HLA-Dw2: A GENETIC MARKER FOR HUMAN IMMUNE RESPONSE TO SHORT RAGWEED POLLEN ALLERGEN Ra5

II. Response after Ragweed Immunotherapy*

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In the preceding paper (1), we reported a significant striking association between Dw2 and IgE and IgG antibody (Ab)[1] responses to Ra5 in ragweed-allergic Caucasian subjects as a result of natural exposure to ragweed pollen. The question arose as to whether this relationship might change after parenteral immunization with much higher dosages of ragweed antigens. Such treatment, known as immunotherapy or hyposensitization, is commonly administered to ragweed-allergic patients to alleviate their allergic symptoms (2). Effective treatment is normally accompanied by high levels of serum IgG Ab toward ragweed antigens, especially the principal component, antigen E (AgE) (3).

Whereas the adult dosage of inhaled Ra5 is probably no more than 60 ng over the 8-wk period of ragweed pollination (4), cumulative annual immunotherapeutic dosages of Ra5 are usually at least one or two orders of magnitude higher. We will show that such immunotherapy induces good immunoglobulin G (IgG) Ab responses to Ra5 in all ragweed-allergic individuals who possess Dw2, whether or not they produced IgE and/or IgG Ab to Ra5 before therapy, whereas the prevalence and level of IgG Ab response in persons lacking Dw2 are both much lower. Despite an extreme degree of heterogeneity in the type of therapy and antigen dosage, the association between IgG Ab response to Ra5 and Dw2 becomes increasingly stronger and more significant over a 3-yr period of therapy.

Materials and Methods

Patients. The study group was comprised of 61 highly ragweed-allergic patients, who were a subset of the 86 patients included in the "clinic group," in the preceding paper (1). As previously stated, there was a bias toward selecting Ra5 responders, and, therefore, the proportion of Ra5+ subjects (23%) was higher than the value of ~9% one might expect in a random sample of ragweed-allergic subjects (cf. data for Westinghouse study group in ref. 1).

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[1] *Abbreviations used in this paper:* Ab, antibody; BSA, bovine serum albumin; BBS, borate-buffered saline, pH 8.3; CIE, crossed immunoelectrophoresis; Creg, cross-reacting group (of HLA antigens); DNV, double-normalized values; Dw2+(−), positive (negative) for HLA specificity Dw2; HLA, human leukocyte antigen; HSA, human serum albumin; Ra5+(−), positive (negative) response to Ra5; RIA, radioimmunoassay.

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TABLE I

Numbers of Available Post-Treatment Serum Samples and Dosages of Antigens for the First 3 yr of Therapy

	Year 1	Year 2	Year 3
Number of patients receiving aqueous ragweed extract*	20	16	5
Cumulative dosages, geometric mean (range) in μg Ra5 equivalents‡	0.75 (0.06—60)	1.4 (0.18—9.9)	8.7 (4.1—12)
Final dosages, geometric mean (range) in μg Ra5 equivalents‡	0.14 (0.01—12)	0.26 (0.08—0.68)	0.37 (0.12—0.54)
Number of patients receiving aqueous allergoid*	32	27	28
Cumulative dosages, geometric mean (range) in μg Ra5 equivalents§	14 (0.53—101)	26 (0.56—695)	24 (1.5—599)
Final dosages, geometric mean (range) in μg Ra5 equivalents§	3.9 (0.12—68)	4.2 (0.09—28)	4.7 (0.46—28)
Number of patients receiving alum-ragweed*	9	7	7
Total number of patients	61	50	40‖

* The changes in the numbers of serum samples in successive years reflect patients switching treatment to a different antigen as well as patients who dropped out of the study.

‡ Cumulative and final dosages of AgE averaged 8.4-fold higher than those of Ra5.

§ Cumulative and final dosages of AgE averaged 16.7-fold higher than those of Ra5.

‖ These 40 individuals received therapy during years 1, 2, and 3, and serum samples were available for all 3 yr. Note that subjects J. G. (Fig. 1 b) and W. R. (Fig. 2 b) were not included because of an incomplete set of serum samples.

Pre- and post-treatment serum samples for each year of therapy were available on almost all of the patients. The sera had been stored at −20° or −70°C between the period of collection and analysis of serum Ab levels.

All 61 individuals had received immunotherapy with ragweed pollen antigens for periods ranging from 1–9 yr, either preseasonally (every 2–4 wk for 12–16 wk before the ragweed season) (5)[2] or on a perennial basis (every 1–2 wk except during the ragweed pollen season, approximately August 15 to October 15 (6, 7). In either mode of therapy, antigen dosages were built up gradually from low levels, depending on the patient's tolerance to the injections. Therefore, antigen dosages varied considerably from patient to patient, as summarized for the first 3 yr of treatment in Table I. Three different types of antigen were used: aqueous ragweed pollen extract (5–7),[2] formaldehyde-modified ragweed ("allergoid;" 5–7),[2] and alum-precipitated ragweed (6). Two of the antigens were well standardized as far as their content of Ra5 and AgE. Reliable standardization could not be performed for the alum-precipitated material, which was used in a few patients.

Different types of antigen and varying antigen dosages were administered, and certain patients switched from one antigen to another, depending on the goal of the particular study in which the patient was a participant. Because several patients dropped out of the studies, the total number of patients for whom post-treatment serum samples were available decreased each year (Table I). A complete set of pre- and post-treatment sera were, however, available on a subgroup of 40 patients who had been treated for 3 successive yr from the start of therapy.

Antigens and Assays for Ab and Total IgE. The Ra5 preparation and radioimmunoassays (RIA) of IgG Ab and IgE Ab responses to Ra5 were as described in the preceding paper (1). IgG Ab responses to ragweed AgE were determined by a similar RIA using a highly purified AgE preparation, as described in detail elsewhere.[2] Intradermal and puncture skin test procedures were performed as previously described (1). Pretreatment total serum IgE levels were also available on all study patients (1).

[2] Marsh D. G., E. Ehrlich-Kautzky, and P. S. Norman. A kinetic study of human immune response to injected ragweed allergen and allergoid. Manuscript in preparation.

HLA Typing and Data Analysis. HLA-A,B,C, and D typings were available on all 61 study subjects and DR typings on 37 of these individuals (1).

In analyzing IgG Ab responses to Ra5 and AgE after each year of therapy, we considered only the first 3 yr of therapy, where complete sets of sera were available on 40 patients (Table I). Our overall approach was similar to that used in the preceding study (1). In the nonparametric statistical analyses (using Fisher's exact test), IgG Ab responses were dichotomized as Ra5+ or Ra5−. In analyzing log[IgG Ab] responses to either Ra5 or AgE by parametric statistics, HLA type, log[pretreatment total IgE], age, sex, and previous immunotherapy (before this study) were categorized as previously described (1). In addition, "type of current immunotherapy" and log[antigen dose] were included as further independent variables. In view of the different lengths of treatment during each year (preseasonal vs. perennial), the "final doses" administered ~2 wk before the post-treatment serum sample, rather than cumulative yearly dosages, were used in computing the log[Ra5] and log[AgE] equivalent doses. With few exceptions, the final doses were also the largest single doses administered in any given year.

Results

IgG Ab Responses. Fig. 1 illustrates the changes in serum IgG Ab responses to Ra5 in 14 immunized individuals who were categorized as Ra5+ both by skin test and IgE Ab before therapy (1). All had significant levels of IgG Ab before treatment. Subsequent IgG Ab responses in the 13 Dw2+ subjects (shown by solid lines) and one Dw2− subject (J. Ko.; dashed line in Fig 1 a) were similar. In the preseasonal treatment group (N = 9), the IgG Ab levels rose significantly after the first period (year 1) of 12–16 wk of treatment, fell between years 1 and 2, when therapy was discontinued, and rose again when therapy was recommenced in year 2. There was a subsequent decrease followed by an increase in IgG Ab levels during years 2–3. In the case of the five patients receiving perennial therapy, all subjects except M. K., who received a very low dosage of ragweed allergen during the 1st yr (cumulative dose,

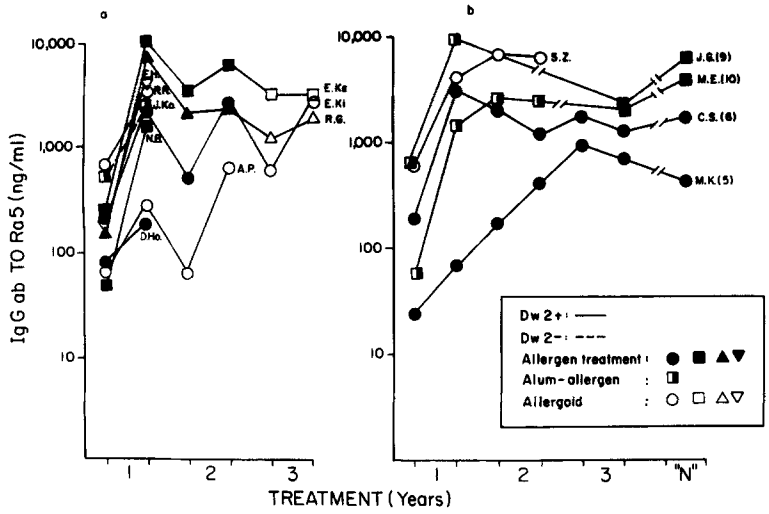

FIG. 1. IgG Ab responses to Ra5 in all patients who were phenotypically Ra5+ by IgE Ab and skin test before therapy with ragweed. (a) Ra5+, preseasonal Rx; (b) Ra5+, perennial Rx. Each patient is designated by his initials and a symbol that also indicates the type of antigen used. Several patients switched treatment (e.g., from allergen to allergoid), indicated by a change in the shading of the patient's symbol. The number of years of treatment is given in parentheses after the patient's initials in the case of perennial therapy (Fig. 1 b).

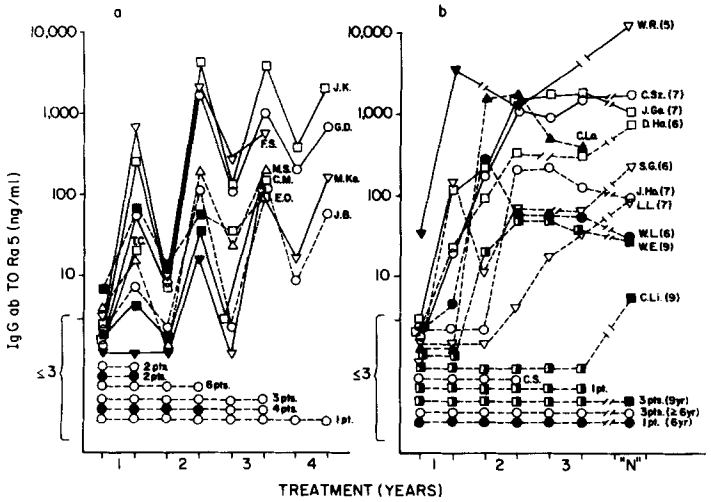

FIG. 2. IgG Ab responses to Ra5 in patients who were phenotypically Ra5− by IgE Ab and skin test before therapy with ragweed. (a) Ra5−, preseasonal Rx; (b) Ra5−, perennial Rx. In the case of nonresponders, the number of patients in each treatment category is indicated. See Fig. 1 for further description of symbols. Individuals typing DR2+,Dw2− were J. B., W. E., C. Li., and C. S.

0.06 μg Ra5; final dose, 0.01 μg Ra5) showed about a 10- to 100-fold rise in IgG Ab level during the 1st yr of therapy. Throughout the whole period of perennial therapy, patients other than M. K. maintained Ab levels of 1,000–10,000 ng/ml, which is the range of peak responses achieved by most subjects treated preseasonally.[3]

Fig. 2 shows the IgG Ab responses to Ra5 after preseasonal or perennial therapy with ragweed for 47 individuals who had no IgE Ab and were skin test negative to Ra5 before therapy (although three individuals, M. S., E. O., and W. R., had detectable IgG Ab before treatment). All 9 Dw2+ individuals as well as 10 (26%) of Dw2− individuals made substantial IgG Ab responses to Ra5 within 2 yr after receiving either preseasonal or perennial therapy. All of the remaining 28 Ra5−,Dw2− subjects except one showed no detectable IgG Ab response throughout the entire treatment period (averaging 4 yr). The one exceptional subject (C. Li.; Fig. 2a) made trace IgG Ab responses (<10 ng/ml) detectable at years 4–9.[4] In the case of one Dw2+ individual (M. Ke.), who received quite low therapeutic dosages (final dose, 0.06 μg Ra5), no response was observed after year 1 of preseasonal therapy, but an IgG Ab response was observed at year 2, as dosages were increased.

In summary, after 1–2 yr of therapy, all 22 Dw2+ subjects (13 Ra5+ and 9 Ra5− by pretreatment IgE Ab) made detectable IgG Ab responses to Ra5, whereas of 39 Dw2− subjects, only 11 subjects (1 Ra5+ and 10 Ra5−) responded similarly.

Univariate Analyses. Table II presents the results of analyses of serum IgG Ab responses to Ra5 at years 0–3 for all treated subjects (group A) and subjects who were phenotypically Ra5− by skin test and IgE Ab before therapy (group B). Individuals within these two groups were classified as Ra5 IgG Ab+ or Ra5 IgG Ab− at each year, according to whether or not they made a detectable IgG Ab response (>3

[3] Peak IgG Ab levels toward AgE were also in this concentration range for most patients.

[4] The significance of these persistent low levels is questionable in view of the failure of the response to increase substantially with continued treatment.

TABLE II

Associations between HLA-B7 and Dw2 and IgG Ab Responses to Ra5 for All Available Treated Patients (Nonparametric Statistics) *

	Number of subjects with IgG Ab phenotype		Percent with B7		*P* values	Relative risks	Percent with Dw2		*P* values	Relative risks
	Ra5+	Ra5−	Ra5+	Ra5−			Ra5+	Ra5−		
Group A, all treated subjects										
Year 0 (N = 61)	17	44	53	23	0.032	3.8	82	18	<0.0001	21
Year 1 (N = 61)	28	33	50	15	0.008	5.6	75	3	<0.0001	96
Year 2 (N = 50)	26	24	46	13	0.014	6.0	62	0	<0.0001	77‡
Year 3 (N = 40)	23	17	43	12	0.041	5.8	57	0	0.0001	45‡
Group B, subjects who were phenotypically Ra5− by skin test and IgE Ab before therapy										
Year 0 (N = 47)	3§	44	33	23	NS‖	1.7	33	18	NS	2
Year 1 (N = 47)	14	33	43	15	NS	4.2	57	3	0.0001	43
Year 2 (N = 42)	18	24	33	13	NS	3.5	44	0	0.0004	40‡
Year 3 (N = 34)	17	17	29	12	NS	3.1	41	0	0.0072	25‡

* *Other associations*: negative associations with A1 at year 3 in group B ($P = 0.03$) and with B15 at years 1 and 2 in group A ($P = 0.05$ and 0.007, respectively).

‡ Determined by the Haldane formula (1, 8) because no Ra5− subjects were Dw2+.

§ Before therapy, low levels of IgG Ab to Ra5 (4 and 7 ng/ml) were detected in two Dw2− subjects and a somewhat higher level (34 ng/ml) in a Dw2+ subject, all of whom had no detectable IgE Ab and were skin test negative. These three subjects in group B, along with 14 Ra5 IgE Ab+,IgG Ab+ individuals, constitute the 17 Ra5+ subjects in group A.

‖ Not significant.

ng/ml) (1), and the data were analyzed by Fisher's exact test. The analyses showed striking associations between Dw2 and presence of IgG Ab to Ra5 for both groups A and B. Less striking associations were observed between IgG Ab responses and B7, none of which was significant in group B. Two other specificities (A1 and B15) were significantly associated with IgG Ab response in one or two analyses (see footnote to Table II). These latter associations were weak and sporadic and are probably not important in view of the large number of analyses performed.

We next compared the geometric mean IgG Ab responses to Ra5 during the first 3 yr of treatment for three subgroups of patients (Ra5+,Dw2+; Ra5−,Dw2+; Ra5−,Dw2−), where the Ra5 response phenotypes were based on IgE Ab responses before therapy (Fig. 3). To maintain consistency within the year to year comparisons, only the 40 subjects who remained on treatment for 3 yr were included. Comparison of the geometric mean IgG Ab responses in the Ra5+,Dw2+ vs. the Ra5−,Dw2+ group showed that the difference in IgG Ab at year 0 remained significant at year 1. The difference was, however, much less marked and no longer significant at years 2 and 3. After only 1 yr of therapy, the geometric mean IgG Ab response to Ra5 for the group that had been phenotypically Ra5−,Dw2− before therapy was significantly lower than the Ra5−,Dw2+ group. Furthermore, the geometric mean difference between these two groups increased from 7-fold at year 1 to over 50-fold by years 2 and 3.

Among the 27 Ra5−,Dw2− subjects, 17 were nonresponders; but the remaining 10 individuals made quite good IgG Ab responses after therapy (Fig. 2). The geometric mean IgG Ab levels in these 10 subjects after each year of therapy for years 1, 2, and

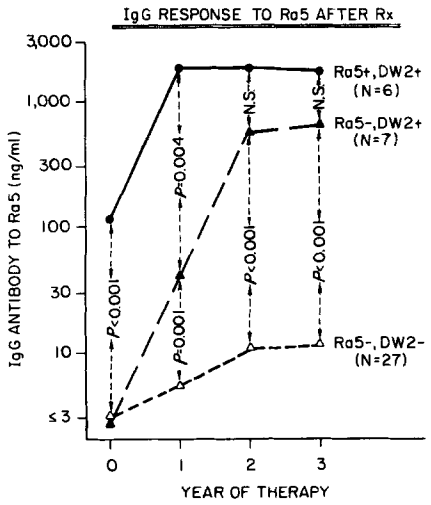

FIG. 3. Geometric mean IgG Ab responses to Ra5 in three subgroups of patients from a total of 40 subjects who had been immunized with ragweed antigens over a period of 3 successive yr. The phenotypes of the groups were designated according to pretreatment IgE Ab (and skin test) responses to Ra5. The significance of differences between the groups before treatment (year 0) and at each successive post-treatment year were calculated by t test.

3 were compared against the corresponding geometric mean Ab levels in the seven Ra5−,Dw2+ subjects. As therapy proceeded, an increasingly greater differential Ab response was observed in the Ra5−,Dw2+ group. The difference between the Ra5−,Dw2+ and Ra5−,Dw2− groups was significant by year 3 (geometric means, 623 vs. 107 ng/ml, respectively; $P = 0.01$).

Multivariate Analyses. We wished to compare both the degree and significance of the association between HLA type (and other independent variables) and IgG Ab responses to Ra5. This was done by multiple regression analysis in which the individual HLA specificities, log[pre-treatment total IgE], age, sex, previous immunotherapy, type of therapy, and log[Ra5 dose] were independent variables, and log[IgG Ab] was the dependent variable. First, we carried out three stepwise multiple regressions in which post-treatment IgG Ab data for all available patients at years 1, 2, and 3 were included, except for a few individuals treated with alum-precipitated ragweed, for whom antigen dosage was not known precisely. The analyses revealed extremely striking associations between Dw2 and log[IgG Ab] at years 1, 2, and 3 ($P < 10^{-10}$ in all cases). In addition, previous immunotherapy was significantly positively associated with log[IgG Ab] at year 1 ($P = 0.01$) but not at years 2 and 3. Further significant associations were observed in one or two of the analyses with A2, A9, B12, B13, B14, B17, B27, Cw2, and Dw1, of which A2, B14, B27, and Cw2 were negatively associated. These associations were generally weak and showed no clear pattern throughout the 3 yr of therapy, except for positive associations with B13 at years 2 and 3 ($P \leq 0.001$ in both cases) and negative associations with A2 at year 2 ($P = 0.005$) and year 3 ($P = 0.002$). Antigen dosage was not significantly correlated with log[IgG Ab] to Ra5 either in the whole study group or the subset of Ra5 IgE Ab+ subjects.

We wished to examine critically the relative influence of each of the variables found

TABLE III

Multiple Regression Analyses: Significant Associations between HLA and Log[IgG] Ab Response to Ra5 after Ragweed Immunotherapy of 40 Patients *

Variable		Period of therapy with ragweed			
		Year 0	Year 1	Year 2	Year 3
Dw2§	b‡	0.595	0.784	0.823	0.874
	P(two-tail)	1.2×10^{-4}	2.9×10^{-8}	1.4×10^{-8}	9.4×10^{-10}
B13‖	b‡	−0.156	−0.010	0.248	0.272
	*P*p(two-tail)	0.24	0.92	0.022	0.008

* Only variables found to be significantly associated with log[IgG Ab] to Ra5 from previous stepwise multiple regressions were included in these analyses. These were: Dw2 (from year 0, 1, 2, and 3 analyses); A2 (from year 0, 2, and 3 analyses); previous immunotherapy and B14 (from year 0 and 1 analyses); B13 (from year 2 and 3 analyses) and A9, B5, B12, B17, B27, Bw35, Cw2, Cw3, and Dw1 (significantly associated in just one of the previous analyses). Besides the cited associations, Bw35 was significantly associated at year 0 (b = 0.291, $P = 0.040$).

‡ Standardized regression coefficient.

§ 13 people had the Dw2 phenotype. The proportions of IgG Ab+ subjects among these Dw2+ individuals at years 0, 1, 2, and 3 were 46%, 92%, 100%, and 100%, respectively.

‖ Eight people had the B13 phenotype. The proportions of IgG Ab+ subjects among these B13+ individuals at years 0, 1, 2, and 3 were 25%, 63%, 75%, and 75%, respectively.

to be significantly associated with IgG Ab response to Ra5 in one or more of the previous stepwise regressions, namely, analysis of pretreatment (year 0) data on all clinic patients (Table II in ref. 1) and the analyses of year 1, 2, and 3 data described above. Therefore, we carried out a series of multiple regression analyses in which only these variables[5] were included in analyses of year 0, 1, 2, and 3 data for the 40 individuals who had been on ragweed therapy over a 3-yr period, as described in Table I.

The results of these analyses (Table III) showed that Dw2 and B13 were significantly associated with log[IgG Ab] to Ra5. These two associations had previously shown the greatest significance in the stepwise regressions. (Bw35 was also weakly associated with log[IgG Ab], but only at year 0; see footnote to Table III). The associations between log[IgG Ab] to Ra5 and Dw2 were particularly striking; they progressively increased between years 0 and 3, both in strength and significance, indicated by changes in the standardized regression coefficient b and *P* values. By year 3, the b value had reached 0.874 (for the range of −1 to +1), with a *P* value of 10^{-9}. The associations between log[IgG Ab] and HLA-B13 were not significant at years 0 and 1 but were significant by years 2 and 3. These trends toward stronger associations between IgG response and Dw2 and B13 as therapy proceeded were also reflected in the increased proportions of responders among Dw2+ and B13+ individuals (see footnotes to Table III). It is also of interest to note that two of the three patients who had IgG Ab but no IgE Ab before treatment (M. S. and L. O.) were B13+, Dw2−; the other similar individual (W. R.) was B13−, Dw2+.

IgE Ab Responses after Therapy. We have seen that IgG Ab responses to Ra5 were induced in certain genetically predisposed individuals after ragweed immunotherapy. Next, we wished to evaluate the possibility that therapy might also induce IgE Ab responses (detectable by RIA and skin testing) in some subjects.

Definitive IgE Ab responses were seen only in the 14 subjects who were sensitive to

[5] The variables included are cited in a footnote to Table III.

Ra5 before therapy and in one Ra5−,Dw2+ subject (W. R.; Fig. 2b). After 5 yr of therapy, patient W. R. had an intradermal skin test end point (8- to 10-mm wheal) at 10^{-6} μg Ra5/ml, and his serum contained ~430 ng IgE Ab/ml. The IgE Ab level could not be measured accurately because of the extremely high IgG Ab level (11,500 ng/ml). This individual was exceptional in that he had quite substantial pretreatment IgG Ab (34 ng/ml) and had the highest total IgE level (5,390 ng/ml) of anyone on the study. An additional Ra5−,Dw2+ subject (C. M.; Fig. 2a), who had the second highest IgE level (468 ng/ml) among Ra5−,Dw2+ subjects, became significantly skin test positive (8- to 10-mm wheal at 10^{-3} μg Ra5/ml by intradermal test) after 3 yr of therapy, although his serum contained no detectable IgE Ab to Ra5.

DR-D Relationships. The relationship between IgG Ab response to Ra5 and DR phenotype was studied in 37 of the 61 study subjects, including 15 of the Ra5 IgE Ab− subjects who produced IgG Ab after therapy. Among Ra5−,Dw2− subjects (pretreatment), we wished to investigate whether there was a greater prevalence of DR2 in people who made substantial IgG Ab responses vs. those who did not. In fact, there was no significant difference in the proportion of DR2 in these two Ra5−,Dw2− subgroups (2 of 10 responders vs. 2 of 7 nonresponders had DR2). As previously stated (1), there was a perfect concordance between DR2 and Dw2 in all Ra5 IgE Ab+ subjects, all of whom made good IgG Ab responses after therapy.

Are the IgG Ab Responses to Ra5 Antigen Selective? The question arose as to whether the IgG Ab responses to Ra5 seen in certain previously Ra5− subjects might be an Ra5-selective phenomenon or simply reflect an overall hyperresponsiveness to ragweed antigens in general. To test this possibility, we analyzed IgG Ab response to ragweed AgE, which is known to be a good immunologic parameter of response toward ragweed therapy (3). The geometric mean post-treatment anti-AgE IgG Ab levels at years 1, 2, and 3 were compared by *t* test in Ra5+ vs. Ra5− subjects (IgE or IgG Ab phenotypes before therapy). At years 1 and 3, there was no significant difference in the geometric mean IgG Ab to AgE between the Ra5+ and Ra5− groups. This was true whether pretreatment response to Ra5 was classified by IgE or IgG Ab ($P > 0.4$ in all cases). At year 2, the responses to AgE were actually greater in Ra5 IgE Ab− than Ra5 IgE Ab+ individuals (geometric means, 3,300 vs. 1,010 ng/ml; $P < 0.001$) and in Ra5 IgG Ab+ vs. Ra5 IgG Ab− subjects ($P = 0.003$). These results strongly suggest that IgG Ab responses to Ra5 do not merely reflect a generalized hyperresponsiveness toward ragweed antigens.

Discussion

Artificial immunization with ragweed antigens in much higher dosages than encountered by natural exposure to pollen induced IgG Ab responses to Ra5 only in certain ragweed-allergic patients. Both the quantitative and qualitative degree of response to Ra5 were significantly associated with HLA-Dw2 throughout treatment ($P < 0.0001$ for all comparisons). By multiple regression analysis, both the strength and significance of the relationship between Dw2 and log[IgG Ab] response to Ra5 were found to increase throughout a 3-yr period of therapy, reaching a significance level of 10^{-9} after the 3rd yr. Immunotherapy did not induce measurable levels of IgE Ab to Ra5 in previously Ra5-insensitive subjects except in one Dw2+ person (W. R.), who had a modest amount of IgG Ab before therapy and a very high total IgE level, perhaps indicative of hyper-IgE responsiveness.

These findings are striking, especially in view of the heterogeneity in the type of antigen, antigen dosage, and dosage regimen used in the patients (Table I). However, the cumulative annual immunotherapeutic dosages of Ra5 are still very low on a body-weight basis in comparison with the "low" doses of protein antigens normally used for immunogenetic studies of *H-2* linkage in mice (9, 10). The respective dosages average ~0.01–0.3 μg/kg for 75 kg adult humans (Table I) vs. 10–100 μg/kg for mice (two 0.1-μg or 1.0-μg doses of antigen on alumina adjuvant). By comparison, the dosages of inhaled Ra5 are no more than ~0.001 μg/kg per yr for adult humans living in the Baltimore area (4). These dosage differences should be considered in extrapolating from animal models to our data in humans. As noted by Provoust-Danon et al. (11), there is clearly a need for further studies of the influence of antigen dosage on the genetics of immune response in animals. The effect of adjuvants and route of immunization are further factors to be considered.

We also found that IgG Ab response to Ra5 in man appears to be an antigen-selective effect. The level of IgG Ab response to the highly immunogenic ragweed allergen, AgE (toward which all subjects responded well), was not significantly related to IgG Ab response to Ra5. Indeed, there was some evidence for a negative relationship.

There are several possible genetic hypotheses that can be invoked to explain these data, including (*a*) that *HLA-Dw2* might be the major *Ir-Ra5* gene in Caucasians; (*b*) there is a separate *Ir-Ra5* gene in linkage disequilibrium with *HLA-Dw2* that results in the association between Dw2 and response to Ra5 seen in Caucasian populations; (*c*) there is an *Ir-Ra5′* gene somewhere in the genome (not necessarily *HLA*-linked) that interacts with, or is dependent on, *HLA-Dw2* for the expression of response to Ra5.

Analogous hypotheses to (*a*) and (*b*), particularly the latter, have been popular explanations for disease-association data and have been extensively discussed elsewhere (12, 13). In guinea pig and mouse experiments, monospecific sera raised against Ia antigens (which include the analogues of human DR) block *Ir*-gene controlled responses in vitro (14–16). These experiments, together with immunochemical analyses of complementing E_α and E_β genes that map in the *I-E* and *I-A* regions of mouse *H-2* (17, 18), suggest an identity between *H-2*-linked *Ir*-gene products and Ia antigens (16). In the light of these findings, a reasonable interpretation is that *DR2* or a gene closely linked to and in strong linkage disequilibrium with *DR2* is the *Ir-Ra5* gene. One might further hypothesize that this gene codes for a cell receptor for an immunodominant determinant on Ra5. If DR2 itself was such a receptor, DR2 rather than Dw2 might be expected to show the more striking association with response to Ra5; in fact, the converse is the case. Perhaps the most probable explanation is that anti-DR2 typing sera recognize a broader range of D specificities than those recognized by Dw2 homozygous typing cells, the latter corresponding more specifically to the product of *Ir-Ra5*.

As an alternative, we feel that explanations based on the third hypothesis deserve serious consideration. One must explain why, on the one hand, only a proportion of Dw2+ subjects respond after natural exposure to extremely limiting doses of inhaled Ra5, whereas, on the other hand, all Dw2+ ragweed-allergic subjects make good IgG Ab responses after injection of much higher (but still immunogenically limiting) doses of Ra5. Among Westinghouse subjects (most of whom had never received immuno-

therapy), only 10 of 29 (34%) of Dw2+ ragweed-allergic subjects and only 1 of 50 (2%) of Dw2+ subjects in the nonragweed-allergic group made IgG and/or IgE Ab responses to Ra5 (1). Nongenetic factors (especially antigen exposure) and IgE-regulating gene(s) must certainly play important roles in determining this differential responsiveness (19). On the other hand, it seems unlikely that these factors are the only determinants among Westinghouse ragweed-allergic subjects, who were selected for their relatively uniform exposure to ragweed pollen (20).

We believe that our observations suggest the possibility that another gene (*Ir-Ra5'*), apart from *Dw2*, may code for a cell receptor facilitating response to the immunodominant determinant on Ra5. This receptor could be the controversial T cell receptor believed to be encoded by Ig heavy-chain genes (16, 21), or it could be expressed on antigen-presenting cells along with the product of the *Dw2* (*Ir-Ra5*) gene. In either case, the postulated product of the *Ir-Ra5'* gene may have affinities for both Ra5 and Ir-Ra5. According to this general postulate, an individual would require two genes, *Ir-Ra5* and *Ir-Ra5'*, to produce IgG Ab (and usually IgE Ab) to Ra5 merely as a result of natural exposure to ragweed pollen. Individuals having *Ir-Ra5* but not *Ir-Ra5'* would require the higher antigenic dosages achieved by artificial immunization to respond. Individuals possessing neither *Ir-Ra5* nor *Ir-Ra5'* would usually not respond to Ra5, even after high-dose immunotherapy (see below for discussion of other possibilities).

The third hypothesis allows for the possibility that the postulated *Ir-Ra5'* gene is not linked to the *HLA* complex, a result that is entirely consistent with data from our family studies (22). Among families selected on the basis of an Ra5-allergic propositus, other family members (including those who are ragweed-allergic) usually do not respond to Ra5, even though they possess the same *Dw2*-containing haplotype as the propositus (22, and unpublished observations). From the data presented here, one would expect that such individuals would respond after ragweed immunotherapy. Indeed, such an experiment might be expected to yield data entirely consistent with immunogenetic experiments in laboratory animals where the effect of MHC-linked genes is well established (16).

We must also explain why certain Dw2− individuals respond to Ra5, including a sizable proportion who make moderate IgG Ab responses after artificial immunization. One possibility is that some of these people possess *Ir-Ra5'*, which may permit weak to moderate responses even when *Dw2* (*Ir-Ra5*) is absent. Alternatively, a few of the Dw2− subjects, who develop IgE and IgG Ab responses as a result of natural exposure and/or respond well after immunotherapy, might well be able to recognize the Ra5 molecule via a secondary determinant(s) or, perhaps, via the primary determinant with a lower affinity. There is some suggestion from the present studies that *HLA-B13* might be associated with a gene conferring response to one such secondary determinant.

In conclusion, our results re-emphasize the usefulness of the allergy model for investigations of the genetics of human immune response (19). It is now clear that studies of response toward the higher antigenic dosages administered as part of accepted immunologic therapy of allergy add important new information to data from studies of ultra-low dose natural exposure. It is particularly appropriate to use a low molecular weight allergen like Ra5 because the number of immunodeterminants is restricted. Also, the allergen should be ultra-pure because of the likelihood that a

person's response to impurities might be interpreted as a response to the major component. The striking relationship between Dw2 and IgE and IgG Ab responses to Ra5 provides strong evidence for the role of an HLA-D specificity in determining immune response in outbred, highly polymorphic human populations. The variable expression of Dw2-associated response to Ra5 under different degrees of immunization suggests the involvement of a further genetic locus, possibly unlinked to *HLA*.

Summary

After artificial immunization (immunotherapy) with ragweed antigens, specific immunoglobulin G (IgG) antibody (Ab) response to Ra5 was significantly associated with HLA-Dw2 ($P < 0.0001$). From a total of 61 treated patients, all 22 Dw2+ subjects made good IgG Ab responses to Ra5 by year 2 of therapy (21 by year 1), even though 8 of them had no detectable IgG Ab and 9 had no detectable IgE Ab before therapy. The prevalence of IgG Ab response among 39 Dw2− subjects was markedly lower; only 11 (28%) responded well after 1–9 yr of therapy. Both by univariate and multivariate statistical analysis, Dw2 was also found to be strongly associated with the quantity of IgG Ab produced. In particular, both the strength and significance of the association between Dw2 and log[IgG Ab] response to Ra5 increased over a 3-yr period of ragweed therapy ($P = 10^{-9}$ by year 3). Multiple regression analysis also revealed a weak association with HLA-B13, which became apparent only after year 2 of therapy. Genetic hypotheses for these findings are discussed. In particular, the possibility of a second *Ir* gene, *Ir-Ra5'*, separate from *HLA-Dw2* and possibly located elsewhere in the genome, is considered.

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