Association of the HL-A7 Cross-Reacting Group with a Specific Reaginic Antibody Response in Allergic Man

Abstract. *A relatively small proportion (17 percent) of individuals highly allergic to ragweed were found to develop marked reaginic (immunoglobulin E–mediated) skin sensitivity to a minor ragweed pollen allergen Ra5 (molecular weight 5200). Sensitivity to Ra5 was significantly associated with the possession of a major histocompatibility antigen of the HL-A7 cross-reacting group. This appears to be the first evidence of a strong association between a specific immune response and a specific group of closely related HL-A antigens in man.*

The development of human atopic allergy is primarily due to the biosynthesis of reaginic antibodies of the immunoglobulin E (IgE) class (*1*). Man's general predisposition to become hypersensitive to diverse components (allergens) which he encounters in low dosage in the environment has long been known to be familial and presumed to be genetically determined (*2, 3*). In part, this genetic control appears to be associated with the general biosynthesis of IgE (of any specificity). However, other genes control the specificity of the IgE antibody molecules actually produced. On the basis of current immunogenetic concepts developed from animal experiments (*4*), and particularly by analogy with Levine's studies in inbred mouse strains (*5*), it seems probable that certain of the latter immune response (Ir) genes would be closely linked to man's major histocompatibility (HL-A) locus.

The recent isolation by Goodfriend and Lapkoff (*6*) of allergen Ra5 (molecular weight 5200 ± 200) from the pollen of short ragweed (*Ambrosia elatior*) added impetus to studies attempting to correlate specific allergies with HL-A type which were already under way in our laboratories (*7*). Allergen Ra5 was found to be physicochemically and immunologically distinct from the major ragweed pollen allergen, antigen E (*6, 8*). In addition to being about one-seventh the molecular size of antigen E, Ra5 was obtained in yields which suggested that its proportion by weight in the pollen was less than one-hundredth that of antigen E (*9*). Under normal conditions of exposure, the mean annual adult dose of antigen E in the Baltimore area has been calculated to be maximally about 1 μg (*3*), suggesting that the corresponding dosage of allergen Ra5 is less than 0.01 μg. Both the extremely low immunizing dosage and the relatively simple chemical structure of Ra5 favor the possibility of observing any linkage which may exist between an HL-A type and allergic sensitivity to this allergen, even in a population as genetically polymorphic as man.

Only individuals who were clinically sensitive to ragweed pollen were selected, indicating that they had both a genetic predilection toward allergy (including, more specifically, ragweed allergy) and had been sufficiently exposed to ragweed pollen to allow the development of IgE-mediated hypersensitivity to its component allergens. Reaginic skin sensitivity of the patients to the major allergen, antigen E, could then be utilized as an index for ragweed sensitivity, in order that patients could be subdivided into groups relatively sensitive and insensitive to the minor allergen Ra5.

Patients (105) were selected on the basis of their high skin and leukocyte sensitivities to antigen E, without knowledge of their HL-A typing. The patients were unrelated; 98 were Caucasian and 7 were Americans of mixed Negro and Caucasian descent. About one-third had previously been treated with ragweed pollen extracts. All were quantitatively skin tested by intradermal injections (0.05 ml) of serial tenfold dilutions of highly purified antigen E (*10*) and Ra5 (*11*), at concentrations of 10^{-7} to 1 μg/ml, in tris-buffered physiological saline stabilized with 0.03 percent (weight to volume) human serum albumin. A patient's sensitivity ratio of Ra5 and antigen E at a given test period was determined from the allergen concentrations eliciting immediate (15 to 20 minute) skin reactions, primarily utilizing reactions graded "2 plus" (*12*). Sensitivity ratios determined in this manner for three individuals who were Ra5 reactive and seven who were Ra5 unreactive corresponded well with ratios determined more precisely by assay of histamine release from leukocytes (*13*).

Leukocytes from all tested subjects were HL-A typed by standard cytotoxic microtechnique (*14*), with 60 serums of well-defined specificity obtained from the NIH serum bank and

Table 1. Comparison of absolute frequencies of HL-A antigens in ragweed-allergic people. Comparisons of *P* were made only for the following 15 serotypes: HL-A1, 3, 11, 10, 2, 9, 13, 12, 5, 7, 8, and W-18, -17, -14 and combined W-10 and "Short" W-10, and the following three "Cregs": HL-A7 (includes all specificities shown in Table 2), HL-A2 (includes W-28), and HL-A5 (includes W-5 and W-18). The frequencies of other serotypes were too low for analysis. A negative association (that is, greater frequency of the HL-A type in the Ra5 insensitive group) is designated by a minus sign.

HL-A serotype	Ra5 positive: Group A ($N = 18$)	Ra5 negative: Group B ($N = 77$)	Ra5 negative: Group B′ ($N = 18$)	Intermediate: Group C ($N = 10$)	*P* values ≤ .1: A vs. B	*P* values ≤ .1: A vs. B′
			1st (La) locus			
1	5	19	4	5		
3	7	20	5	1		
11	2	5	1	0		
10	0	16	4	2	— .08	
2	10	37	9	4		
W-28	1	3	0	0		
9	1	16	4	2		
W-19	1	4	1	1		
			2nd (Four) locus			
13	0	6	4	0		
12	3	29	6	4		
W-18	1	10	2	1		
5	2	7	0	0		
W-5	0	0	0	0		
W-15	0	1	1	0		
W-17	3	4	0	2		
"Short" W-10	1	0	0	0		
W-10	2	5	1	0		
W-27	0	3	0	0		
"Short" W-27	0	2	1	0	.02* .006†	.03 .007
7	9	15	2	2		
W-22	0	1	0	0		
"Short" W-22	1	0	0	0		
8	3	20	6	6		
W-14	2	7	1	0		

* HL-A7 alone. † HL-A7 "Creg."

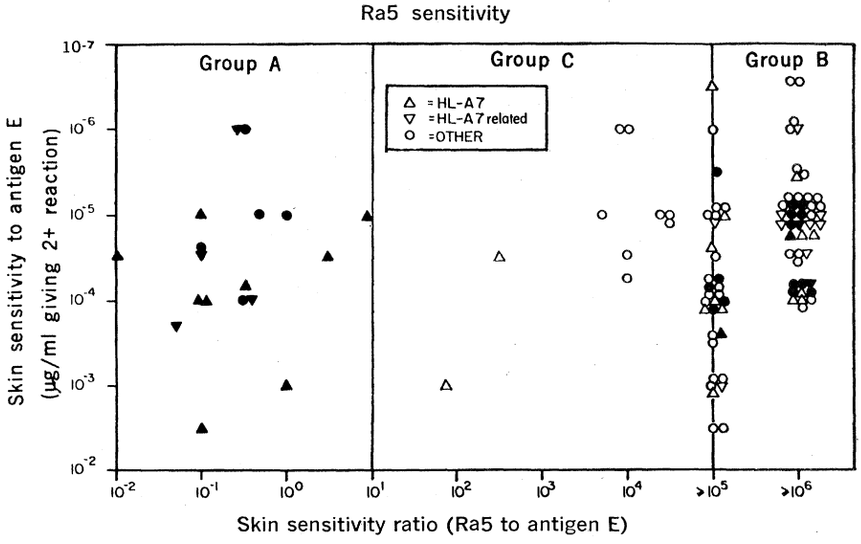

Fig. 1. Comparison of skin sensitivity to ragweed's major allergen, antigen E, with the ratio of skin sensitivities to ragweed allergens Ra5 and antigen E in 105 people highly allergic to ragweed. Individuals of group A were highly sensitive to Ra5, those of group B were insensitive to Ra5, and those of group C had intermediate Ra5 sensitivity. The closed symbols represent individuals of group B which were matched (18 pairs) with those of group A according to the criteria cited in the text.

private sources. All typings were performed in duplicate, and we repeated most of them at a later date to be certain that no ambiguities existed, and to include further defined typing serums as they became available. The HL-A specificities investigated are cited in Table 1. A more detailed description of the typing of HL-A7–related antigens is presented in Table 2.

Figure 1 shows skin sensitivities to antigen E plotted against the ratio of Ra5 sensitivity to antigen E sensitivity. Of the 105 individuals only 18 (17 percent) were classified as highly Ra5-sensitive (group A) and possessed sensitivity ratios less than or equal to 10 on a weight basis ($\leqq$ 73 on a molar basis). Also, 77 people (73 percent), classified as Ra5-insensitive (group B), possessed sensitivity ratios greater than or equal to 100,000 on a weight basis. The remaining 10 individuals (group C) had sensitivity ratios between the limits of groups A and B.

For the purpose of comparing HL-A antigen frequencies in Ra5-sensitive as compared to Ra5-insensitive individuals, we preferred to analyze data from patients of group A as compared to group B where the difference of 10,000-fold in sensitivity ratios (Ra5 to antigen E) unquestionably reflects widely different capabilities of the two ragweed-allergic groups to synthesize reaginic antibody against the minor ragweed allergen Ra5 (*15*). For a second comparison, we individually matched from group B a group of 18 people (group B′) as closely as possible with members of group A, with respect to race, age, skin and leukocyte sensitivities to antigen E, and immunotherapy with ragweed extract.

Using chi-squared statistical analysis, with Yates' correction for small sample size, we found a significant elevation of the HL-A7 antigen frequency in the highly Ra5-sensitive group A relative to the frequencies of this antigen in either of the Ra5-insensitive groups B or B′ ($P = .02$ and .03, respectively) (Table 1). When the combined frequencies of antigens comprising the HL-A7 "Creg" [cross-reacting group (*16*)] were similarly compared, the differences between groups A and B and groups A and B′ were more significant ($P = .006$ and .007, respectively). Excluding all American Blacks and one Indian from the analysis, the corresponding P values for comparison of HL-A7 "Creg" antigens were .01 and .006, respectively.

Taking a less conservative difference in Ra5 : antigen E sensitivity ratio of $\geqq 100$ (rather than $\geqq 100{,}000$ used in the analyses given above) to define the the Ra5-sensitive and Ra5-insensitive groups, we compared the 20 individuals possessing sensitivity ratios between 10^{-2} and 10^{3} (Fig. 1) with the 77 unmatched or 20 matched Ra5-insensitive controls with ratios $\geqq 10^{5}$. The elevations of HL-A7 and HL-A7 "Creg" in the expanded Ra5-sensitive group relative to controls were of exceptionally high significance (*HL-A7*: unmatched, $P = .003$; matched, $P = .006$; *HL-A7*

Table 2. Analysis of our typing of the HL-A7 cross-reacting group of antigens. Reaction grading was as follows: ++, strongly positive cytotoxicity (conventional 3–4 plus); +, moderately strong cytotoxicity (conventional 1–2 plus); ±, trace reaction; −, no reaction.

HL-A specificity*	McCutcheon	Cutten	Melnikoff	Bass	Hutter	Cowan	Kawano	Fontanel†	B-1†	McCartin†
"Short" W-10	−	−	−	−	−	−	+	−	+	−
W-10	−	−	−	−	−	−	++	+	++/+	++
W-27	++	±	−	−	++/+	++	−	−	−	−
"Short" W-27	±	−	−	−	±/−	++	−	−	−	−
HL-A7	++	++	++	++	++	++	−	−	−	−
W-22	++	+	++	++	+	−	−	±	−	−
"Short" W-22	++	−	++	−	−	−	−	−	−	−

* Cross-reactions within the HL-A7 complex are grouped together on the basis of our experience and the results of other investigators (*16, 20, 21*). Decreasing cross-reactivity is represented by small → large brackets. Specificities designated as "short" were recognized by only two out of four or five serums of the designated typing. † Serums known as "Fontanel" and "B-1" also contain antibodies reacting with HL-A10; serum "McCartin" reacts strongly with HL-A13 and moderately with HL-A12. Positive reactions with these serums were disregarded where cells typed for HL-A10 or HL-A13 or HL-A12, or some combination thereof, respectively.

Creg: unmatched, $P = .002$; matched, $P = .001$). These results are, however, more subject to error than those cited in Table 1 (*15*). A total of 41 allergic people possessed an antigen of the HL-A7 "Creg" in the present study; of these, 13 (32 percent) were highly Ra5-sensitive.

Serotypes HL-A10, 9, and 13 showed some degree of negative association with Ra5 sensitivity (although none were significant at the $P = .05$ level). For 2nd locus antigens, this is not unexpected since specificities showing negative associations are allelic with those showing positive associations. Negative associations for 1st locus antigens can, in part, be explained in terms of the linkage disequilibria between 1st and 2nd locus antigens (*21*).

We have shown a highly significant association between an allergic individual's ability to develop marked skin sensitivity to allergen Ra5 (following exposure to ragweed pollen) and his having the major histocompatibility antigen HL-A7 or one of the group of immunologically related, presumably chemically closely similar, antigens comprising the HL-A7 "Creg." Skin sensitivity to a particular allergen has previously been shown to correlate well with an allergically predisposed individual's ability to synthesize specific IgE antibody after inhalation of immunogenically limiting low doses of the allergen (*1, 3, 17*). More recently, in passive leukocyte and skin sensitization experiments (*13*), we have shown that allergic sensitivity to Ra5 is IgE-mediated. Thus, we have demonstrated an association between a specific immune response and HL-A serotype in man. Even though most P values are highly significant, in view of the fairly small population sample, it will be necessary to confirm our results in a second series of patients.

The genetic locus controlling responsiveness to Ra5 appears to be analogous to the mouse Ir-1 locus, which maps closely to, and normally segregates with, the K locus of the major histocompatibility (H-2) region of the mouse (*4, 18*). Furthermore, since HL-A7 "Creg" antigens form part of the 2nd locus of HL-A, there appears to be a good correlation between the K locus of H-2 and the 2nd locus of HL-A. This conclusion is compatible with several findings that man's susceptibility to certain immunological diseases is associated with HL-A antigens of the 2nd locus (*20*). It seems probable that this genetic control is operative predominantly, if not exclusively, at the thymus-derived (T) cell level (*4*), rather than in the bone marrow–derived (B) cells where the IgE antibody is actually synthesized.

We have presented strong evidence for a histocompatibility-associated Ir gene or genes in man. Under extremely limiting immunogenic stimulation with allergen Ra5, there is a highly significant association between immune response and a specific group of closely related HL-A antigens, a situation analogous to that described for inbred animal strains. Further studies of specific allergic hypersensitivities in man should forward our understanding of the genetic control of immune responsiveness.

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9. Short ragweed pollen contains approximately 5000 μg of antigen E per gram of pollen (radial immunodiffusion analysis). The maximum yield of Ra5 so far obtained is 15 μg per gram of pollen.
10. The sample of antigen E was supplied by Dr. T. P. King, Rockefeller University. Gel electrophoresis revealed that it consisted essentially of fraction IV-C with minor proportions of other isoallergens of the antigen E complex. It was homogeneous by ultracentrifugal analysis and gave a single precipitin line on immunodiffusion analysis against hyperimmune rabbit antiserum to antigen E and antiserum to whole ragweed extract.
11. The sample of Ra5 gave a single protein-staining band on acrylamide gel disc electrophoresis at *p*H 4.5 and 6.5 when 90-μg samples were used. It was homogeneous by equilibrium ultracentrifugal analysis ($\log_{10} c$ plotted linearly against r^2) and gel filtration. It gave a single precipitin line against hyperimmune rabbit antiserum to Ra5, but no line against antiserum to antigen E.
12. A skin reaction of a 0.8- to 1.0-cm diameter wheal without pseudopodia and with erythema of 2.0- to 4.0-cm diameter (variable from individual to individual) was graded as "2 plus." In some cases it was necessary to interpolate the allergen concentration yielding a 2-plus reaction from larger and smaller reactions at two allergen concentrations differing by tenfold.
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15. Two types of experimental problems exist: (i) possible trace, physicochemically undetectable, allergenic impurities in Ra5, totaling, say, 0.1 percent, might lead to the erroneous impression that certain Ra5 insensitive individuals are weakly sensitive to Ra5; (ii) positive skin sensitivity end points are subject to an error of ± one tenfold dilution.
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Retzius Cells: Neuroeffectors Controlling Mucus Release by the Leech

Abstract. *Mucus release from the skin of leeches is under the control of the pair of large Retzius cells in each segmental ganglion. The rate of mucus release increases with the impulse activity of Retzius cells and with the concentration of their putative neurotransmitter, 5-hydroxytryptamine, to which the skin is exposed.*

The largest cells among the approximately 175 pairs of bilaterally symmetrical neurons in the iterated segmental ganglion of the leech ventral nerve cord are the two "kolossal" cells of Retzius (*1*). Because their large size renders them accessible to study, Retzius cells have been the subject of histological, histochemical, electron microscopic–microchemical, neuropharmacological,