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SPECIFIC IgE ANTIBODY RESPONSES TO RAGWEED ALLERGENS Ra5S AND Ra5G ASSOCIATED WITH DISTINCT HLA-DR β GENES*

K. M. COULTER, W. H. YANG,† G. DORVAL, M. A. DROUIN,† C. K. OSTERLAND and L. GOODFRIEND

Thorp Division of Immunology, Royal Victoria Hospital, Montreal, Quebec, Canada; and †Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada

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Abstract—Previous studies have established that sensitivity (IgE antibody response) to Ra5S, a 5000 mol. wt protein of short ragweed pollen, is significantly associated with host possession of HLA-DR2. The same allele was implicated [Goodfriend *et al.* (1985) *Molec. Immun.* **22**, 899–906] in sensitivity to Ra5G, a 4400 mol. wt homologue of Ra5S in giant ragweed pollen, based on frequency of *co*-sensitivity to both proteins. However, data reported here generated in HLA-DR assays of *mono*-sensitive individuals demonstrate that sensitivity to Ra5S and Ra5G is associated with separate alleles: DR2 and DRw52 respectively. Results consistent with the same sensitivity/DR associations were obtained in immunoabsorption studies with sera from co-sensitive individuals. As HLA-DR2 and DRw52 have identical alpha but different beta chain types (β_1 and β_3), it was considered that IgE antibody responses to Ra5S and Ra5G are associated with distinct DR-β genes.

INTRODUCTION

Environmental proteins of diverse size and structure constitute a plethora of incitants to which genetically susceptible individuals mount allergic responses. Among these, low mol. wt proteins, such as Ra5S‡ of short ragweed pollen, were suggested (Goodfriend *et al.*, 1973; Marsh *et al.*, 1973) as potentially efficacious for detecting immunodominant HLA-associated *Ir* genes controlling the development of specific allergic responses. For a review of earlier studies based on this premise, see Marsh *et al.* (1973, 1975) and Goodfriend (1975, 1976). More recently, several studies have established that sensitivity to Ra5S is significantly associated with host possession of the HLA-Dw2/DR2 allele (Bias *et al.*, 1979; Marsh *et al.*, 1982; Coulter, 1983). In a report from this laboratory (Goodfriend *et al.*, 1985), the same allele was implicated in sensitivity to Ra5G, a homologue of Ra5S in giant ragweed pollen, based on freqency of co-sensitivity to these proteins. However, cross-reactivity of Ra5G with IgE antibodies formed to Ra5S may have provided a spurious basis for the observed co-sensitive frequency (Coulter *et al.*, 1986) and hence an apparent association of DR2 with Ra5G sensitivity. Accordingly, for further analysis, we focused on our observation that some ragweed-sensitive individuals are cutaneously sensitive to Ra5G but not Ra5S and vice versa (Goodfriend *et al.*, 1985). Cutaneous tests simultaneously with Ra5S and Ra5G were therefore continued to expand the groups uniquely sensitive to each allergen for subsequent HLA-DR typing. The validity of the sensitivity/DR associations observed was tested in the co-sensitive case by P–K tests with co-sensitive sera prior to and following absorption with solid phase Ra5S and collation of the test results with HLA-DR type.

MATERIALS AND METHODS

Ra5S and Ra5G

The allergens were isolated from short and giant ragweed pollen (Greer Laboratories, Durham, NC) to column chromatographic and disc electrophoretic homogeneity (Lapkoff and Goodfriend, 1974; Goodfriend *et al.*, 1985). Minor contaminants in both allergen preparations were removed by HPLC in a C18 μBondapak analytical column (Waters Associates, Missisauga, Ontario). For cutaneous tests, the purified allergens (Fig. 1) were dissolved to 0.1 mg/ml bicarbonate buffered saline with phenol (BBS; Hollister-Stier), Millipore filtered and stored at −20°C. Final dilutions were made in 0.03% HSA diluent (Pharmacia, Montreal).

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‡In the WHO nomenclature, *Amb a V* corresponds to Ra5S, *Amb t V* to Ra5G.

Abbreviations: BBS, bicarbonate buffered saline; HPLC, high performance liquid chromatography; HSA, human serum albumin; i.d., intradermal; Ir, immune response; MHC, major histocompatibility complex; P–K, Prausnitz–Kustner; RAST, radioallergosorbent test.

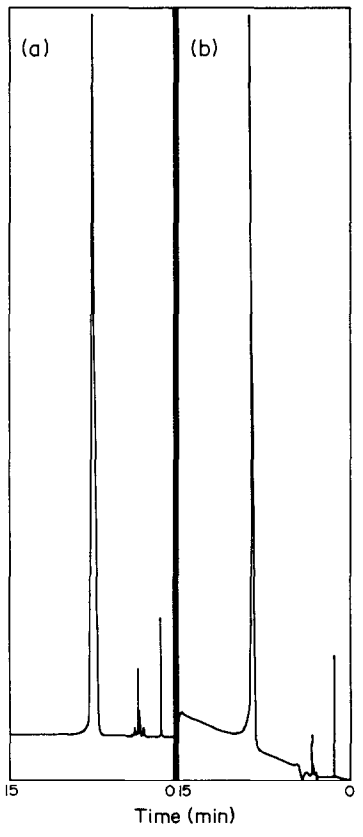

Fig. 1. HPLC purified Ra5G (a) and Ra5S (b). The ordinate represents absorbance at 214 nm; minor peaks eluting after the zero time signal are due to sample buffer.

Patient study group/direct skin tests

The study group was derived from (mixed) ragweed-sensitive patients attending the allergy clinics of the Royal Victoria Hospital (Montreal), the Ottawa Civic Hospital, the Ottawa General Hospital, and the Allergy Consultants of Ottawa. Patients were screened for cutaneous reactivity to Ra5S and Ra5G by i.d. injection of 0.02 ml of each allergen at 0.1 μg/ml; HSA diluent served as negative control. Tests were considered positive 20 min post-injection for wheal and flare diameters minimally 5 and 10 mm, respectively.

Of an initial survey population of 595 ragweed-allergic patients in the Ottawa region, 10% reacted to Ra5S, 5% to Ra5G and 2.9% reacted to both. From these and additional patients obtained in this series, consenting Caucasian volunteers were recalled for blood donations following repeat skin tests performed as described except for the use of a reduced dosage of Ra5S (0.01 μg/ml).

HLA typing

Groups skin test positive to each allergen were age and sex matched as closely as possible with a group of Caucasian ragweed-allergic individuals skin test negative to both. Peripheral blood samples were coded in a double blind manner. Mononuclear cell suspensions were prepared by Ficoll-Paque flotation (Pharmacia, Montreal); *B* cells were purified using Leuko-Pak leucocyte filters (Fenwal Laboratories, Deerfield, IL) and adjusted to 3×10^6/ml RPMI-1640 (Gibco). HLA antigens (DR1—w10, w52, w53; DQw1—3) were assayed in Terasaki plates according to standard cytotoxicity methods of the supplier (One Lambda, Los Angeles). Statistical significance of the data was computed using the two-tailed Fisher Exact Test (Sokal and Rohlf, 1969). *P* values were adjusted for multiple comparisons by the number of alleles assayed (Hawkins, 1981).

Immunoabsorption/P–K tests

An Ra5S-sorbent was formed by coupling the allergen to CNBr-activated Sepharose 4B (Pharmacia, Montreal; 25 μg Ra5S/ml beads); Sepharose 4B served as control. Control and Ra5S-sorbents (300 μl each) were separately incubated with 0.75 ml serum with gentle mixing for 2 hr. After centrifugation, the supernatants were assayed by RAST (Goodfriend *et al.*, 1985) for IgE anti-Ra5S antibodies. While control absorptions had no detectable effect on serum RAST values, a single absorption with Ra5S sorbent removed more than 90% of the Ra5S-specific IgE antibodies in all sera except one, for which two absorptions were required.

For the P–K tests, 0.05 ml control and test supernatants were injected i.d. into the back of a non-allergic volunteer (L.G.) and challenged 24 hr later with 0.02 ml of Ra5S (0.01 μg/ml) or Ra5G (1.0 μg/ml). Reactions were read as for the direct skin tests.

RESULTS

1. By cutaneous tests of ragweed-allergic individuals we obtained a group of 16 sensitive to Ra5G but not to Ra5S and a group of 23 sensitive to Ra5S but not to Ra5G. The latter permitted additional assessment of any allele that might be found associated with the Ra5G-sensitive group. Both groups were compared for incidence of HLA-DR specificities with a group of 61 ragweed-sensitive individuals cutaneously insensitive to both allergens.

As shown in Table 1, all members of the Ra5G-sensitive/Ra5S-insensitive group carried the HLA-DRw52 specificity. The incidence (100%) was significantly higher ($P = 0.002$) than the value (61%) obtained for the control group of Ra5S/Ra5G-insensitive individuals. No significant association was observed for HLA-DR2 nor any of the remaining HLA-DR specificities, after correction for the number of DR specificities assayed. It is noteworthy, however, that the DR5 allele was present at appreciably higher than control frequency.

In contrast, the incidence of HLA–DRw52 obtained for the Ra5S-sensitive/Ra5G-insensitive group was 57% (Table 2), close to the 54.2% reported for

Table 1. Association between HLA-DR alleles and Ra5G sensitivity

HLA Allele[a]	Patient population: Ra5G+ ($N = 16$)	Ra5S−/Ra5G− ($N = 61$)	P	Adjusted P^b
DRw52	16 (100%)	37 (16%)	0.001	0.002
DR5	10 (63%)	17 (28%)	0.01	N.S.[c]
DR2	2 (14%)	9 (16%)	N.S.	N.S.

[a]Results for HLA-DR alleles not shown in the table were not significant before correction.
[b]Adjusted for multiple comparisons.
[c]Not significant.

Table 2. Associations between HLA-D region alleles and Ra5S sensitivity

HLA Allele[a]	Patient population: Ra5S+ ($N = 21$)	Ra5S−/Ra5G− ($N = 61$)	P	Adjusted P^b
DR2	17 (81%)	9 (15%)	6.3×10^{-8}	6.3×10^{-7}
DRw52	12 (57%)	37 (61%)	N.S.[c]	N.S.
DQw1	19 (90%)	23 (38%)	1.9×10^{-5}	5.7×10^{-5}

[a]Results for HLA-D regions alleles not shown in the table were not significant before correction.
[b]Adjusted for multiple comparisons.
[c]Not significant.

the general Caucasian population (Juji, 1980). Only the DR2 allele (as found previously) as well as DQw1 were significantly associated with Ra5S sensitivity ($P = 6.3 \times 10^{-7}$; $P = 5.7 \times 10^{-5}$ respectively).

2. To test the validity of the Ra5G/DRw52, Ra 5S/DR2 associations in the co-sensitive case, sera from 8 of 23 co-sensitive individuals were selected on the basis of cutaneous reactivity, but blind with respect to their previously determined HLA-DR types. The sera were individually absorbed with Sepharose 4B (control) and Ra5S–Sephrose 4B; sera from two individuals sensitive to Ra5G alone (and typing HLA-DRw52) were included as controls for specificity of the Ra5S-sorbent. Supernatants corresponding to control and specific absorptions of each serum were assayed for P–K (IgE antibody) activity to Ra5S and Ra5G. As shown in Table 3, the Ra5S-sorbent completely removed all P–K activity (IgE antibodies) directed to Ra5S in all of the co-sensitive sera. While without effect on the P–K activity to Ra5G in the two control (Ra5G mono-specific) sera, the Ra5S-sorbent abolished activity to Ra5G in five of the eight co-sensitive sera. These results indicated that the cutaneous reactions elicited by Ra5G in these individuals were due to cross-reactivity with tissue-fixed IgE antibodies to Ra5S (cf. Coulter *et al.*, 1986) but the possible presence of IgE antibodies to Ra5G cross-reacting with Ra5S was not excluded. The P–K activities to Ra5G in the three remaining co-sensitive sera were largely intact (there was a reduction for the serum of donor 7), indicating the presence of Ra5G-specific IgE antibodies. As shown in the final columns of Table 3, seven of the eight co-sensitive individuals with Ra5S-combining IgE antibodies carried the DR2 allele, while three with Ra5G-specific IgE antibodies carried DRw52, in conformity with the findings for the mono-sensitive groups.

DISCUSSION

Although not our primary purpose, we confirmed the findings of several studies (Bias *et al.*, 1979; Marsh *et al.*, 1982; Coulter, 1983) that cutaneous

Table 3. Effect of immunoabsorption with Ra5S–Sepharose 4B on P–K activity of co-sensitive sera

Serum Donor[a]	Absorbent: Sepharose 4B Challenge Ra5S	Sepharose 4B Challenge Ra5G	Ra5S–Sepharose 4B Challenge Ra5S	Ra5S–Sepharose 4B Challenge Ra5G	HLA-DR 2	HLA-DR w52
1	49[b]	20	0	0	−	+
2	56	20	0	0	+	−
3	35	20	0	0	+	+
4	30	16	0	0	+	−
5	25	20	0	0	+	−
6	30	12	0	12	+	+
7	16	42	0	16	+	+
8	56	30	0	30	+	+
9	0	15	0	30	−	+
10	0	21	0	20	−	+

[a]Donors 1–8 inclusive were skin test positive to Ra5S and Ra5G; donors 9 and 10 to Ra5G only.
[b]Values in the table refer to products of wheal diameters (mm^2).

sensitivity to Ra5S, reflecting host synthesis of IgE antibodies to this minor ragweed allergen, is significantly associated with the HLA-DR2 allele. Our major finding is that, without exception, individuals cutaneously *in*sensitive to Ra5S but sensitive to Ra5G, typed HLA-DRw52. The observed frequency of the DRw52 allele for this group was significantly different from control and published values. The validity of the Ra5G/DRw52 association was supported by the observation of a normal frequency value for DRw52 in the group cutaneously sensitive to Ra5S but *in*sensitive to Ra5G.

The sensitivity/DR relationships (Ra5S/DR2 and Ra5G/DRw52) were deduced by delineation of two groups of ragweed-allergic individuals uniquely sensitive to one or other of the proteins. The same sensitivity/DR relationships appear to extend to the case of individuals *co*-sensitive to both (Table 3). Thus, seven of eight co-sensitive individuals with Ra5S-binding IgE antibodies were DR2 positive, as would be expected for an Ra5S skin test positive population (Table 2.) In addition, of the eight co-sensitive individuals, three possessed Ra5G-specific IgE antibodies and type DRw52, while the remainder (with Ra5S-combining IgE antibodies) showed a DRw52 distribution conforming to an Ra5S-sensitive population (Table 2). Although of limited size, the co-sensitive group was chosen on the basis of cutaneous sensitivity and blind with respect to DR type, so that it seems likely that the findings will prove of general validity. It should be noted that the immunoabsorption step depleted the serum samples of all (RAST detectable) Ra5S-binding IgE antibodies: as the latter might include antibodies formed to Ra5G able to cross-react (bind) to Ra5S, it is not excluded that formation of such antibodies is associated with the DR2 allele.

The findings for two additional alleles are worth noting: DQw1 was significantly associated with sensitivity to Ra5S and the frequency of DR5 was appreciably elevated in the Ra5G-sensitive group. The functional significance of these associations is unclear: the former may reflect linkage disequilibrium between the DQ and DR subregions, the latter the operation of an allele within the DR subregion less immunodominant than DRw52 in the formation of Ra5G-specific IgE antibodies.

At all events, our data strongly implicate DR2 and DRw52 as gene products immunodominantly operative, [perhaps by interaction with immunogen (cf. Schwartz, 1985)] in the formation of IgE antibodies respectively specific to the S and G forms of Ra5. As the corresponding genes are constituted of a common alpha gene but distinct beta genes (β_1 and β_3, respectively) (Giles and Capra, 1985), this would imply that the human IgE antibody responses to Ra5S and Ra5G are respectively associated with possession of DR2 β_1 and DRw52 β_3 genes.

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