

Basal Serum IgE Levels and HLA Antigen Frequencies in Allergic Subjects

II. Studies in People Sensitive to Rye Grass Group I and Ragweed Antigen E and of Postulated Immune Response (*Ir*) Loci in the HLA Region*

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Abstract. In allergic Caucasian subjects, IgE-mediated responsiveness to the rye grass Group I (Rye I) antigen is significantly associated with possession of HLA-B8 ($p=0.001$). Analysis of this association relative to basal total serum IgE level showed a marked relationship in Rye I-negative subjects, where the frequency of B8 was much higher at high than at low IgE levels. In Rye I-positive subjects, there was evidence for the converse situation. In the case of people sensitive to ragweed antigen E, we found no association with any HLA specificity, even in patients with low IgE levels. The *HLA*-associated specific IgE responses to rye grass Group I, and those we have previously reported for ragweed Ra3 and Ra5, lead us to speculate about the possible locations within the *HLA* complex of putative *Ir* loci controlling recognition of these allergens. Further, we postulate the existence of two *I* regions. *I-1*, which contains “*Ir-Rye I*” and “*Ir-Ra5*”, and *I-2*, which contains “*Ir-Ra3*”. Alternative explanations of our findings are also discussed in detail.

Introduction

We previously found that both HLA type and basal total serum IgE level are important determinants in the expression of specific IgE-mediated responses in allergic people (Marsh *et al.* 1973a, Marsh *et al.* 1974, Marsh *et al.* 1975, Goodfriend *et al.* 1977). Evidence for a relationship between HLA frequency and basal IgE level in unrelated allergic subjects is particularly strong in the study of Ra3 sensitivity reported in the preceding paper (Marsh *et al.* 1977). In this case, association between specific IgE-mediated response and HLA type is clearly evident in people with low total serum IgE levels, but is masked in people with high IgE levels.

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In the present study, we wished to explore further associations between specific IgE-mediated responses and HLA type, and, in particular, relationships between HLA frequency and total IgE level. We therefore elected to study IgE responses to two important pollen allergens—rye grass Group I (Rye I; M.W. 27,000) and ragweed antigen E (AgE; M.W. 37,800), both of which are more complex, and allergenically more important, than Ra3. We will show that the regulation of basal serum IgE level is an important factor to be considered in studying *HLA* relationships in specific IgE response to Rye I, but not to AgE. In the case of Rye I, there is a significant association between specific immune response and the possession of HLA-B8, which is more pronounced in people with total IgE levels falling in the lowest IgE quartile of our study group. On the other hand, in the case of the most complex allergen, AgE, there is no evidence for an association with any HLA type, even in individuals with genetically limiting, low total IgE levels.

Analysis of the associations between responsiveness to Rye I and the possession of HLA-B8 and A1, and comparisons with the data presented for people sensitive to Ra3, provide a basis for speculation about the map positions of putative *Ir* genes in the human. Such arguments take into account the linkage disequilibria among loci of the *HLA* complex.

Materials and Methods

Subject Selection. Our study subjects were selected on a basis similar to those reported in the preceding paper (Marsh *et al.* 1977), except that the emphasis in the present selection was for marked clinical sensitivity to grass and/or ragweed pollens—not specifically ragweed. The study group consisted of 214 Caucasians of European extraction (131 M, 83 F) and 17 American Blacks (8 M, 9 F). Ages ranged from 11 to 59 years (mean = 30 ± 9 years). As in the previous study, approximately 25% of our study subjects had been on immunotherapy at some time with grass and/or ragweed pollen extracts, although fewer than 10% had received therapy during the previous 2 years.

Antigens. The rye Group I (Rye I) allergen was purified from the pollen of perennial rye grass (*Lolium perenne*), according to the method of Johnson and Marsh (1965), with modifications (Marsh 1975). Antigen E (AgE) was purified from the pollen of short ragweed (*Ambrosia elatior*) by the method of King and coworkers (1964). Antigen E was initially kindly supplied by Dr. T.P. King, Rockefeller University, but later it was purified in our laboratory from material obtained through Dr. John Nutter (Marsh *et al.* 1977). The homogeneity of these antigen preparations was established as described in the preceding paper and by Johnson and Marsh (1965). By agarose and starch gel electrophoresis, the Rye I preparation was found to consist of the 2 major isoallergens, I-B and I-C and traces of the minor isoallergens, I-A and I-D (Johnson and Marsh 1965, Marsh 1975). Similarly, by acrylamide and agarose gel electrophoresis, as well as by isoelectric focusing, the AgE preparations were found to contain predominantly the AgE-B and AgE-C isoallergens with smaller amounts of other antigenically closely related isoallergens. Rye I and AgE have been shown previously to be completely noncrossreacting antigenically and allergenically (Lichtenstein *et al.* 1969, Marsh 1975).

Quantitative Assay of IgE-Mediated Sensitivity. Immunoglobulin E-mediated skin sensitivity to both Rye I and AgE was assayed in 183 Caucasian and 15 Black patients by intradermal titration, as described in the preceding paper. Subjects' leukocyte histamine release sensitivities to both antigens were determined in 87 Caucasian and 7 Black patients, using the *in vitro* leukocyte histamine release assay of Lichtenstein and Osler (1964), as modified by May and coworkers (1970).

(These data were kindly supplied by Dr. L.M. Lichtenstein.) A patient's sensitivity is expressed as the antigen concentration which elicits release of 50% of the total cellular histamine. The leukocyte histamine release is considered to be more precise than skin testing in that replicate tests agree to within $\pm 10\%$ (L.M. Lichtenstein, personal communication).

The available sensitivity data on our 214 Caucasian and 17 Black patients are summarized in Table 1. The people who were skin tested represent a somewhat less selected sample of our patient population than those who were studied by histamine release. Nevertheless, there was a definite bias in the skin-test data against testing people who were sensitive to Rye I, but not to antigen E, because of the idiosyncracies of the various clinical and immunological studies from which the patients were collected. In the case of leukocyte histamine release, patients who were clinically allergic to only 1 of the 2 types of pollen (ragweed or grass) were generally assayed only with the antigen from the pollen to which they were sensitive (AgE or Rye I). Hence, these patients are an even more selected subset of our patient group than those who were skin tested. While either the skin-test or the leukocyte data have inherent biases when considered separately, when considered together they represent a good cross-section of Rye I and AgE sensitivities in the types of allergic patient being studied by the Johns Hopkins Clinical Immunology Division (see *Discussion*).

The foregoing considerations provide a rationale for grouping together our skin-test and leukocyte histamine release data. This is possible using allergen sensitivity ratios (Rye I/AgE), but not absolute sensitivity measurements for a single allergen. In 40 patients sensitive to Rye I and AgE who were assayed by both skin testing and histamine release, the Rye I/AgE ratios by the 2 methods correlated very well ($r=0.76$; $p<0.001$), as previously reported for sensitivity ratios involving other allergens (Marsh *et al.* 1975, Marsh *et al.* 1977). Conversely, the relationship between absolute skin-test and leukocyte histamine release sensitivities to a particular allergen varies from person to person, with a small proportion of highly allergic people being quite insensitive by the leukocyte assay (Conroy *et al.* 1977).

Thus, analysis of ratio data has certain advantages in the present case (see also Marsh *et al.* 1977). Another advantage of the Rye I/AgE ratio is that one can easily compare groups of patients who are similarly sensitive to both allergens, sensitive only to Rye I, and sensitive only to AgE. A disadvantage of using sensitivity to AgE as the denominator is that Rye I and AgE come from different pollens; therefore, in contrast to the situation with Ra3, response to AgE does not adjust so well for individual variations in allergen exposure. Calculation of possible allergen dosages (Marsh *et al.* 1973b, Marsh 1975) suggests that the mean annual dosages of grass Group I and ragweed AgE are similar for people experiencing similar degrees of outdoor exposure during the grass and ragweed pollen seasons. The use of ratios might be invalid if a significant relationship should exist between response to AgE and a specific HLA type, or if people who respond to AgE have an atypical distribution of total IgE levels. It was therefore necessary to investigate both of these possibilities.

Hence, we have elected to present detailed analyses of both absolute sensitivities toward Rye I and AgE and the Rye I/AgE sensitivity ratio data. Absolute sensitivity data will be presented only for skin tests where we have a larger, and somewhat less biased, patient subgroup than for the leukocyte assay. Sensitivity ratio data will be presented for our total patient group, generally giving preference to histamine release measurements (where available), since these measurements are somewhat more precise than skin tests.

Other Assays. HLA typing and total serum IgE analysis were performed precisely as described in the preceding paper.

Results

Our remarks will be confined to the large Caucasian group of patients unless otherwise stated, since the Black group is too small for most analyses. The frequencies of HLA antigens (Albert *et al.* 1973) and total IgE levels (see below) are different in the two races, which must, therefore, be considered separately.

Table 1. Summary of Skin-Test (ST) and Histamine Release (HR) Data on the Total Allergic Sample of 214 Caucasians and 17 Blacks

	Number of Patients Skin-Tested			Number of Patients Assayed by HR			Number of Patients Assayed with Both Ags. by ST and HR
	Both Ags.	AgE only	Rye I only	Both Ags.	AgE only	Rye I only	
Caucasian	183	25	6	87	75	10	57
Black	15	1	0	7	8	1	4

Absolute IgE-Mediated Skin Sensitivity Measurements

Quantitative intradermal skin testing with both Rye I and AgE was performed on 86 percent of our patients (Table 1). All of these people had a "2-plus" skin sensitivity endpoint (8–10 mm wheal with erythema) to one or both allergens at concentrations of 10^{-3} $\mu\text{g/ml}$ or less. The corresponding distributions of skin sensitivity to Rye I and AgE in the 183 Caucasians tested with both antigens are presented in Figure 1. For both antigens, there were maxima in the distributions at 10^{-4} $\mu\text{g/ml}$ (approximately 3×10^{-10} M for both antigens) and minima at 10^{-2} $\mu\text{g/ml}$. Complete (or almost complete) lack of reactivity (2-plus reaction at ≥ 1 $\mu\text{g/ml}$) was uncommon to AgE, where it was found in only seven percent of our patients; but 29 percent of the study group were unreactive to Rye I. Similar results were obtained for the much smaller Black sample.

The observed distribution of HLA antigens in Caucasian subjects who were either highly skin-sensitive to Rye I (sensitivities 10^{-7} to 10^{-3} $\mu\text{g/ml}$) or relatively insensitive to Rye I (sensitivities 10^{-2} to ≥ 1 $\mu\text{g/ml}$), and the corresponding data for AgE, are presented in Table 2. There were significant positive associations between Rye I sensitivity and the possession of HLA-B8 ($p=0.0009$) and, to a lesser extent, with HLA-A1 ($p=0.05$). The phenotype A1,B8 was also significantly associated with sensitivity to Rye I ($p=0.03$). In addition, there were negative associations between Rye I sensitivity and HLA-A9, BW15, BW17, and BW40. The strong negative association ($p=-0.0004$) between Rye I sensitivity and A9 is not unexpected in view of the negative association between A9 and B8 found in Caucasians (Albert *et al.* 1973).

There were no associations significant at the $p < 0.1$ level for the comparison of the AgE-sensitive versus the AgE-insensitive group. While A9 was more than twice as common in the AgE-sensitive as in the AgE-insensitive subjects, this probably reflects the pronounced negative association between Rye I response (present in the AgE-negative people) and A9. The frequencies of A1 and B8 were similar in both AgE-sensitive and AgE-insensitive groups.

Analysis of HLA Distributions in Subgroups Differing in Rye I/AgE Ratio

The distribution of Rye I/AgE sensitivity ratios in the 214 Caucasians was essentially trimodal (Fig. 2). Group A ($N=32$) was comprised of individuals

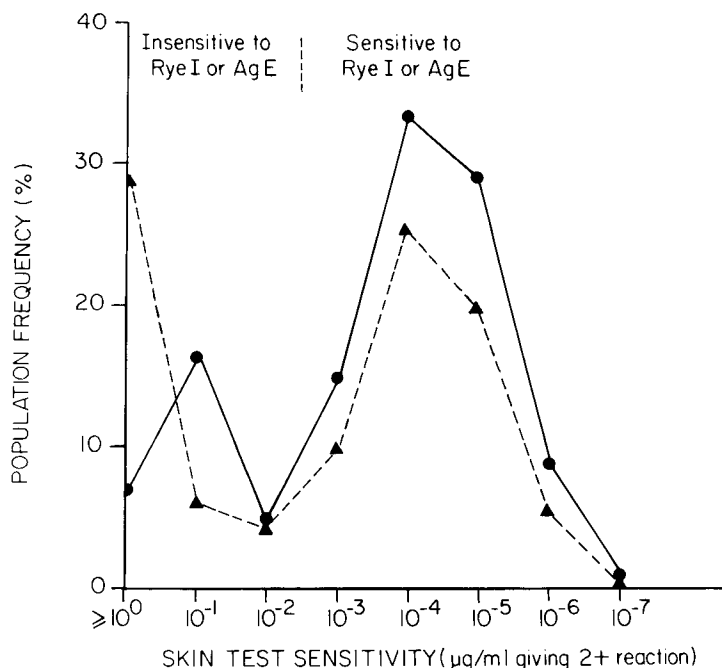


Fig. 1. Distributions of skin sensitivity to allergens Rye I (dashed line) and AgE (solid line) in 183 allergic Caucasian individuals

highly sensitive to Rye I but completely, or almost completely, insensitive to AgE; group B ($N=116$) comprised the majority of our study patients, who were highly sensitive to both Rye I and AgE; group C ($N=66$) was a relatively large group of subjects who were highly sensitive to ragweed AgE but insensitive to Rye I. The distribution frequency for the sample of 17 American Blacks was found to be quite similar to that of our Caucasian subjects.

Although varying widely in their relative sensitivity to ragweed AgE, groups A and B had a common high relative sensitivity to the Rye I allergen. The combination of these two groups will be referred to as the "Rye I-positive (*Rye I*+ phenotype)". In sharp contrast, group C was completely, or almost completely, insensitive to Rye I, but was highly sensitive to AgE. It will be referred to as the "Rye I-negative (*Rye I*-) phenotype". By analogous reasoning, combined groups B and C will be denoted as the "*AgE*+ phenotype," and group A the "*AgE*- phenotype."

The distributions of HLA antigen frequencies among Caucasian groups A through C, and for *Rye I*+ versus *Rye I*- phenotypes and *AgE*+ versus *AgE*- phenotypes, are presented in Table 3. Statistical analysis revealed significant threefold elevations of HLA-B8 in Rye I-sensitive, relative to Rye I-insensitive subjects—specifically, for the comparisons of: group B (sensitive to both antigens) versus group C (sensitive only to AgE); group A (sensitive only to Rye I) versus group C; and the overall *Rye I*+ group versus the *Rye I*-group. In the first and third comparisons, specificity HLA-A1 was significantly

Table 2. Comparison of HLA Frequencies in Caucasian Subjects Defined by their Absolute Skin-Test Sensitivities to Rye I and AgE

HLA	Rye I				Antigen E				Normal Population Frequencies ^a (N=1309)	<i>p</i> values ^b	
	Sensitive ^a (N=111)		Insensitive ^a (N=72)		Sensitive ^a (N=160)		Insensitive ^a (N=23)			Rye I-Sens. versus Rye I-Insens.	
<i>A locus</i>											
1	38	34.2	15	20.8	45	28.1	8	34.8	26		0.05
2	61	55.0	30	41.7	78	48.8	13	56.5	52		0.08
3	31	27.9	19	26.4	43	26.9	7	30.4	23		
9 ^c	10	9.0	21	29.2	29	18.1	2	8.7	20		−0.0004
10 ^d	18	16.2	9	12.5	23	14.4	4	17.4	12		
11	13	11.7	7	9.7	16	10.0	4	17.4	12		
W19 ^e	11	9.9	9	12.5	18	11.3	2	8.7	26		
28	2	1.8	4	5.6	5	3.1	1	4.3	9		
<i>B locus</i>											
5	10	9.0	11	15.3	19	11.9	2	8.7	10		
7	28	25.2	15	20.8	35	21.9	8	34.8	23		
8	36	32.4	8	11.1	37	23.1	7	30.4	19		0.0009
12	39	35.1	31	43.1	59	36.9	11	47.8	27		
13	5	4.5	6	8.3	11	6.9	0	0.0	5		
14	9	8.1	5	6.9	13	8.1	1	4.3	8		
18	9	8.1	6	8.3	14	8.8	1	4.3	9		
27	4	3.6	3	4.2	7	4.4	0	0.0	10		
W15	0	0.0	4	5.6	4	2.5	0	0.0	10		−0.05
W16	2	1.8	2	2.8	4	2.5	0	0.0	5		
W17	3	2.7	9	12.5	12	7.5	0	0.0	10		−0.02
W22	3	2.7	2	2.8	5	3.1	0	0.0	5		
W35	7	6.3	1	1.4	6	3.8	2	8.7	13		
W40	8	7.2	12	16.7	18	11.3	2	8.7	12		−0.04
<i>Phenotypes</i>											
A1,B8	25	22.5	7	9.7	26	16.3	6	26.1	14		0.03
A2,B12	30	27.0	13	18.1	37	23.1	6	26.1	16		
A3,B7	16	14.4	10	13.9	22	13.8	4	17.4	10		

^a Percentage frequencies cited in italics. Normal U.S. Caucasian population frequencies are from Albert *et al.* 1973

^b Chi-squared analysis. Yates' correction applied for the analyses of BW15 and BW17. Only values of $p \leq 0.1$ are cited

^c Includes specificities recognized as AW23 and AW24 (see Marsh *et al.* 1977)

^d Includes specificities recognized as AW25 and AW26 (see Marsh *et al.* 1977)

^e Includes specificities recognized as A29, AW30, AW31, and AW32 (see Marsh *et al.* 1977)

elevated by 1.7- to 1.8-fold, and the A1,B8 phenotype was significantly elevated by 2.6- to 2.7-fold in the Rye I responder groups. These latter findings presumably reflect the marked linkage disequilibrium between A1 and B8 in Caucasians (Albert *et al.* 1973, Bodmer 1973). Four other HLA types—A9, BW15, BW17, and BW40—were negatively associated with sensitivity to Rye

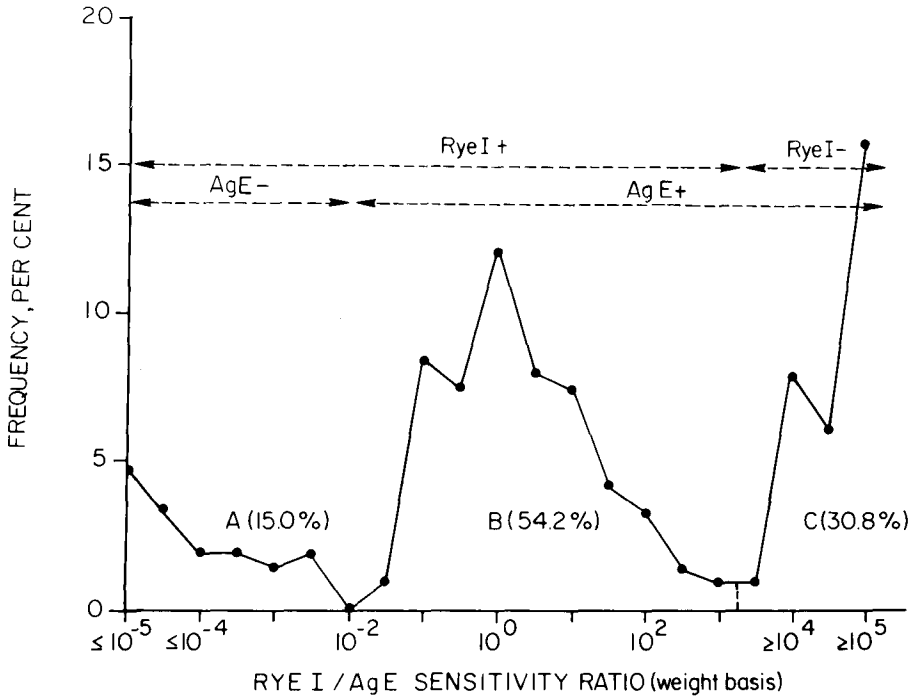


Fig. 2. Distribution of Rye I/AgE allergen sensitivity ratios in 214 allergic Caucasian subjects. See text for description of groups A–C and the *Rye I*+, *Rye I*–, *AgE*+, and *AgE*– phenotypes

I. These results are similar to those found for comparisons using absolute Rye I sensitivity data (*cf.* Tables 2 and 3).

There were no significant positive associations between AgE responsiveness and any HLA type. However, BW35 appears to be negatively associated. Specificities HLA-B13 and B27 were more common in *AgE*+ than to *AgE*– phenotypes. A larger study would be necessary to establish whether these differences will prove to be significant, but we think this unlikely (see *Discussion*).

Total Serum IgE Levels

Figure 3 shows the distributions of total serum IgE levels for Caucasians falling in groups A through C, based on the sensitivity ratio data. There were no significant differences [unpaired t-test on $\log(\text{IgE})$] between the distributions in IgE level for any pair of groups A–C; for the *Rye I*+ versus the *Rye I*– phenotype; or for the *AgE*+ versus the *AgE*– phenotype. However, there was a tendency for the IgE levels in Caucasian subjects sensitive only to Rye I (group A) or AgE (group C) to have slightly (but not significantly) lower total IgE levels than the doubly sensitive group B (geometric mean IgE levels = 211, 210, and 218 for groups A, C, and B, respectively). The IgE distributions for Rye I responders and AgE responders, based on absolute skin sensitivity

Table 3. Distribution of HLA Antigens in 214 Allergic Caucasian Subjects Based on Rye I/AgE Sensitivity Ratios

HLA	Group A ^a (N=32)		Group B ^b (N=116)		Group C ^c (N=66)		Normal Population Freqs. ^a (N=1309)	<i>p</i> values ^b			Groups A + B versus Group C (<i>Rye I</i> + versus <i>Rye I</i> -)	Groups B + C versus Group C (<i>AgE</i> + versus <i>AgE</i> -)	
								Group B versus Group A	Group B versus Group C	Group A versus Group C			
<i>A locus</i>													
1	9	28.1	41	35.3	13	19.7	26		0.03		0.04		
2	18	56.3	57	49.1	27	40.9	52						
3	10	31.3	28	24.1	20	30.3	23						
9 ^c	5	15.6	15	12.9	17	25.8	20		-0.03		-0.03		
10 ^c	7	21.9	21	18.1	7	10.6	12						
11	3	9.4	13	11.2	7	10.6	12						
W19 ^c	3	9.4	9	7.8	10	15.2	26						
28	1	3.1	2	1.7	4	6.1	9						
<i>B locus</i>													
5	2	6.2	15	12.9	9	13.6	10						
7	11	34.4	22	19.0	16	24.2	23	-0.06				-0.09	
8	10	31.3	37	31.9	7	10.6	19		0.001	0.01	0.001		
12	11	34.4	39	33.6	27	40.9	27						
13	1	3.1	8	6.9	5	7.6	5						
14	3	9.4	9	7.8	6	9.1	8						
18	3	9.4	13	11.2	5	7.6	9						
27	0	0.0	7	6.0	3	4.5	10						
W15	0	0.0	0	0.0	4	6.1	10		-0.03		-0.01		
W16	2	6.2	2	1.7	2	3.0	5						
W17	1	3.1	3	2.6	8	12.1	10		-0.02		-0.01		
W22	0	0.0	4	3.4	2	3.0	5						
W35	4	12.5	5	4.3	0	0.0	13			0.02	0.09	-0.04	
W40	3	9.4	7	6.0	11	16.7	12		-0.02		-0.02		
<i>Phenotypes</i>													
A1,B8	6	18.8	29	25.0	6	9.1	14		0.009		0.01		
A2,B12	6	18.8	27	23.3	11	16.7	16						
A3,B7	7	21.9	12	10.3	8	12.1	10						

^a Percentage frequencies are cited in italics. The data for normal Caucasian frequencies are from Albert *et al.* (1973)

^b Determined by chi-squared analysis with Yates' correction where appropriate (*i.e.*, for comparisons involving BW15, BW17, and BW35). Only values of $p \leq 0.1$ are cited. Minus sign indicates negative correlation with HLA frequency of the first group under comparison

^c See footnotes to Table 2

In the Black sample ($N=17$), the proportions of HLA-A1, HLA-B8, and the A1,B8 phenotype were, respectively, as follows: Group A ($N=3$), 67%, 33%, 33%; Group B ($N=7$), 14%, 0%, 0%; Group C ($N=7$), 14%, 14% and 14%

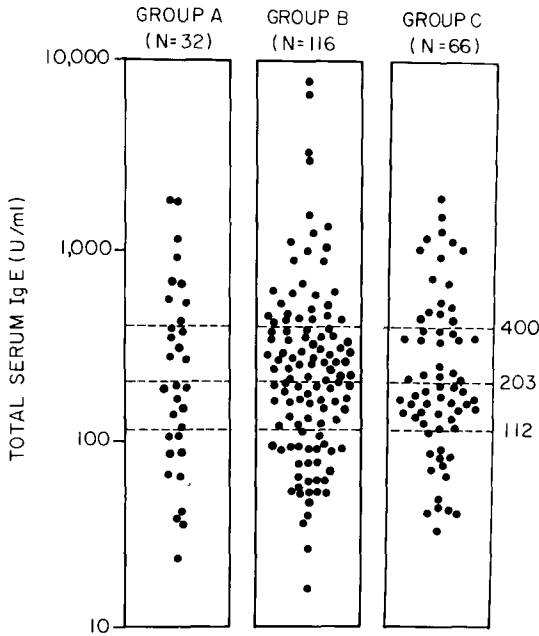


Fig. 3. Distributions of total serum IgE levels in allergic Caucasian groups A-C (see Fig. 2 and text). Quartile divisions for the total population ($N=214$) are given by dashed lines

measurements, were experimentally indistinguishable from the overall allergic group and from the data previously reported for allergic subjects (Marsh *et al.* 1974).

The distribution of IgE levels for the 17 allergic Black subjects was higher than for the 214 Caucasians (geometric mean IgE=370 versus 214 U/ml). This difference was significant ($p<0.05$) when log (IgE) levels were compared by t-test. In a study of Americans unselected for allergy, Grundbacher (1975) showed that Blacks have significantly higher total IgE levels than Caucasians. See also Marsh (1976) for further discussion of possible reasons for racial differences in total IgE levels.

Relationship Between HLA Frequencies and Total Serum IgE Levels

There was no clear evidence for modality in any of the distributions for any group or subgroup in either the sensitivity ratio or the absolute sensitivity data. As in the preceding study (Marsh *et al.* 1977), we therefore elected to test whether basal IgE level influenced specific IgE response. In the case of the sensitivity ratio data (Fig. 3), the quartile divisions occurred at 112, 203, and 400 U/ml; in the absolute sensitivity data, the quartile divisions were at 107, 197, and 377 U/ml.

Since our strongest positive association was between responsiveness to Rye I and the possession of HLA-B8, we first investigated the relationship between

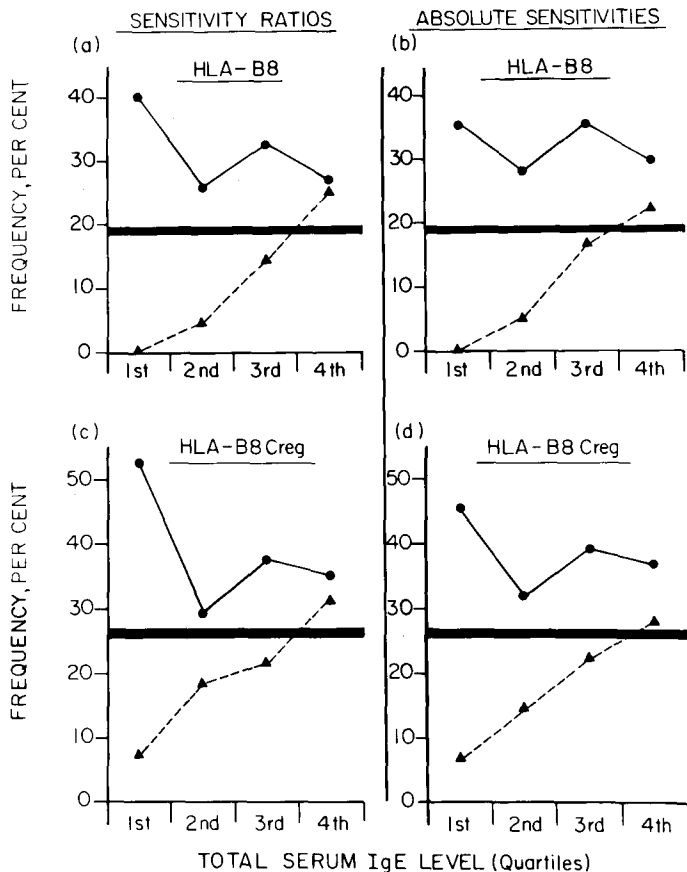


Fig. 4a-d. Distributions of HLA-B8 and the HLA-B8 Creg (B8 and/or B14 in the phenotype) in allergic Caucasian subjects, according to IgE quartile (see text). Rye I responders are represented by solid curves and Rye I nonresponders by dashed curves. **a** and **c** show curves for *Rye I+* and *Rye I-* phenotypes, based on sensitivity ratio data (Fig. 2), and **b** and **d** show curves for Rye I-sensitive and Rye I-insensitive people, based on absolute sensitivity data (Fig. 1). Normal U.S. Caucasian HLA frequencies are indicated by heavy horizontal lines (from Albert *et al.* 1973)

the frequency of this HLA type and total IgE level in Rye I responders and nonresponders. Figure 4a shows the relationship for the *Rye I+* phenotype (solid line) and the *Rye I-* phenotype (dashed line), based on the sensitivity ratio data, and Figure 4b shows the corresponding data for absolute sensitivity measurements. In both cases, the most striking observations are in the Rye I nonresponder patients, where there are marked increases in the frequency of B8 as IgE levels increase (dashed lines). The lowest IgE level in a Rye I-negative, B8-positive patient is 155 U/ml. Above this level, the B8 frequency approaches that normally found in American Caucasians (heavy horizontal lines). In Rye I responders, there is some evidence for the converse trend in Figure 4a, but not in Figure 4b. In Figure 4a, the frequency of B8 is over twice the Caucasian frequency in the first quartile. The relationships between

Table 4. Comparisons of HLA Frequencies for Rye I-Positive Individuals with Low Total IgE versus Rye I-Negative Individuals (Sensitivity Ratio Data)^a

HLA	<i>Rye I+</i> Phenotype ^b		<i>Rye I−</i> Phenotype ^b		<i>p</i> Values ^c			
	First IgE quartile ^d (<i>N</i> =40)		First quartile ^d (<i>N</i> =14)	All quartiles ^d (<i>N</i> =66)	Column 2 versus Column 3	Column 2 versus Column 4		
A1	15	37.5	1	7.1	13	19.7	0.07	0.06
B8	16	40.0	0	0.0	7	10.6	0.008	0.0002
A1,B8 (phenotype)	11	27.5	0	0.0	6	9.1	0.05	0.008
B14	6	15.0	1	7.1	6	9.1	Not significant	
B8 Creg	21	52.5 ^e	1	7.1	13	19.7	0.004	0.0002

^a Comparisons restricted to Caucasians. In the Black sample, the proportions of HLA-A1, HLA-B8, the A1,B8 phenotype, B14, and B8 Creg were, respectively, as follows: Rye I+, first quartile (*N*=1), 100%, 100%, 100%, 0%, 100%; Rye I-, first quartile (*N*=2), 0%, 0%, 0%, 50%, 50%; Rye I-, all quartiles (*N*=7), 14%, 14%, 14%, 14%, 29%

^b See Fig. 2 for definition of Rye I+ and Rye I- phenotypes

^c Determined by chi-squared analysis, with Yates' correction applied where appropriate. No other HLA comparisons were significant at the $p \leq 0.1$ level

^d Percentage frequencies cited in italics

^e The incidence of the B8 Creg does not equal that of B8 plus B14, since one individual has both B8 and B14 in his phenotype

IgE level and the frequencies of HLA-A1 and the A1,B8 phenotype (not shown) follow the same trends as B8, but to less remarkable degrees.

A further interesting finding is that HLA-B14 is much more frequent in the first than in the second, third, and fourth quartiles in patients responding to Rye I. In the analyses based on ratios, the frequency of B14 in the first quartile (15 percent) is 2.7-fold greater than in the combined second, third, and fourth quartiles ($p=0.06$); in the absolute sensitivity data, the B14 frequency is 3.2-fold greater in the first quartile than in the higher quartiles ($p=0.06$). Since B14 and B8 are serologically related and constitute the B8 cross-reacting group (Creg), it also seems appropriate to plot the frequency distributions for the B8 Creg (Figs. 4c and 4d). In this case, there is evidence for mirror-image relationships between the curves for Rye I responders and nonresponders on either side of the normal Caucasian population frequency for the B8 Creg phenotype. For the ratio data (Fig. 4c), the frequency of B8 Creg is significantly higher ($p=0.04$) in Rye I+ individuals in the first quartile than in Rye I+ individuals with higher IgE levels. An investigation of other possible HLA-IgE relationships revealed no evidence for any other than those discussed above.

The above analyses compare HLA frequencies with IgE level within groups of people of a particular sensitivity classification. We next compared frequencies of HLA antigens between Rye I responders with limiting low IgE levels in the first quartile and Rye I nonresponders (Table 4). For simplicity, we present detailed analyses only of the sensitivity ratio data, since this is more representative of our total patient sample. However, the analyses of the absolute sensitiv-

ity measurements show virtually identical results. First, in comparing *Rye I*+ (first quartile) with *Rye I*− (first quartile), there are significant elevations of HLA-B8, the A1,B8 phenotype, and B8 Creg in the responder group (Table 4, column 5). Comparing the *Rye I*+ (first quartile) group with the total *Rye I*− sample, we again find significant elevations in B8, the A1,B8 phenotype, and B8 Creg (Table 4, column 6).

Interrelations between total serum IgE level and HLA antigen frequency were also investigated, for both AgE responders and nonresponders (in terms of sensitivity ratio and absolute sensitivity), but no trends were apparent for any HLA type. Furthermore, people with limiting low total serum IgE level in the AgE responder groups showed no significant differences in HLA antigen frequencies when compared with AgE nonresponders (either individuals with IgE levels in the first quartile or the whole group).

Discussion

As in the preceding study, we consider only highly allergic individuals who strongly express IgE-mediated sensitivity by skin test and/or leukocyte histamine release assay. The present investigation concerns people of widely varying sensitivities to the highly purified allergens rye grass Group I (*Rye I*) and ragweed antigen E (AgE). In the allergic Caucasian group, we find the most frequent HLA association with B8, often as the A1,B8 phenotype; whereas there is no evidence for a positive association between AgE sensitivity and any HLA type. As discussed in *Materials and Methods*, we have investigated IgE-mediated responsiveness to *Rye I* in terms of *Rye I*/AgE ratio for our total patient group (214 Caucasians, 17 Blacks), and absolute skin sensitivity to *Rye I* in a somewhat biased subset comprising 86 percent of our patients. In both cases, the associations between B8 and responsiveness to *Rye I* are so strong ($p \leq 0.001$) that they are unlikely to be chance observations, even considering that analyses have been made for 22 different HLA specificities.

In the Ra3 study (Marsh *et al.* 1977), we found a pronounced relationship between the frequency of HLA-A2 and total serum IgE level in patients responding to Ra3. The frequency of A2 was significantly greater for Ra3 responders having limiting low IgE levels than for those with higher IgE levels. The atypical patients, who are highly allergic even though they possess IgE levels below 100–130 U/ml (roughly corresponding to the first IgE quartile), were designated “low IgE phenotypes.” In the present case, the analogous relationship between B8 and IgE level in *Rye I* responders is not quite so clearcut. There is some evidence for a relationship in the *Rye I*/AgE sensitivity ratio data for our total Caucasian sample (Fig. 4a)¹, but not for patients classified according to absolute skin sensitivity to *Rye I* (Fig. 4b). The probable reason for this discrepancy lies in selecting the subset of patients to be skin tested (see *Materials and Methods*), where there was a bias against testing people

¹ An earlier preliminary report of this data (Marsh 1976), in which weak *Rye I* responders were excluded from the analysis, shows a more pronounced relationship

who responded only to Rye I. The particular people who were excluded had lower than average IgE levels and a high frequency of B8. This type of sampling problem points clearly toward a future need to study specific allergic responses in the population as a whole, rather than in people who are readily available as a result of other studies.

In the case of Rye I nonresponders, the frequency of B8 is zero in people having the low IgE phenotype, but increases to approximately its normal Caucasian population frequency at higher IgE levels (Figs. 4a and 4b). This finding is consistent with the view that people with low phenotypes require an immune response gene associated with B8 in order to be able to respond to Rye I. Thus, the negative correlation—*i.e.*, absence of B8—would mean absence of the *Ir*-Rye I+ allele of the gene. It should be noted that this relationship between IgE and B8 in the nonresponders is not a reflection of AgE responsiveness; it holds when considering absolute sensitivity to Rye I (Fig. 4b), and no similar or inverse relationship between B8 frequency and IgE level is found in the AgE absolute sensitivity data.

A further interesting aspect of our IgE-HLA data in the Rye I study is the evidence that similar relationships hold for B14 as B8. These HLA antigens are serologically related (they form the B8 Creg), presumably reflecting a structural similarity. Plots of B8 Creg frequency versus IgE level show striking mirror-image curves for *Rye I*+ and *Rye I*− individuals on either side of the normal Caucasian population frequency of B8 (Fig. 4c).

The present data, therefore, further point to the existence of two distinct allergy phenotypes which can be classified, in large part, according to basal IgE level (see preceding paper for further discussion).

Of additional interest are the associations we have found between responsiveness to Rye I and the phenotype A1,B8, and responsiveness to Ra3 and A2,B12. These data, together with inspection of the patients' phenotypes and genotypes (where known), are consistent with respective association with the two of the most common Caucasian haplotypes, A1,B8 and A2,B12. However, the HLA association with Rye I sensitivity is stronger with the *B*-locus antigen, B8; whereas the association with Ra3 sensitivity is stronger with the *A*-locus antigen, A2. *B*-locus associations are the ones most frequently found between *HLA* and disease susceptibility (McDevitt and Bodmer 1974, Möller 1975). The incidence of B8 in Rye I-sensitive subjects (32 percent) is similar to the frequencies of B8 found in certain diseases. Some diseases appear to be more strongly associated with DW3 (Dausset and Svejgaard 1976), a specificity of the *D* region, which is in linkage disequilibrium with B8 (Kissmeyer-Nielsen 1975). It remains to be seen whether the association with specific IgE responsiveness to Rye I will prove to be stronger with DW3. It seems probable that the observed weaker associations between Rye I sensitivity and HLA-A1 ($p = 0.05$) arise from the linkage disequilibrium between A1 and B8.

We believe that the studies of sensitivity to Rye I and Ra3 provide a model for studying gene interaction in human immune response, and may shed some light on the possible location of hypothesized *Ir* loci within the *HLA* complex. We envision that the polygenic regulation of human immune response includes *HLA*-linked loci, which control specific responses, and non-*HLA*-linked loci,

which regulate the biosynthesis of different immunoglobulin classes and indirectly affect the synthesis of specific antibodies of that class (Marsh 1976, Marsh and Bias 1977)². It is also possible that certain of these regulatory loci affect more than one immunoglobulin class.

While multiple gene loci are essential for any given immune response, we will consider primarily the hypotheses of a single IgE-regulating locus, and of a number of *HLA*-linked *Ir* loci which control all specific immune response functions, including IgE production. First, the IgE-regulating locus determines whether a person is a low or high IgE phenotype, the low IgE phenotypes having a more restricted capacity to synthesize IgE antibodies. Second, the *Ir-Rye I+* and *Ir-Ra3+* alleles of postulated *Ir-Rye I* and *Ir-Ra3* loci are absolute (but not necessarily sufficient) requirements for the synthesis of specific IgE antibodies against Rye I and Ra3 in people having low IgE phenotypes. In such people, the concurrence of phenotypes *Ra3+* and A2 (93 percent) is much greater than for *Rye I+* and B8 (40 percent), which possibly suggests a closer map relationship between *Ir-Ra3* and the *A* locus than between *Ir-Rye I* and the *B* locus, based on the linkage disequilibrium within *HLA*. If this relationship is valid, one can utilize the relative degrees of positive associations with alleles at these and other loci to estimate the map positions of the hypothesized *Ir* loci.

This hypothesis suggests that there may be at least two *I* regions in *HLA*: *I-1* containing *Ir-Rye I*, and *I-2* containing *Ir-Ra3*. The map position of *I-1* would be close to the *B* locus, while *I-2* would be even closer to the *A* locus. Consistent with this hypothesis, the *Ir-Ra5* gene would be included in the *I-1* region, since sensitivity to the ragweed Ra5 allergen is more closely associated with a *B*-locus specificity, B7 (Marsh *et al.* 1973). We must emphasize, however, that further definition of the *low IgE*, *Rye I+* and *low IgE*, *Ra3+* phenotypes and rigorous genetic analysis of families will be required to establish these points.

The concept of two *I* regions is compatible with the data of Mann and coworkers (1976), who suggested that human "Ia-like" specificities expressed on B lymphocytes may map in two regions, one close to the *A* and the other close to the *B* locus of *HLA*. The first of these regions might be coincident with the strong MLR (D) region and the second with the weak MLR (MLR-W) region. It is also known that *Ir* genes and MLR specificities map in the *I* region of the mouse *H-2* complex. While only one *I* region has been well-defined in the mouse, there is recent evidence for *H-2*-linked *Ir* loci mapping outside the *D-K* region close to the *Tla* locus (Young *et al.* 1976).

The above discussions, based on a model of *Ir* loci in linkage disequilibrium with the serologically defined loci of *HLA* (*cf.* Bodmer 1973), provide only one approach to explaining our findings. To demonstrate linkage of gene loci, concordant inheritance within families must be demonstrated. So far, this has not been possible in humans because of the extremely complex multifactorial control of immune response, as discussed in detail elsewhere (Bias and Marsh

² The hypothesized *HLA*-linked *Ir* genes may not have absolute specificity for a particular antigenic determinant. Also, they may be gene complexes rather than discrete loci

1975, Black *et al.* 1976). While there have been two studies (Levine *et al.* 1972, Blumenthal *et al.* 1974) attempting to show linkage between specific IgE response to AgE and familial *HLA* type, the conclusions of the authors are unconvincing, for reasons which have been documented extensively elsewhere (*e.g.*, Bias and Marsh 1975, Marsh 1975, Marsh and Bias 1977). In the mouse, the existence of many important *H-2*-linked *Ir* genes was established because of their position between the *D* and *K* loci. The products of *D* and *K* could then be used as real markers, and recombinants were readily detectable. Furthermore, since congenic lines were often used, Ig-regulating genes and the many other loci necessary to produce an immune response did not interfere with gene expression. Obviously, the human data will not be so clearcut, and we must obtain sufficient data to test alternative hypotheses. The alternatives, as we see them, are as follows.

1. The serologically defined specificities themselves (or closely related specificities) act as receptors for the major (immunodominant) Rye I and Ra3 determinants. Individuals (particularly those with high IgE phenotypes) who respond to Rye I or Ra3, but who do not have B8 or A2, respectively, would be recognizing minor determinants on the molecules.

2. There are *Ir-Rye I* and *Ir-Ra3* genes which control recognition of the immunodominant determinants somewhere in the genome and interact with, or are dependent on, the products of certain alleles at the *HLA-B* or *A* loci for their expression.

3. There are functional interactions between the products of certain *HLA-B* and/or *HLA-A* alleles and the products of alleles of linked (or unlinked) *Ir* loci which lead to improved immune function.

The first hypothesis is supported by our findings (particularly in people with low IgE phenotypes) of associations between A2 Creg and Ra3 response (Table 5 in Marsh *et al.* 1977) and B8 Creg and Rye I response, as well as our previous observations of the association between the B7 Creg and Ra5 response (Marsh *et al.* 1973, Marsh *et al.* 1975, Goodfriend *et al.* 1977). That is, we tend to find secondary associations with related HLA antigens in the same Creg as the HLA specificity involved with the primary association. There are precedents for the second alternative from human studies of *ABO*-secretor and disease associations (Glynn *et al.* 1959, Clarke *et al.* 1959, Clarke *et al.* 1960, Glynn and Holoborow 1969). The last of the three alternatives, although more complex, is also compatible with our data. In this case, we can retain the theory of *HLA*-linked *Ir* genes as discussed, but add the concept that the immune function of specific *Ir* gene products is somehow improved by interaction with the products of certain related groups of *A*- or *B*-locus specificities, probably on the cell surface (*cf.* Marsh 1975, Marsh and Bias 1977).

In conclusion, we feel that we can begin to approach the problem of *Ir* gene mapping by studying unrelated subjects specially selected for their high allergic expressivity. Such people must be endowed with an appropriate combination of genes and prior allergenic exposure. This approach, however, is presently limited by the lack of rigorous proof that *HLA*-linked *Ir* genes exist, and by the assumption that linkage disequilibrium can be used to infer the probable map relationships of *Ir* and other loci within this region.

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