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Immune responsiveness to *Ambrosia artemisiifolia* (short ragweed) pollen allergen *Amb a* VI (Ra6) is associated with HLA-DR5 in allergic humans

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Abstract. The relationship between HLA type and specific immune responsiveness toward ultrapure *Ambrosia artemisiifolia* (short ragweed) pollen allergen *Amb a* VI (Ra6) was explored in a genetic-epidemiologic study of groups of 116 and 81 Caucasoid subjects who were skin-test positive (ST^+) toward common environmental allergens. Specific immune responsiveness to *Amb a* VI was assessed by measuring serum IgE and IgG antibodies (Abs) by double Ab radioimmunoassay in both ST^+ groups. Significant associations were found between IgE Ab responsiveness to *Amb a* VI and the possession of HLA-DR5; *P* values for the two groups were, respectively, 7×10^{-7} and 1×10^{-3} by nonparametric analyses, and 4×10^{-11} and 5×10^{-8} by parametric analyses. The levels of significance for the associations between HLA-DR5 and IgG Ab responsiveness were highly dependent on the extent of ragweed immunotherapy (Rx) within the patient group; by parametric statistics, the associations were 10^{-11} for the group that had received relatively little Rx and 2×10^{-3} for the group that had received more intensive Rx. These results provide further striking evidence for the existence of specific *HLA*-linked human *Ir* genes involved in responsiveness toward inhaled allergens and illustrate the usefulness of the allergy model in studies of the genetic basis of human immune responsiveness. Extension of these studies to investigation of structure-function relationships involved in antigen recognition by Ia molecules and the T-cell receptor will lead to a better understanding of human susceptibility toward immunologic diseases.

Abbreviations used in this paper: Ab, antibody; *Amb a* VI, *Amb a* V, new IUIS nomenclature for *Ambrosia artemisiifolia* pollen allergens nos. 6 and 5 (short ragweed Ra6 and Ra5) (Marsh et al. 1986b); *Lol p* II, III, new IUIS nomenclature for *Lolium perenne* pollen allergens II and III (perennial rye grass, Rye II and Rye III) (Marsh et al. 1986b); BBS, borate-buffered physiologic saline; BSA, bovine serum albumin; DARIA, double-antibody radioimunoassay; Ia, immune-associated; PAGE, polyacrylamide gel electrophoresis; RIST, radioimmunosorbent test; Rx, immunotherapy; SDS, sodium dodecyl sulfate; ST, skin test

Introduction

Previous studies of atopic humans who are skin-test positive (ST^+) to pollen allergens have revealed significant associations between particular HLA-D types and specific immune responsiveness toward each of several highly purified pollen allergens (Marsh et al. 1981). These studies have also shown evidence that investigation of human immune responsiveness toward inhaled atopic allergens provides a particularly good immunogenetic model (Marsh 1976). In the past, we have emphasized the specific model of immune responsiveness toward *Amb a* V (short ragweed Ra5, M_r 5000)* and its homologs from other *Ambrosia* (ragweed) species which exhibit particularly striking associations with HLA-Dw2 and DR2 in Caucasoid populations (Marsh 1986). For example, several studies (Marsh et al. 1982a, b, Goodfriend et al. 1985, Roebber et al. 1985, Blumenthal et al. 1985) have shown that 90–95% of IgE antibody positive ($IgE\ Ab^+$) subjects to *Amb a* V possess Dw2 and DR2; whereas among ragweed-allergic subjects having no IgE Ab to *Amb a* V, the proportions of Dw2 and DR2 are 20–25%, which are similar to the normal U.S. Caucasoid population frequencies for these specificities.

The aim of the present study was to investigate whether a similarly striking HLA association may exist for specific immune responsiveness toward another short ragweed pollen allergen, *Amb a* VI (Ra6). This allergen (M_r 11 500) exists in two chromatographic forms, A and B, each of which contains two closely related molecular species which have slightly different electrophoretic migration rates at pH 8.5 (Roebber et al. 1983). Since the various isoallergens cannot be distinguished antigenically or allergenically, the present studies were performed with a single, antigenically ultrapure preparation of *Amb a* VIA.

* The new IUIS allergen nomenclature (Marsh et al. 1986b) will be used throughout this article. The old name for each allergen is given in parentheses at the first citation

Materials and methods

Study subjects. Two groups of allergic, adult Caucasoids were studied. *Westinghouse subjects* ($N=116$; 89% male; mean age 37 ± 10 SD years; age range 20–56 years) were employees of the Westinghouse Electric Corporation, Hunt Valley, Maryland, who formed part of our extensive genetic-epidemiologic study of allergy (Freidhoff et al. 1981, 1984). All of these subjects were chosen because they were ST^+ to one or more of the crude allergen preparations described in the next section (see Freidhoff et al. 1983 for details). Eighty-three (72%) were ST^+ to short ragweed. *Clinic patients* ($N=81$; 81% male; mean age 38 ± 10 SD years; age range 20–60 years) had very high levels of clinical sensitivity and ST positivity to short ragweed and/or rye pollen extracts; 91% were highly sensitive to short ragweed by both criteria. These patients had been used for a variety of clinical and immunological studies of pollen hay fever in which both clinical and immunological sensitivity had been fully documented (Norman and Lichtenstein 1978, Norman et al. 1981, 1982). Of the Westinghouse subjects 12% (14/116) reported having received ragweed pollen extract immunotherapy (Rx) from their private physicians at some time during their lives; 7 of these had received Rx during the past 5 years. Normally, the Rx regimens used by private physicians employ relatively low antigen dosages. In the clinic group, 25% (20/81) had received ragweed Rx (10 during the past 5 years), usually at the Johns Hopkins Medical Institutions, where high-dose Rx is the norm. These percentages reflect an approximately twofold higher prevalence of Rx in the clinic group. However, because of differences in the therapeutic regimens, the antigen dosages (and therefore patient IgG Ab levels) would also be expected to be much higher in the treated clinic patients than the treated Westinghouse subjects.

Antigens. The ultrapure *Amb a* VIA preparation (M_r 11 500 by gel filtration analysis) isolated from *Ambrosia artemisiifolia* (short ragweed) pollen was identical with that used in a previous study (Roebber et al. 1983). It was homogeneous when tested by crossed immunoelectrophoresis against a highly potent anti-crude ragweed serum and by SDS-PAGE (Fig. 1), as well as by PAGE at pH 4.3 (Roebber et al. 1983). It gave two antigenically indistinguishable bands on agarose electrophoresis at pH 8.5 (Roebber et al. 1983). The amino acid composition (108 residues) and the sequence of the first 12 NH_2-terminal residues are known (Roebber et al. 1983). The preparation contained no detectable carbohydrate. The following crude allergen extracts were used for puncture skin testing of the Westinghouse subjects: *Ambrosia artemisiifolia* (short ragweed) pollen, *Lolium perenne* (perennial rye) pollen, cat dander, dog dander, house dust, and *Alternaria* spp. The allergen preparations used for skin testing were standardized and characterized as previously discussed (Freidhoff et al. 1983). Allergen concentrations and criteria for ST positivity were chosen which best discriminated between clinically allergic and nonallergic subjects (Freidhoff et al. 1981).

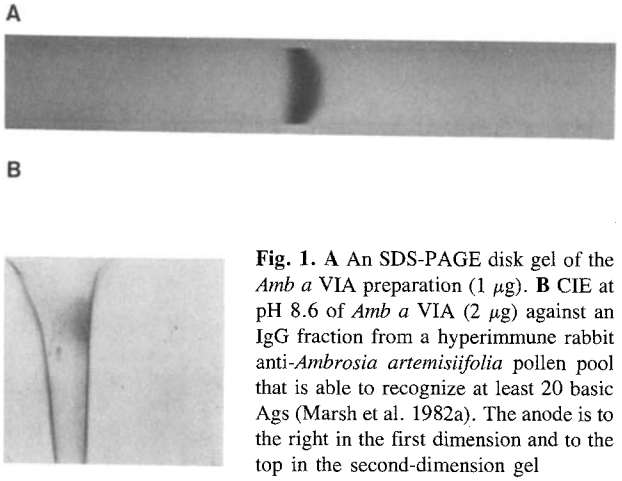

Fig. 1. A An SDS-PAGE disk gel of the *Amb a* VIA preparation (1 μg). **B** CIE at pH 8.6 of *Amb a* VIA (2 μg) against an IgG fraction from a hyperimmune rabbit anti-*Ambrosia artemisiifolia* pollen pool that is able to recognize at least 20 basic Ags (Marsh et al. 1982a). The anode is to the right in the first dimension and to the top in the second-dimension gel

Measurement of IgG and IgE serum Abs to Amb a VI by DARIA. The *Amb a* VI preparation was radiolabeled with ^{125}I using a modified chloramine T procedure (Marsh et al. 1982a), to yield a specific activity of ca. 50 000 cpm/ng. Microprecipitates of denatured antigen were removed by filtration (0.45 μm membrane) to minimize subsequent "background noise" in the RIAs. The radiolabeled antigen was diluted to 1 μg/ml in 5% BSA in BBS at pH 8.0, and stored at −70 °C. The BSA served the dual purpose of minimizing autodegradation of the antigen on storage and nonspecific adsorption of the radioactive antigen to the plastic assay tubes during RIA. The DARIA methodology was similar to that described for analysis of serum Abs to *Amb a* V (Ra5) (Marsh et al. 1982a). In brief, the ^{125}I-labeled *Amb a* VI antigen (100 μl, ca. 10 ng/ml in 20% normal goat serum) was allowed to incubate for 4 h at 23 °C with a diluted sample (100 μl) of the allergic human serum to be analyzed. Serum dilutions of 1/10–1/1000 were used for the study of IgG Abs and dilutions of 1/2 to 1/10 for the study of IgE Abs. (The dilution selected depended on the estimated Ab level.) Negative control sera were obtained from people having no specific Ab toward the test antigen. For the IgG assay, a 1/10 dilution of serum from a nonresponder person was used to prepare dilutions greater than 1/10. For the IgE assay, 50 μl of IgE myeloma serum (serum PS, diluted 1/100) was added as a "carrier." A slight excess of goat anti-human IgG (γ-chain specific) or goat anti-human IgE (ε-chain specific) was added, as appropriate, for the respective assays. The immune complexes were allowed to precipitate for 18 h at 4 °C; the precipitates were washed four times with BBS at pH 8, counted in a gamma counter, and the Ab contents computed by comparison against serial dilutions of a standard serum of known Ab content (Mulligan et al. 1966, Roebber et al. 1983). All analyses were carried out in triplicate in at least two experiments. If the coefficients of variation within or between experiments were greater than 10%, the assays were repeated. The negative controls were always less than 0.3% of B_{max}. (B_{max} is the maximum precipitable cpm using a high-titer serum from an immunized goat, with rabbit anti-goat IgG as the immunoprecipitant). A test serum was considered to be "positive" for IgE or IgG Ab when statistical analysis (*t* test) revealed consistently significant elevations in the cpm for the test sera versus the negative control. Because of some variability in the cpm for sera with very low or negative Abs, we were able to assign a serum Ab level as "unequivocally positive" only when the mean cpm was approximately twice that of the negative control; this corresponded to an Ab level of 0.4 ng/ml in the diluted sample. The most concentrated sera tested were 1 : 2 dilutions for IgE Ab and 1 : 10 for IgG Ab, respectively; therefore, the corresponding lowest detectable levels in undiluted sera were 0.8 ng/ml for IgE Ab and 4 ng/ml for IgG Ab.

Total serum IgE levels. Total serum IgE levels were analyzed by a variation of the "direct RIST" assay (Schellenberg and Adkinson 1975), utilizing Sepharose-anti-IgE beads as the immunosorbent. The beads were incubated with the patient's serum (or a standard serum or a negative control), washed, and reincubated with purified ^{125}I-anti-IgE Ab (ε-chain specific). The beads were then washed and counted, and the quantity of IgE in the patient's serum was computed by reference to a titration curve for the standard serum. The standard serum used in each series of experiments had previously been standardized against the WHO IgE reference. All analyses were performed in triplicate in at least two assays and discrepant values (having coefficients of variation > 10%) were repeated. Levels of total IgE are expressed in ng/ml, where 2.42 ng ≡ 1 IU.

HLA typing. The standard two-stage microcytotoxicity test (Amos et al. 1969) was used for typing HLA-A, -B, and -C specificities using 140 well-defined HLA typing sera standardized against International Work-

shop reagents. HLA-DR and -DQ typing was performed according to our modification (Bias et al. 1981) of the double-fluorescence staining method of van Rood and co-workers (1975), using 70 sera of well-defined specificity with which we were able to identify specificities DR1-DRw10, DRw52, and DRw53, and DQw1, 2, and 3. In regard to DR5 typing, all of the $DR5^+$ subjects possessed the more common DRw11 subspecificity; no one having the rare DRw12 subspecificity was observed.

Data analyses. Nonparametric analyses. Each group of allergic subjects was divided into two subgroups according to whether a serum was classified as "positive" (≥ 0.8 ng/ml) or "negative" (<0.8 ng/ml) for IgE Ab toward *Amb a* VI, according to the rationale discussed above. The study groups were also similarly divided according to their IgG Ab levels ("positive" being ≥ 4 ng/ml). Using Fisher's exact test, we then looked for significant differences in the proportions of each HLA specificity among the respective "positive" and "negative" subgroups. Since some HLA-A, -B, and -C typings were not available on the clinic patients, we were able to analyze only for associations between responsiveness to *Amb a* VI and HLA-DR and -DQ in the clinic group. Odds ratios were used as estimates of relative risk (Marsh et al. 1982a). Fisher's exact test was also used to investigate the significance of differences in the proportions of treated and untreated subjects having IgG and IgE Abs. *Parametric analyses.* For all parametric tests, "negative" IgE and IgG Abs were included as the logs of the lowest detectable Ab levels (see above). Student's *t* test (two tail) was used to analyze for differences in the IgG and IgE Ab levels between certain study groups and subgroups. Stepwise multiple regression analysis was used to investigate further the significance of the associations found by the nonparametric analyses. For *each* study group, we performed two analyses, using log [IgE Ab] and log [IgG Ab] to *Amb a* VI as the *dependent* variables. Each of the HLA-DR specificities and age, log [total serum IgE], ragweed Rx, and sex were used as the *independent* variables in all of these analyses. The HLA-A, -B, and -C specificities were also included as independent variables in the analyses of the Westinghouse group. Reported ragweed Rx by the patient's clinician was categorized as "2" where injections had been administered within the past 5 years, "1" for injection given >5 years ago, and "0" for no previous Rx.

Results

The proportions of individuals positive to ultrapure *Amb a* VI in both the Westinghouse and the clinic groups were about twice as high by serum IgG Ab as by IgE Ab measurements (Table 1, column 2). Within the group carefully selected for *clinical* allergy, i.e., clinic subjects, the proportions of individuals positive to *Amb a* VI by serum IgE and IgG Abs were almost twice the respective proportions in the Westinghouse group. Within each study group, the proportion of IgE Ab^+ people was higher among subjects who had received ragweed Rx (Rx^+) than those who had not (Rx^-), although the difference was significant only for the Westinghouse subjects (Table 1, column 5). The corresponding proportions for IgG Ab positivity in Rx^+ than Rx^- subjects were, as expected, significantly higher in the Rx^+ subjects in both study groups. This effect was more striking in the clinic than the Westinghouse group, as seen also by parametric analysis using the *t* test (Table 1, last footnote). These findings, which reflect the much more aggressive ragweed Rx among treated clinic than among treated Westinghouse subjects (noted in *Materials and Methods*), are highly relevant in interpreting the data for the two study groups (see below).

Figure 2A and B show the relationship between log [IgE Ab] and log [IgG Ab] for the two study groups; Rx^+ patients are shown by solid symbols, and different symbols are used for $HLA\text{-}DR5^+$ subjects (□) and $DR5^-$ subjects (○). There were several subjects whose immune response phenotype was "IgG Ab^+, IgE Ab^-," but none was "IgE Ab^+, IgG Ab^-." Among IgG Ab^+

Table 1. Proportions of subjects positive to *Amb a* VI by serum IgE Ab and IgG Ab

	Total group	Rx^+	Rx^-	*P* values*
Westinghouse subjects:	(*N*=116)	(*N*=14)	(*N*=102)	
IgE $Ab^{\dagger}$	11%‡	36%‡	8%	0.02
IgG $Ab^{\dagger}$	23%	57%	19%	0.007
Clinic patients:	(*N*=81)	(*N*=20)	(*N*=61)	
IgE $Ab^{\dagger}$	19%	30%	15%	Not significant
IgG $Ab^{\dagger\|}$	40%	75%	28%	0.0005

* Comparison of Ab positivity in Rx^+ and Rx^- groups (Fisher's exact test, two-tail)

† Among the IgE Ab^+ subjects, the geometric mean IgE Ab in the Westinghouse subjects was somewhat higher than in the clinic patients (8.6 ng/ml vs 5.6 ng/ml, respectively; *P* not significant). Among the IgG Ab^+ subjects, the geometric mean IgG Ab levels to *Amb a* VI for the clinic group was higher than that of the Westinghouse group (122 ng/ml vs 64 ng/ml, respectively; *P* not significant)

‡ In a preliminary report of this work (Marsh et al. 1986a), we noted slightly higher proportions of IgE Ab^+ and IgG Ab^+ subjects, as a result of classifying people with apparent Ab levels in the range of 0.2–0.4 ng/ml (in diluted serum samples) as "positive." Subsequent analyses revealed that such low levels could not be confirmed reproducibly. Thus, the cut-point finally selected was 0.4 ng/ml (in diluted samples; see *Materials and Methods*)

‖ Among the IgG Ab^+ clinic patients, the geometric mean IgG Ab level to *Amb a* VI was much higher among Rx^+ than among Rx^- subjects (261 vs 58 ng/ml; $P<0.02$). The corresponding difference between Westinghouse Rx^+ and Rx^- subjects (98 vs 53 ng/ml) was not significant

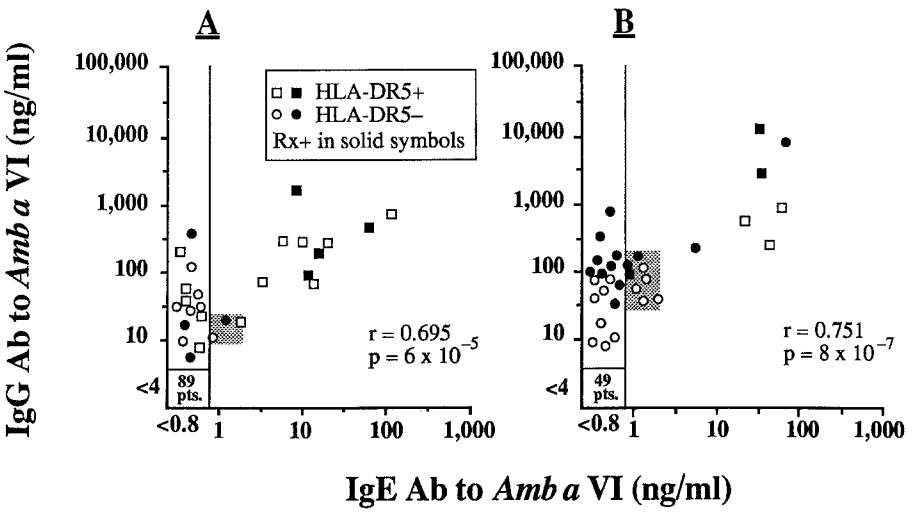

Fig. 2A and B. Relationships between IgE Ab and IgG Ab levels to *Amb a* VI for (**A**) 116 Westinghouse and (**B**) 81 clinic subjects. The numbers of patients (pts.) who had undetectable IgG Ab (<4 ng/ml) and IgE Ab (<0.8 ng/ml) are indicated. The *shaded areas* include people having IgE Ab levels in the range of 0.8–2.0 ng/ml. The correlation coefficients and *P* values are shown for log [IgE Ab] vs log [IgG Ab] for all the Ab^+ subjects (27 Westinghouse and 32 clinic). When the Rx^+ subjects were excluded, the correlation coefficients were $r=0.737$ ($P=3\times10^{-4}$) and $r=0.788$ ($P=2\times10^{-4}$) for 19 untreated Westinghouse and 17 untreated clinic subjects, respectively

subjects (includes *all* IgE Ab^+ subjects), there were strong ($r\approx0.7$), significant ($P<0.0001$) associations between log [IgE Ab] and log [IgG Ab] for both study groups. The correlation coefficients were slightly higher after the treated subjects had been excluded (see legend to Fig. 2). Similar strong correlations between log [IgE Ab] and log [IgG Ab] levels toward several different allergens (including *Amb a* VI) have previously been observed (Marsh et al. 1982a, Platts-Mills 1982, Roebber et al. 1983, Freidhoff et al. 1986).

By nonparametric analysis of the proportions of the different HLA types between *Amb a* VI^+ and *Amb a* VI^- subgroups, the most striking and consistent associations were between IgE Ab and IgG Ab positivity and HLA-DR5 (Table 2). Within each group of subjects studied, the associations with DR5 were stronger and more significant for IgE Ab than IgG Ab. The associations with DR5 for both IgE Ab and IgG Ab were stronger and more significant for Westinghouse than for clinic subjects. Among the Westinghouse IgE Ab^+ subgroup, the two atypical $DR5^-$ subjects had the lowest IgE Ab levels to *Amb a* VI (0.9 and 1.2 ng/ml); conversely, the 11 $DR5^+$ subjects in this subgroup had a geometric mean IgE Ab level of 12.6 ng/ml (range, 1.9–117 ng/ml). (The shaded areas in Fig. 2A and B include IgE Abs of 0.8–2.0 ng/ml.) Among Westinghouse IgE Ab^+ subjects, the two $DR5^-$ subjects also had the lowest IgE Ab/total IgE ratios. For both study groups, the geometric mean IgE Ab and IgG Ab levels were always significantly higher among $DR5^+$ than $DR5^-$ subjects (all *P* values < 0.02). There was a total of 13 $DR5^+$, IgG Ab^- subjects for the combined study groups (Table 2); only one of these had ever received ragweed Rx (12 years ago from a private physician).

Among the Westinghouse subjects, we also found significant positive associations with DQw3 for IgE and IgG Abs to *Amb a* VI ($P=0.0009$ and 0.02, respectively), which presumably result from the strong linkage disequilibrium between DR5 and DQw3 in Caucasoid populations. The same trends were found in the clinic patients, but they were not significant. There were also negative associations between IgE Ab or IgG Ab positivity and HLA-A3, -DR2, -DR6, -DR7, -DQw1, and DQw2 within one of the study groups. Since *none* of these associations

Table 2. Associations between IgE and IgG antibody response to *Amb a* VI and HLA-DR5 in two groups of allergic subjects

Response	Proportions of $DR5^+$		Relative risks	*P* values*
	Amb a VI^+	*Amb a* VI^-		
Westinghouse subjects ($N=116$):				
IgE Ab	11/13 (85%)	14/103 (14%)	35	7×10^{-7}
IgG Ab	16/27 (59%)	9/89 (10%)	13	1×10^{-6}
Clinic patients ($N=81$)				
IgE Ab	6/15 (40%)	4/66 (6%)	23	1×10^{-3}
IgG Ab	6/32 (19%)	4/49 (8%)	2.6	9×10^{-2}

* Fisher's exact test: two-tail in the case of the analyses of the Westinghouse subjects and one-tail in the case of the clinic patients
The Caucasian population frequency of HLA-DR5 is 19.5% (Engelfriet et al. 1980)

could be confirmed in the other group, it would appear that they are not of biological importance. Given the large number of statistical comparisons, one might anticipate some sporadic chance associations.

To examine the significance of the observed HLA-DR5 associations further, we carried out step-wise multiple regression analyses on data for log [IgE Ab] and log [IgG Ab] to *Amb a* VI for both study groups. This approach has the following advantages: (a) the relative influence of several variables known to be associated with immune responsiveness to allergens (Freidhoff et al. 1981, 1984, Barbee et al. 1981, Freidhoff 1987) can be investigated; (b) log [Ab] levels to *Amb a* VI can be treated as *metric* traits, thereby giving appropriately greater weight to the higher Ab levels, and minimizing the influence of choosing artificial cut-points between "positive" and "negative" subjects (the choice of which is largely determined by the sensitivity of the DARIA). Multiple regression analyses of the data are justified since, among responders, the frequency distributions of log [IgE Ab] and log [IgG Ab] to *Amb a* VI were found not to be significantly different from normal distributions in either of the two study groups. Also, for each of the groups, the distribution of log [total IgE] was not significantly different from a normal distribution.

The multiple regression analyses (Table 3) provided confirmation of the DR5 associations with IgE and IgG Ab responsiveness to *Amb a* VI. In the Westinghouse subjects, we also observed significant negative associations with age for both log [IgE Ab] and log [IgG Ab], reflecting a decreased immune responsiveness with increasing age among adults, that we and others have noted in earlier studies of allergy (Freidhoff et al. 1981, 1984, Barbee et al. 1981, Freidhoff 1987). Log [total IgE] was also weakly associated with log [IgE Ab] to *Amb a* VI in the Westinghouse group ($P=0.04$). The results of multiple regression analyses in which only ragweed-positive subjects were included were almost identical with those shown in Table 3 (data not shown). The relative influences of the $DR5^+$ and Rx^+ phenotypes on log [IgG Ab] in the two study groups were of particular interest. In the case of the Westinghouse subjects (where the influence of Rx was clearly weaker than in the clinic patients), we found that the association of log [IgG Ab] with DR5 was very striking ($P=10^{-11}$), whereas the association with Rx was much less significant ($P=0.002$). Conversely, in the clinic patients, where the influence of Rx was much higher, the association of log [IgG Ab] with DR5 was much weaker and less significant ($P=0.002$) than was the corresponding association with Rx ($P=3\times10^{-8}$).

Table 3. Variables showing significant associations with log [IgE Ab] and log [IgG Ab] levels to *Amb a* VI in stepwise multiple regression analyses*

Independent Variable	P values†	
	log [IgE Ab]	log [IgG Ab]
Westinghouse subjects ($N=116$):		
HLA-DR5	4×10^{-11} (+)	1×10^{-11} (+)
Age	3×10^{-3} (−)	8×10^{-3} (−)
Ragweed Rx	--	2×10^{-3} (+)
Log [total IgE]	4×10^{-2} (+)	--
Clinic patients ($N=81$):		
HLA-DR5	5×10^{-8} (+)	2×10^{-3} (+)
Ragweed Rx	—	3×10^{-8} (+)

* The following independent variables were included in the analyses of the Westinghouse data: all HLA-A, B, C, and DR types, log [total IgE], age, sex and ragweed immunotherapy (Rx). These same variables were also used for the clinic patients, except that the HLA-A, B, and C data were excluded since they were not available on all subjects

† Positive (+) and negative (−) associations are indicated

Discussion

We have demonstrated striking associations between HLA-DR5 and IgE and IgG Ab responsiveness to *Amb a* VI in two groups of allergic Caucasoids. There was almost an absolute association between DR5 and serum IgE Ab responsiveness to *Amb a* VI in the Westinghouse group; only 2 of 13 responder subjects who possessed the lowest IgE Ab levels typed $DR5^-$ (Table 2, Fig. 2A). By nonparametric analysis, the association between DR5 and serum IgE Ab responsiveness in the clinic group was weaker but significant. It can, however, be seen in Figure 2B that 7/9 (78%) of the IgE Ab^+, $DR5^-$ subjects' serum IgE Abs cluster at levels around 0.8–2.0 ng/ml; the remaining two IgE Ab^+, $DR5^-$ subjects had both received immunotherapy, which may have induced IgE Ab production. Ragweed Rx certainly influenced both the strength and significance of the association between IgG Ab to *Amb a* VI and DR5. The influence of much higher levels of Rx was particularly marked in the clinic patients, and was most evident in the parametric statistical analyses of the data (Table 3).

The complex interrelationships found in two different study groups between the levels of IgE and IgG Abs to *Amb a* VI and HLA, ragweed Rx and other variables (age and log [total IgE] with IgE Ab; Table 3) illustrate the importance of utilizing a comprehensive genetic-epidemiologic approach in defining the important factors that control human immune responsiveness to allergens (Marsh 1976).

Based on the current understanding of Ag presentation, our results are consistent with the following hypothesis: on the surface of the antigen-presenting cell (APC), a particular Ia molecule, associated with the serologically defined specificity HLA-DR5, controls the immune

recognition of a major Ia recognition site [''agretope'' (Heber-Katz et al. 1983)] on the *Amb a* VI molecule. Recent computer analyses of the structures of antigen-derived peptides, which contain immunodominant T-cell recognition sites for mouse T-cell clones, have shown that most of such peptides are ''amphipathic'' in nature [i. e., contain alternating hydrophilic and hydrophobic subregions (Berzofsky 1986, Spouge et al. 1987)]. Amphipathic peptides may be favored structures for presentation by the APC because they are stabilized in the amphipathic lipid bilayer of the APC. Such considerations also lend support to the concept of major Ia recognition sites on Ag molecules. It is presently not clear whether the DR5-associated Ia molecule, with which this postulated Ia recognition site interacts, is encoded by either *DR* or *DQ* α-β gene pairs, because of the strong linkage disequilibrium between certain alleles of these two *HLA-D* subregions (Baur et al. 1984). The DNA sequences of several genes within a single *DR5* haplotype are currently under study by Schwartz and his collaborators (Tieber et al. 1986, Didier et al. 1986); but, at the population level, the extent of polymorphism within the DR_β, DQ_α, and DQ_β genes present in *DR5* haplotypes is still unknown.

The postulated major Ia recognition site on *Amb a* VI would be the one predominantly recognized at the level of the APC under conditions of ultralow dose, natural exposure to the antigen. ''Minor'' Ia recognition sites on *Amb a* VI would be more likely to be recognized by other Ia molecules after artificial immunization (ragweed Rx) with much higher dosages of *Amb a* VI, leading to a ''dilution'' of the DR5 association. [The *Amb a* VI dosages are not well defined, but based on previous computations of allergen dosages (Marsh 1975, Marsh et al. 1982b) and the concentration of *Amb a* IV in the pollen (Roebber et al. 1983), the adult dosages of *Amb a* VI are probably considerably less than 0.2 μg/year by natural exposure and of the order of 1–200 μg/year for different levels of ragweed Rx — the Westinghouse subjects being at the lower, and the clinic subjects at the higher, end of this range.]

The association found in the present study is closely analogous to the extremely strong association found previously between Dw2 (the MLR-defined subspecificity of DR2 common in Caucasoids) and responsiveness to *Amb a* V (Marsh et al. 1982a, Blumenthal et al. 1985, Marsh 1986). In the case of *Amb a* V (M_r 5000), which is about half the size of *Amb a* VI, 90–95% of IgE responders were found to possess DR2/Dw2. The HLA-DR3/Dw3 associations with responsiveness to the rye grass pollen *Lol p* II and *Lol p* III (both M_r 11 000; Freidhoff et al. 1985, Ansari et al. 1987), which have similar relative masses to *Amb a* VI, were significant although less striking than that between DR5 and responsiveness to *Amb a* VI. The strengths of the various HLA associations probably reflect several interacting factors, including the following: (a) the numbers and population frequencies of different alleles of *DR*-region (and, possibly *DQ*-region) polymorphic genes that encode the relevant Ia molecules (Baur et al. 1984); (b) the number of major Ia recognition sites on the allergen molecule; (c) the intensity of allergenic exposure. Molecular biological and cellular immunological studies are in progress to define the relative importance of the first two of these factors with the aim of understanding these structure-function relationships at the molecular level.

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