All samples except group IV were at least 95% pure by published physicochemical and immunological criteria (4–6, 12, 16, 17). Serial 10-fold dilutions (10⁻² to 1 kg/ml) of each allergen were prepared in sterile Tris-buffered physiological saline stabilized with 0.03% (w/v) human-serum albumin. The concentration of each allergen eliciting a 2-plus reaction (0.8–1.0 cm wheal; 2.0– to 4.0 cm erythema) 15–20 min after intradermal injection (0.05 ml) was taken to be the person’s sensitivity to the allergen (7).

HL-A Typing. The leukocytes of all family members were typed, in duplicate, by standard leukocytotoxotic microassay (18), with 90 or more defined sera capable of recognizing 31 different HL-A serotypes.

RESULTS AND DISCUSSION

Definition of Low and High IgE and Calculation of Gene Frequencies. The general tendency for allergic people to have high and nonallergic people to have low serum IgE levels is well established (19, 20), but some nonallergic people have atypically high and some allergic people have atypically low IgE levels. We will define the cut-off between high and low IgE as the level at which the combined percentages of such atypical subjects is minimized. First, the cumulative percent frequencies of IgE levels for nonallergic and allergic subjects was plotted (Fig. 1). The difference curve for these data was then calculated (dashed line). The maximum of this curve, at 95 ± 5 U/ml, gives our best estimate of the IgE cut-off level. Only 21% of the nonallergic subjects have IgEs above and only 22% of the allergic subjects have IgEs below this level. Our estimate of 95 U/ml is in excellent agreement with a value of 91 ± 5 U/ml, calculated by replotting data of Gleich et al. (20), taking 1 U/ml = 2.42 ng of IgE/ml (21).

In preliminary family studies (22), the mode of inheritance of high IgE levels, with or without demonstrable allergy, most closely approximated a Mendelian recessive pattern. Dr. T. A. Waldman, N.I.H., kindly supplied data on the serum IgE levels of 74 unselected adults living in the nearby Washington, D.C. area. A plot of his data showed that 27.5% of the population possessed IgE levels over 95 U/ml, from which we calculated gene frequencies of hypothetical alleles, R and r, to be 0.48 and 0.52, respectively, assuming Hardy–Weinberg equilibrium (23). Using analogous data obtained by Dr. G. J. Gleich, Mayo Clinic, Minnesota (ref 3; and data on 79 unselected adults, personal communication), we determined the frequencies of R and r to be 0.45 and 0.55. The close agreement between the data, for different populations living under different exposure conditions, is encouraging.

Test for Recessive Inheritance of High IgE. We analyzed three types of mating: type A, low × low giving at least one high IgE (Rr × Rr); type B, low × high giving at least one high IgE (Rr × rr), and type C, high × high (Fig. 2). Types A and B were analyzed by the “bias of ascertainment” method (23). In four families (types D and E) having all low children, it is not possible to determine whether the low parents are RR homozygotes or Rr heterozygotes.

Individuals with IgE levels near the 95 U/ml cut-off point could not readily be assigned as “high” or “low” for the following reasons: uncertainty in the precise value of the cut-off; year-to-year variation in basal IgE levels in allergic people; effect of known allergic stimulation in allergic members of families B7, B9, E3, C4, and C5; accuracy of individual IgE determinations. For samples taken at the most appropriate time of year, we estimate that there is an uncertain “gray region” extending about 10 U/ml below (arising from possible errors) and 26 U/ml above (arising from errors plus annual variation in basal IgE) the cut-off point. Allergic individuals falling in this region were assigned as “low,” and nonallergic individuals as “high” (see legend to Fig. 2). Of the five families bled at least appropriate times of the year, IgE levels could readily be assigned as high or low in B7, C4, and C5. Family B9 had one allergic and one (as yet) nonallergic child, having IgE levels of 102 U/ml and 88 U/ml, respectively (Fig. 3). In view of the boosting effect of allergenic stimulation, and the clearly defined distribution of IgE levels in this family, these two levels were taken to be low. All the children of family E3 were allergic and all, in-
female child died after A10, low. IgE

For families of types A, B, and C, we found a good agreement between the observed and predicted incidence of high IgE levels in the siblings (Fig. 2), especially considering the uncertainty in assigning people whose IgE levels were in the gray region, 85-121 U/ml. As predicted, all 25 siblings of the 5 type C families had high IgE levels. The incidences of high children in types A and B families are both slightly higher than one standard deviation above the theoretically predicted values. This may be due to a bias in selecting several families (especially B6 and B9) because they had more than one highly allergic child, or may result from the relatively small number of families studied. The apparent imbalance of male children with high IgE levels (42 boys compared with 22 girls) results from selecting most families from an allergy study in a boys' school. We saw no sex difference in the distribution of IgE levels in the children's parents or the 205 allergic adults studied, in agreement with other reports on adults and children (14, 20). Thus, we have every reason to believe that the IgE gene is autosomal. The small number of families with mating types D and E reflect the selection only of families with at least one allergic child.

Thus, the inheritance of a high serum IgE level appears to approximate closely to a simple Mendelian recessive trait.

We also suggest that the same conclusion is directly applicable to the experiments of Schwartzman et al. (24) in dogs. While they did not have reagents to measure canine IgE levels, they clearly demonstrated that matings of dogs having multiple allergic symptoms and skin reactivities (characteristic of high IgE levels in man) gave rise only to offspring with similar allergic manifestations. Analysis of their data on matings analogous to type B also gave results consistent with our hypothesis.

The gene product of the dominant R allele may regulate the rate of IgE biosynthesis in or secretion of IgE from plasma cells. Other possibilities for control include: regulation at the T-cell level (see ref. 25), differentiation of B-cells into IgE-producing plasma cells, and removal of circulating IgE by degradation or cellular adsorption. Whatever the mechanism, only the rr homozygotes would have their serum IgE levels determined by a less efficient regulator, the gene product of the r allele**.

Control of Specific IgE Response. The induction of specific IgE antibody synthesis (and resulting allergy) will depend

** It is quite possible that more than two alleles of this gene, and/or that further IgE-regulating genes, may be involved in determining IgE levels in certain people, particularly those having very high or very low levels.
on whether the individual has the necessary Ir genes (including those associated with HL-A), which determine recognition of the stimulating allergen in immunogenically limiting dosage. In order to ascertain the relative importance of (a) HL-A associated Ir genes and (b) the IgE-regulating gene, in determining specific IgE responsiveness in man, we made a detailed study of 24 pairs of highly allergic siblings from seven families. Three families were tested with eight allergens and the remainder with either five or six allergens. A sibling pair was considered to be concordant in sensitivity to a particular allergen when the ratio of skin sensitivities in the two members was less than or equal to 100, and discordant when this ratio was 1000 or greater.

Table 1 summarizes concordance of specific sensitivities with HL-A haplotype within pairs of highly allergic siblings. Each comparison represents one specific sensitivity in one of the pairs. Since not every allergic child is sensitive to every allergen, we made two types of comparison: column 1, irrespective of whether both children were negative to the allergen in question, and column 2, where at least one member of the pair gave a 2-plus reaction with the allergen solution at a concentration of $10^{-1}$ µg/ml or lower. We found no significant differences between the levels of concordance in overall specific sensitivities for HL-A identical pairs (group A), pairs sharing one HL-A haplotype (group B), and HL-A distinct pairs (group C).

In Table 2, the concordance in skin sensitivities to specific allergens is examined for pairs of highly allergic siblings who have either similar or very different IgE levels. Columns 1 and 2 present the same types of evaluation as presented in Table 1. In Table 2, we find a much greater overall concordance in specific sensitivities in group F, where both members of the pair have high IgE levels, than in group E, where the IgE levels are very different. For the more critical comparisons in column 2, the difference is highly significant by chi-squared analysis with Yates' correction ($P = 0.001$). Low-low pairs (group D) are sensitive to fewer allergens than high-high pairs, but there is not yet sufficient data to permit a valid comparison of group D with the other groups.

The importance of genetic regulation of total IgE level, relative to HL-A associated Ir genes, in determining specific sensitivity is further illustrated by Family B9 (Fig. 3). Of five highly allergic siblings, two have inherited HL-A haplotypes A and D; the other three have the alternative haplotypes B and C. For siblings nos. 3, 4, and 5 (genotypes BC, AD, and BC), there is a similar overall pattern of specific skin sensitivity to the eight highly purified allergens. If HL-A associated Ir genes were predominant determinants of

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**Table 1. Influence of HL-A genotype on the concordance of specific skin sensitivities within sibling pairs of eight families**

<table>
<thead>
<tr>
<th>Haplotypes of sibling pair</th>
<th>No. of sibling pairs</th>
<th>Concordance in specific skin sensitivity†</th>
</tr>
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<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Identical*</td>
<td>9</td>
<td>46/66 (70%) 14/24 (41%)</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One different*</td>
<td>6</td>
<td>32/45 (71%) 11/24 (46%)</td>
</tr>
<tr>
<td>Group C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both different*</td>
<td>9</td>
<td>48/65 (74%) 19/36 (53%)</td>
</tr>
</tbody>
</table>

* Groups include data from one family where the father was probably homozygous (see Fig. 4 of ref. 8). If this family is excluded, the analyses are not significantly different.
† See text.

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**Table 2. Influence of genetic control of basal IgE level on the concordance of specific skin sensitivities within sibling pairs**

<table>
<thead>
<tr>
<th>IgE levels of sibling pair</th>
<th>No. of sibling pairs</th>
<th>Concordance in specific skin sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-low</td>
<td>3</td>
<td>18/21 (86%) 3/6 (50%)</td>
</tr>
<tr>
<td>Group E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-high</td>
<td>11</td>
<td>52/83 (63%) 12/43 (28%)</td>
</tr>
<tr>
<td>Group F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-high</td>
<td>10</td>
<td>56/72 (78%) 29/45 (64%)</td>
</tr>
</tbody>
</table>
children nos. 6 and 8) with high IgE levels may not have clinical allergy or significant skin sensitivity to grass and ragweed pollen allergens. The young children may not yet have received sufficient allergenic exposure (see ages of onset of symptoms in siblings nos. 1, 2, 4, and 5); on the other hand, some members (particularly the father) may lack essential Ir genes or other "allergy genes." Second, some individuals have very low total IgE levels and yet are allergic (see sibling no. 1), but usually such individuals are sensitive to fewer allergens than people with high IgE levels. Third, there is no evidence of linkage between HL-A haplotype and IgE level. Finally, there is a clear failure to demonstrate parent-to-child transmission of IgE-mediated sensitivity to any allergen, ruling out the possibility of a single gene model to explain allergy.

Thus, in most allergic families, a gene regulating basal serum IgE level appears to mask the role played by hypothesized Ir genes linked to HL-A haplotype, in controlling the expression of specific IgE-mediated sensitivities to a number of different allergens. This conclusion contradicts the recent findings of Levine et al. (27), claiming to demonstrate genetic linkage between HL-A haplotype and skin sensitivity to ragweed antigen E in eight allergic families. We have several reservations about their results, discussed in detail elsewhere (3, 4). Despite an attempt to rule out the influence of allergic "expressivity" genes, we feel that most of their findings (particularly on several five-member families with two allergic members) could well be explained primarily by genetically determined differences in IgE level rather than HL-A haplotype. Also, the use of the highly complex multideterminant allergen, antigen E, which crossreacts with other ragweed allergens (4), complicates analysis of specific Ir genes.

We are left with the following dilemma: our family studies emphasize the importance of the IgE-regulating gene relative to the hypothesized HL-A-linked Ir genes in determining specific IgE-mediated response, but previous population studies (7, 5) showing association of HL-A7 Creg with response to the low molecular weight allergen, Rα5, point to the reverse. We believe that particularly for the more complex and more abundant allergens, the products of different alleles of Ir genes (perhaps controlling recognition of different carrier determinants at the T-cell level) may well be involved, especially where an individual has a high capacity to synthesize IgE. In such cases, the effects of Ir genes associated with single (or a limited number) of HL-A types would be masked by the influence of the IgE-regulating gene. This prediction is borne out in a recent population study showing a clear interrelation between Ir genes associated with HL-A8 Creg and the IgE-regulating gene in determining sensitivity to the rye grass group I allergen (28, 4). Future family studies investigating chromosomal linkage between HL-A type and specific IgE response to complex allergens should most appropriately be directed toward the relatively rare allergic families where all members have low IgE levels.

We thank Drs. S. H. Hsu, L. M. Lichtenstein, and P. S. Norman for providing some of the sera and clinical data, Drs. T. P. King and L. Goodfriend for giving ragweed allergens, and E. Hill, E. Jarrett, E. Weathers, P. Black, E. Siekierski, M. Askey, and the staff of the Immunogenetics Laboratory for assistance. Supported by NIH Grants AI 09565 and GM 10189 and a grant from the John A. Hartford Foundation, and by RCDA AI 50504 (to D.G.M.). This is Publication no. 108 of the O'Neill Research Laboratories.