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Immunology

Association of HLA antigens and total serum immunoglobulin E level with allergic response and failure to respond to ragweed allergen Ra3

(*IR* genes/*Is* genes/*Ig* regulator gene)

DAVID G. MARSH*†‡, GARY A. CHASE§, LINDA R. FREIDHOFF*†, DEBORAH A. MEYERS*†, AND WILMA B. BIAS¶

*Laboratory of the Howard Hughes Medical Institute in the Division of Clinical Immunology, ¶Division of Medical Genetics, Department of Medicine, School of Medicine and §Department of Health Services Administration, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205; and †O'Neill Laboratories, Good Samaritan Hospital, Baltimore, Maryland 21239

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ABSTRACT **We have applied rigorous statistical analysis to human immune response and HLA data in allergic subjects having varying sensitivity to the minor ragweed allergen, Ra3. By using regression analysis on a series of subsets of Ra3 responders and nonresponders, we obtained data supporting the concept of discrete "responder" and "nonresponder" phenotypes. We found significant *positive* associations between Ra3 response and total serum IgE and the presence of HLA antigens of the A2 crossreacting group (A2 and A28), and significant *negative* associations with antigens of the A3 crossreacting group (A3 and A11). Our results suggest that alleles of the *HLA-A* locus may function either as immune response or immune suppressor genes or that such alleles are epistatic to the response or suppressor genes. Our data add further support to previous findings that HLA-associated specific IgE response is strongly influenced by genetic regulation of basal IgE level.**

Recent research in laboratory animals—particularly inbred mice—has revealed that specific immunity toward foreign macromolecules is controlled in large part by a delicate balance between immune response (*Ir*) and immune suppressor (*Is*) genes linked to the major histocompatibility complex (1, 2). Because of the homology between the major histocompatibility complexes of mammalian species, it is reasonable to postulate that *Ir* and *Is* genes will be found within the human *HLA* region. However, because of the complex genetic and environmental factors governing human immune function, mapping of an *Ir* (or *Is*) gene to *HLA* has yet to be demonstrated by standard linkage analysis (3). It seems clear that other loci not linked to the major histocompatibility complex interact by various mechanisms in controlling immune response in animals and probably humans (reviewed in ref. 4).

We have focused not on widely studied (often highly complex) models of disease susceptibility, but rather on the genetics of specific immune function—namely, IgE and IgG antibody production and cellular immunity—all of which are readily measured in subjects responding to environmental allergens (5). Our previous studies of unrelated allergic individuals revealed significant associations between *specific* IgE responses and specific major HLA types and HLA crossreacting groups (Cregs)‖ (6–9). We were especially interested in the association between response to ragweed allergen Ra3 and HLA-A2 as a potential prototype of gene interaction. This association was particularly striking in individuals having genetically regulated low basal total serum IgE levels (8). It seemed that possession of HLA-A2 or A2 Creg (A2 and A28, and perhaps the more weakly crossreactive A9 group) might be a requirement *sine qua non* for an IgE response to Ra3 in atypical allergic subjects having low IgE phenotypes. This assertion, however, was based on the assumptions that one could distinguish between Ra3+ and Ra3− phenotypes, and between "low IgE" and "high IgE" phenotypes, by means of rationalized cutpoints.

We now explore HLA and IgE associations with Ra3 sensitivity in a more rigorous fashion utilizing standard statistical analyses in which data for basal IgE and Ra3 response are considered at their face value in the total group and a *series* of subsets of "Ra3 nonresponder" and "Ra3 responder" allergic subjects. In the total group, we show that both positive (for HLA-A2 Creg) and negative (for A3 Creg) associations exist for sensitivity to Ra3 and that IgE level is an important correlate of the Ra3 immune response phenotype. Within the various Ra3 nonresponder and responder subsets, these associations are greatly reduced or disappear altogether, providing evidence for the concept of Ra3+ and Ra3− phenotypes. Our data further suggest the possibility of interaction between three genes in determining Ra3 sensitivity: an IgE regulator gene (not associated with or linked to HLA), an *Ir* gene associated with A2 Creg, and an *Is* gene associated with A3 Creg.

SUBJECTS, MATERIALS, AND METHODS

Study Subjects. Subjects were sampled from a pool of allergic volunteers participating in a multifaceted clinical effort at the Good Samaritan Hospital, as described (8). The present study was confined to 139 unrelated Caucasian subjects of European extraction, 137 of which were the same as those on the two phases of the previous study. In brief, all of these individuals were highly allergic clinically to ragweed or grass pollens or both. There was an excess of male subjects (ratio, 3:2) and ages ranged from 12 to 59 years, with a mean of 32 ± 11 (SD) years.

Immunochemical Materials and Methods. Ragweed antigen Ra3 (M_r 12,100) was the preparation from Lawrence Goodfriend described previously (8). It was found to be homogeneous physicochemically and immunologically by using standard techniques of electrophoresis and immunoelectrophoresis (8), although traces of an impurity could be detected by subsequent crossed immunoelectrophoresis utilizing high concentrations of antigen and a potent anti-Ra3 serum (unpublished data).

Allergic (IgE-mediated) skin sensitivity to Ra3 was assayed

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Abbreviations: *Ir*, immune response (gene); *Is*, immune suppressor (gene); Creg, crossreacting group (of HLA antigens).

‡ To whom reprint requests should be addressed at the Good Samaritan Hospital, Baltimore, MD 21239.

‖ Groups of serologically related HLA specificities.

in our patients by intradermal titration using a series of antigen concentrations increasing by 10-fold in the range of 10^{-7}–1 μg/ml (8). Skin-test sensitivity was expressed as the concentration that elicited a 2-plus reaction (8- to 10-mm wheal with erythema) 15–20 min after injection of 0.05 ml of allergen solution.

Total serum IgE levels were determined as described (6) by a competitive binding double antibody radioimmunoassay technique, employing ^{125}I-labeled Fc fragment of human IgE and rabbit anti-IgE serum (Fc specific). Wherever possible, sera were drawn at times of the year when IgE levels were usually at their lowest (basal) values (10). IgE concentrations were expressed in international units/ml, where 1 international unit = 2.42 ng.

HLA Typing. Serological typing for the *HLA-A* and *-B* locus specificities shown in Table 1 was performed by standard leukocytotoxicity microassay using between 60 and 90 sera obtained from the National Institutes of Health and private sources (8). Before the present analyses were performed, about 30 of the individuals were retyped with additional antisera in order to identify antigens not recognized by the initial typing because appropriate reagents were unavailable. We concentrated on retyping subjects from the early stages of the previous study, particularly those with "blanks" or antigens that could be subclassified ("split").

Because many HLA antigens occur in quite low population frequencies, and because we have previously observed marked associations with HLA Cregs, certain analyses were performed by combining the HLA antigens into the following groups: (*i*) A1; (*ii*) A2 and A28; (*iii*) A9, AW23, and AW24; (*iv*) A3 and A11; (*v*) A10, A25, A26, AW19 (A29, AW30, AW31, AW32, and AW33); (*vi*) B5, B18, and BW35; (*vii*) B7, B27, and BW22; (*viii*) B12, B13, and B40; (*ix*) B8 and B14; and (*x*), a category of "other" relatively infrequent HLA types, B15, BW16, and B17.

Analytic Methods. The distribution of Ra3 sensitivity in our study sample was markedly nonnormal (Fig. 1). We were not able to normalize the data by standard transformation techniques. It is conjectured that the distribution is essentially a mixture of two major groups ("insensitive" and "sensitive") with the variability in Ra3 sensitivity within each group being the result of undetermined genetic and nongenetic interactions and random errors of measurement and sampling. It is unclear whether the hump of weakly sensitive subjects represents hyperresponders to trace impurities in the Ra3, weak responders to Ra3 itself, errors of measurement and sampling, or a complex mixture of all of these possibilities.

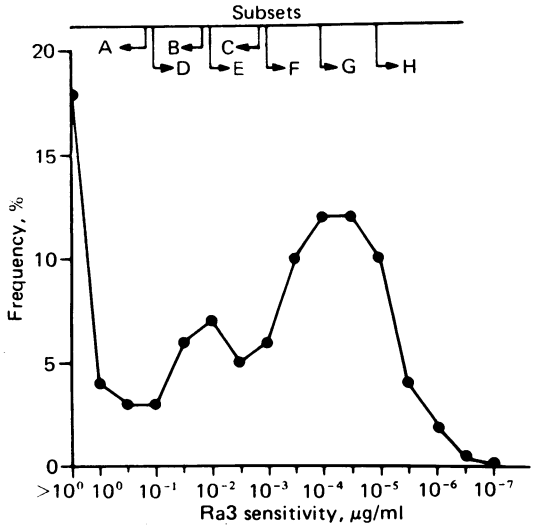

FIG. 1. Frequency distribution of Ra3 sensitivity in 139 allergic Caucasian subjects. Sensitivity defined by μg Ra3/ml producing a 2-plus endpoint in the range of 10^{-7}–10^{0} μg/ml. The insensitive categories of 10^{0} and $>10^{0}$ μg/ml were not precisely defined and were considered as 10^{0} μg/ml in the statistical analyses. Log [IgE] and HLA distributions for subsets A–H are analyzed in Table 3.

We decided to apply linear regression techniques to the whole distribution and then to several possible "nonresponder" and "responder" subsets defined by a series of cutpoints in Ra3 response (Fig. 1). Recognizing that the criteria for normality in the distribution of Ra3 response was not met, especially for the whole study group, we used nonparametric methods (Spearman rank order and χ^2 analyses) to validate the principal conclusions of these analyses.

The Biomedical Data Processing (BMDP) statistical package operating on a DEC-10 computer was used for the multiple regression analyses (11). We performed preliminary analyses to ascertain the most important factors affecting Ra3 response. Beginning with a series of 22 separate multiple regression analyses, we examined the relative influence of log [total IgE] and each individual HLA antigen on the outcome of Ra3 response.** Then, in a single multiple regression analysis we looked at the following 14 variables: sex, year of birth, month bled, log [IgE], and HLA type (divided into the 9 Cregs and one "other" category described in the previous section). The first set of analyses suffers from the drawback that several HLA antigens occur in low population frequencies, making it difficult to detect possible significant associations without studying several hundred subjects. In the second analysis, this drawback is reduced and other factors are considered at the expense of possibly masking individual HLA effects.

Additional analyses focused primarily on ascertaining the relationship between log [IgE] and individual HLA-A2 Creg and A3 Creg antigens in predicting Ra3 response, because these variables were found to have the greatest effect. First, we employed the multiple cutpoint approach discussed above in order to study the distribution of log [IgE] and A2 and A3 Cregs in different "nonresponder" and "responder" categories. Second, we explored the relationship between these variables by studying the distributions of Ra3 sensitivity and IgE levels within HLA phenotypes containing A2 Creg or A3 Creg antigens, and neither A2 nor A3 Cregs.

RESULTS AND DISCUSSION

The results of the preliminary regression analyses are shown in Tables 1 and 2. Table 1 gives the results from 22 separate analyses looking at each individual HLA type with log [IgE], and Table 2 shows data from a single multiple regression in which sex, age, month bled, log [IgE], and the HLA Cregs are considered as variables. In each analysis, HLA variables were defined as the presence or absence of a specificity. For example, the regression coefficient, *b*, of −1.3 for HLA-A1 means that, on average, the presence of A1 was associated with a decrease in Ra3 sensitivity of 1.3 units.

The presence of individual A2 Creg antigens (A2 and A28) in the HLA phenotype was found to be a significant *positive* predictor and the presence of A3 Creg antigens (A3 and A11), a significant *negative* predictor of Ra3 sensitivity (Table 1). These findings were confirmed by a single multiple regression analysis shown in Table 2, where $P = 0.003$ for A2 Creg and P

** A separate analysis was performed for each antigen except for the A9, A10, and AW19 groups where we were not able to differentiate between splits in all patients with the sera available at the time of typing.

Table 1. Data from 22 regression analyses examining effect of individual HLA specificities and log [Total IgE] on Ra3 response

HLA locus*	Frequency, %	Association with HLA b†	P‡
A			
1	30	−1.30	
[2	47	8.91	0.006
[28	4	20.8	0.015
[3	26	−10.3	0.005
[11	12	−10.9	0.030
9, 23, W24	18	0.009	
[10, 25, 26	13	1.13	
[W19, (29–33)	14	3.97	
B			
[5	9	6.10	
18	5	2.12	
[W35	9	−1.65	
[7	25	−4.26	
27	6	−2.63	
[W22	4	3.58	
[12	37	7.69	0.020
13	5	−10.3	
[40	14	−9.08	0.052
[8	29	3.39	
[14	6	−6.55	
15	6	9.15	
W16	4	−16.5	0.037
17	6	−6.13	

* Creg groups used in analyses in Table 2 are bracketed.
† b = regression coefficient for the HLA antigen. The regression coefficients for log [IgE] were in the range of 11.6–13.2 for all of the 22 analyses. Corresponding P values were all ≤ 0.001.
‡ Only values of $P \leq 0.1$ cited (two-tailed test).

= 0.007 for A3 Creg. Although 14 possible predictors of Ra3 response were tested in a single linear regression, log [IgE], HLA-A2 Creg, and Creg A3 were the only significant predictors. The significant P values for variables in a single multiple regression analysis do not suffer from the objections raised by examining the same variables in separate analyses. Our finding that, for all of the analyses shown in Tables 1 and 2, log [IgE] was strongly associated with Ra3 sensitivity indicates that Ra3 response increases significantly with increasing total IgE level. Sex, age, and the month in which the samples were taken were not significantly correlated with Ra3 response (Table 2).

Table 2. Multiple regression analysis examining effects of sex, age, month bled, log [IgE], and HLA Cregs on Ra3 sensitivity

Variable	b*	P†
Sex	0.60	
Year of birth	0.15	
Month bled	0.38	
Log [IgE]	13.4	<0.001
HLA-Al	1.71	
HLA-A2,A28	12.1	0.003
HLA-A3,11	−11.6	0.007
HLA-A9,23,W24	2.43	
HLA-A10,25,26,AW19 group	3.43	
HLA-B5,18,W35	1.77	
HLA-B7,27,W22	0.28	
HLA-B12,13,W40	−1.49	
HLA-B8,14	−0.49	
HLA-B15,W16,17	−0.55	

* Regression coefficient.
† Only P values ≤ 0.1 cited (two-tailed test).

In addition, B12 (a specificity in linkage disequilibrium with A2) showed a positive association, and BW16 and B40 showed negative associations ($P < 0.05$ in all cases; Table 1). When one considers the number of analyses performed, these weak associations are probably not significant. To investigate these possible weak associations further, we studied a subsample of 40 individuals obtained by excluding all subjects with A2 Creg or A3 Creg antigens or both in order to eliminate *A*-locus associated effects. When this was done, no significant effect of either B12 ($b = 3.1$, $P = 0.58$) or B40 ($b = -10.1$, $P = 0.19$) remained. The negative regression coefficient for B40 ($b = -10.1$) hints at a possible significant association if larger samples could be studied.

The next series of analyses (Table 3) focused on ascertaining the relative influence of component A2 and A3 Creg specificities and log [IgE] on the degree of Ra3 response within various subsets of our study group, defined by a series of cutpoints in Ra3 sensitivity (Fig. 1). For example, in the analysis of group D we considered the association between the magnitude of Ra3 sensitivity and the HLA and log [IgE] variables *only* for subjects possessing sensitivity endpoints at $\leq 10^{-1}$ μg/ml. Group E is included in group D, and group F with group E, etc. The cutpoints were chosen to cover a wide range of possible nonresponder and responder categories, with and without the difficult-to-define "hump" of weak responders (Fig. 1). By this procedure we explored whether it might be possible to define groups of nonresponders and responders, each of which is relatively homogeneous in terms of associations of Ra3 response with log [IgE] and HLA-A2 and A3 Creg specificities.

Although strong associations were found between Ra3 response and A2, A28, A3, A11, and log [IgE] for the *total sample* [indicated by large positive or negative regression coefficients, b, with significant P values (see Table 3)], the associations were markedly reduced or nonexistent for the various subsets. The only significant associations ($P < 0.05$) were found in the nonresponder subsets, groups B and C. In clear nonresponders (group A), as well as all the responders subsets (groups D–H), no significant associations were found. With regard to defining categories of nonresponders, the problem arises with the inclusion of weak responders—specifically, those having sensitivity endpoints at $\leq 10^{-1}$ μg/ml and $\geq 10^{-3}$ μg/ml (group C minus group A). This sensitivity range corresponds precisely to the "hump" of weak responders in the Ra3 sensitivity distribution (Fig. 1). Therefore, by applying cutpoints throughout the distribution, we have defined a region (sensitivity $\leq 10^{-1}$ and $> 10^{-3}$ μg/ml) that appears to divide the sample into two categories of response to Ra3. This suggests the overall sample is a mixture of two populations and supports our concept of responder and nonresponder phenotypes (8). Within either population a significant regression between Ra3 response and IgE level and between Ra3 response and HLA A2 Creg or A3 Creg is not seen. It is the combining of these two populations (which differ in Ra3 response, IgE level, and possession of HLA A2 Creg and A3 Creg) that generates the significant regression coefficients seen in the analysis of the total sample.

Because the distribution of Ra3 sensitivity was not normal (Fig. 1), we utilized nonparametric statistics to confirm the three major Ra3 associations—with IgE level, A2 Creg, and A3 Creg. By the conservative Spearman rank test, sensitivity to Ra3 was shown to be significantly associated with total IgE level ($r_s = 0.33$; $P < 0.001$). We utilized the χ^2 test to confirm the positive HLA-A2 Creg and negative A3 Creg associations by using the two most logical cutpoints defined by the Ra3 distribution (Fig. 1) and the analyses in Table 3—namely, where the Ra3$^+$ phenotype was defined by sensitivities of $\leq 10^{-1}$ μg/ml and $\leq 10^{-3}$ μg/ml. Significant associations were obtained for A2

Table 3. Analysis of the association of Ra3 sensitivity with A2 and A3 Creg specificities and log [IgE] in a series of nonresponder and responder subgroups

		Ra3 nonresponders*			Ra3 responders*				
Variable	Total sample*	$>10^{-1}$ A	$>10^{-2}$ B	$>10^{-3}$ C	$\leq 10^{-1}$ D	$\leq 10^{-2}$ E	$\leq 10^{-3}$ F	$\leq 10^{-4}$ G	$\leq 10^{-5}$ H
HLA-A2	9.1	−0.35	1.6	2.7	2.9	3.1	1.6	−0.51	−0.27
	0.003								
HLA-A28	19.6	—	—	—	11.5	9.7	5.3	5.7	3.2
	0.02				**0.07**	**0.08**		**0.09**	
HLA-A3	−9.3	−0.91	−2.0	−6.3	−5.5	−3.4	−2.9	−3.3	−2.1
	0.008			**0.02**	**0.08**			**0.10**	
HLA-A11	−11.4	0.85	−0.89	−4.9	−3.3	−2.8	−3.8	−5.1	−2.5
	0.02							**0.08**	
Log [IgE]	13.9	0.34	6.2	9.4	3.1	3.3	3.1	1.7	2.0
	<0.001		**0.006**	**<0.001**					
% of sample	100%	23%	30%	42%	77%	70%	58%	46%	22%

Regression coefficients (b) and in regular type and P values (for $P \leq 0.1$) are in heavy type (two-tailed test).
* Given as sensitivity (μg/ml producing 2-plus reaction).

Creg and A3 Creg in both sets of analyses (Table 4, part A). To investigate further the relative influence of A2 and A3 Cregs as determinants of Ra3 sensitivity, we divided our sample into the following phenotypic classes: I. A2 Creg, X (where X $\not\equiv$ A3 Creg); II. A3 Creg,Y (where Y $\not\equiv$ A2 Creg); III. A2 Creg,A3 Creg; and IV. Z,Z′ (where Z and Z′ $\not\equiv$ A2 or A3 Creg). As expected, the A2 Creg,X phenotype was significantly *positively* and the A3 Creg,Y phenotype was significantly *negatively* associated with Ra3 sensitivity by χ^2 analysis, and no significant associations were observed for the Z,Z′ phenotype, using either the 10^{-1} or the 10^{-3} μg/ml Ra3 sensitivity cutpoint. For the heterozygous A2 Creg,A3 Creg phenotype, there was evidence of a negative (but not significant) association with Ra3 response using the 10^{-3} μg/ml cutpoint but not the 10^{-1} μg/ml cutpoint (Table 4, part B).

These findings led us to explore the Ra3 sensitivity distributions within each of the phenotypic categories I–IV. As shown in Fig. 2, these distributions provide further evidence for the strong *positive* association of Ra3 sensitivity with A2 Creg,X and strong *negative* association with A3 Creg,Y. There was an almost complete association of A2 Creg,X with response to Ra3 (over 90% had sensitivities $<10^{-1}$ μg/ml). On the other hand, despite the negative association with A3 Creg,Y, most subjects with this phenotype exhibited *some* response to Ra3 (58% had sensitivities $<10^{-1}$ μg/ml). Of particular interest was the suggestive evidence of a bimodal distribution of Ra3 sensitivity in the small category of A2 Creg,A3 Creg heterozygotes, with roughly half the subjects having a strong response (Ra3 sensitivity $\leq 10^{-3.5}$ μg/ml) and the other half having weak or no response (Ra3 sensitivity $\geq 10^{-2}$ μg/ml). The geometric means for the total IgE levels for the two distribution modes were very similar—225 international units/ml for Ra3+ and 217 international units/ml for Ra3−—suggesting that factors other than IgE level modulate Ra3 sensitivity in people having the A2 Creg,A3 Creg phenotype.

In all categories except A2 Creg,A3 Creg, total IgE level *was* a significant predictor of Ra3 sensitivity using the Spearman rank test (see legend to Fig. 2). Not surprisingly, the strongest correlation ($r_s = 0.51$; $P = 0.001$) was found in the Z,Z′ category where strong HLA influences were absent. To investigate further the effect of IgE level on Ra3 response for the subset of individuals possessing A2 Creg (combined categories I and III) versus the remainder of the sample, we performed linear regression analyses of log [IgE] on Ra3 response. A significant difference ($P < 0.001$) between the estimates of the regression coefficients (b) of the two groups was found ($b = 8.5$ and 17.9 for A2 Creg and non-A2 Creg, respectively), reflecting the high frequency of A2 in subjects with low IgE levels. Overall, these results emphasize the marked effect of total IgE level in influencing specific IgE-mediated response to Ra3 in most allergic subjects.

These studies have provided rigorous analyses of human immune response data by utilizing standard statistical techniques including multiple regression analysis, a technique not previously applied to such data. A novel feature was the use of regression analysis on a series of subsets of Ra3 responders and

Table 4. Tests of association between Ra3 sensitivity and phenotypes containing A2 Creg or A3 Creg or both

		Cutpoint at 10^{-1} μg/ml*			Cutpoint at 10^{-3} μg/ml*		
Category	Phenotype	Ra3+ ($N = 107$)	Ra3− ($N = 32$)	P†	Ra3+ ($N = 81$)	Ra3− ($N = 58$)	P†
A.	A2 Creg	56.1	31.2	0.01	59.3	37.9	0.01
	A3 Creg	29.9	56.2	−0.006	27.2	48.3	−0.01
B.‡ I.	A2 Creg,X	41.1	12.5	0.003	46.9	17.2	0.0003
II.	A3 Creg,Y	15.0	37.5	−0.005	14.8	27.6	−0.06
III.	A2 Creg,A3 Creg	15.0	18.7	NS	12.3	20.7	NS
IV.	Z,Z′	29.0	31.2	NS	25.9	34.5	NS

NS, not significant ($P > 0.1$).
* Frequencies of Ra3+ and Ra3− phenotypes given as percentages.
† χ^2 analysis. Negative signs indicate negative associations.
‡ X $\not\equiv$ A3 Creg; Y $\not\equiv$ A2 Creg; Z and Z′ $\not\equiv$ A2 Creg or A3 Creg.

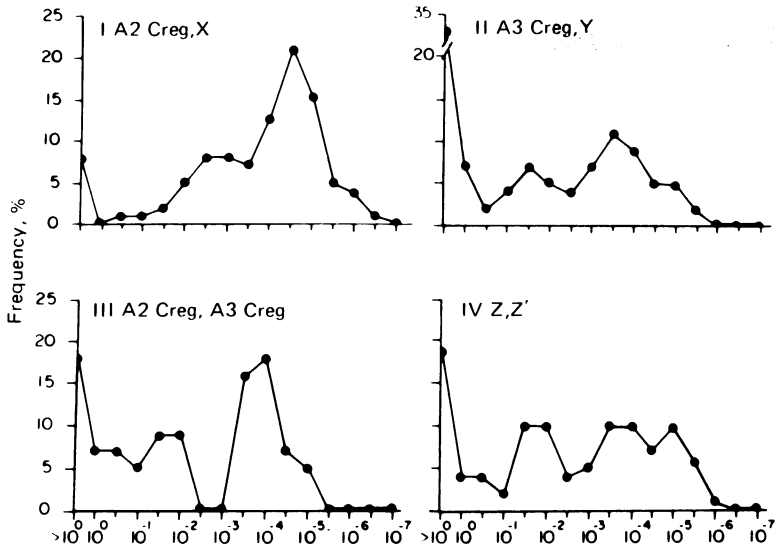

FIG. 2. Frequency distributions of Ra3 sensitivity in allergic subjects within each of the following phenotypic categories: I. A2 Creg,X (where X ≠ A3 Creg) (N = 48); II. A3 Creg,Y (where Y ≠ A2 Creg) (N = 28); III. A2 Creg,A3 Creg (N = 22); and IV. Z,Z' (where Z and Z' ≠ A2 Creg or A3 Creg) (N = 41). Spearman rank tests for the correlation of total IgE with Ra3 sensitivity in each category gave the following results: I. $r_s = 0.32$, $P = 0.02$; II. $r_s = 0.42$, $P = 0.03$; III. $r_s = 0.29$, $P = 0.2$; IV. $r_s = 0.51$, $P = 0.001$.

nonresponders, the results of which supported our previous hypothesis of Ra3 responder and nonresponder phenotypes. We have demonstrated significant positive associations of Ra3 allergic sensitivity with both total IgE level and HLA-A2 Creg antigens (A2 and A28) and significant negative associations with HLA-A3 Creg antigens (A3 and A11) (Tables 1 and 2). This suggests that immune suppression as well as immune response is associated with *A*-locus crossreacting groups. Response to Ra3 in individuals who possess both A2 Creg and A3 Creg alleles appears to be distributed into two groups—responders and nonresponders—although testing of a larger sample of heterozygotes is needed to clarify the issue.

These results have extended and confirmed our previous findings (8) of association between Ra3 sensitivity and HLA-A2. Our previous studies showed that the association with A2 is strongest in individuals with low IgE phenotypes (<120 international units/ml), where over 90% of Ra3 responders possess A2. The present analyses have revealed that the high frequency of A2 in subjects with low IgE is reflected in a significant difference ($P < 0.001$) in the estimates of the slopes of the regression lines of IgE level on Ra3 response for the group possessing A2 Creg versus the group not possessing A2 Creg. Because there is now ample evidence that basal serum IgE levels are genetically determined (10, 12, 13), we conclude that this genetic effect exerts a greater influence on Ra3 response in people *without* A2 Creg than those *with* A2 Creg.

How are these findings relevant to arguments concerning the existence of genes that govern immune response and immune suppression in humans? Given the relatively small chromosomal segment comprising the major histocompatibility complex of man and the likelihood that the population exists in a state of genetic disequilibrium, the associations are consistent with hypothesized *HLA*-linked *Ir* and *Is* genes. In view of the marked association *both* of response *and* failure to respond with *HLA* Cregs, the possibility exists that *A*-locus alleles may, themselves, function as *Ir* or *Is* genes. However, we believe that a more probable explanation is that such alleles are epistatic[††] to specific *Ir* or *Is* genes (for further discussion of Creg associations, see also refs. 4 and 9). Thus, the serological crossreactivity detected by HLA typing may reflect a similarity of function with respect to facilitating either response or suppression of response toward Ra3. Interacting with a hypothesized *Ir* gene is a gene, *R/r*, which regulates IgE level (10, 13). In hyper-IgE responders (genotype *rr*), any influences of *HLA*-associated genes on Ra3 response usually appear as secondary effects to the IgE-regulator gene. Family studies need to be performed in order to show definitively whether IgE response and suppression of IgE response to Ra3 are inherited in Mendelian fashion and to investigate *Ir* and *Is* linkage relationships with HLA loci.

[††] Interaction between genes situated at different loci, where one gene influences the expression of another gene.

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1. Benacerraf, B. & Germain, R. N. (1978) *Immunol. Rev.* **38**, 70–119.
2. Katz, D. H. (1977) *Lymphocyte Differentiation, Recognition and Regulation*, (Academic, New York), pp. 530–630.
3. Bias, W. B. & Marsh, D. G. (1975) *Science* **188**, 375–377.
4. Willcox, H. N. A. & Marsh, D. G. (1978) *Immunogenetics* **6**, 209–225.
5. Marsh, D. G. & Bias, W. B. (1978) *Immunological Diseases*, ed. Samter, M. (Little, Brown, Boston), 3rd Ed., pp. 819–831.
6. Marsh, D. G., Bias, W. B., Hsu, S. H. & Goodfriend, L. (1973) *Science* **179**, pp. 691–693.
7. Marsh, D. G., Bias, W. B., Santilli, J., Schacter, B. & Goodfriend, L. (1975) *Immunochemistry* **12**, 539–543.
8. Marsh, D. G., Goodfriend, L. & Bias, W. B. (1977) *Immunogenetics* **5**, 217–233.
9. Marsh, D. G. & Bias, W. B. (1977) *Immunogenetics* **5**, 235–251.
10. Marsh, D. G., Bias, W. B. & Ishizaka, K. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3588–3592.
11. Draper, N. R. & Smith, H. (1966) *Applied Regression Analysis* (Wiley, New York).
12. Bazaral, M., Orgel, H. A. & Hamburger, R. N. (1974) *J. Allergy Clin. Immunol.* **54**, 288–304.
13. Gerrard, J. W., Rao, D. C. & Morton, N. E. (1978) *Am. J. Hum. Genet.* **30**, 46–58.