

Short communication

Grass pollen immunotherapy induces highly cross-reactive IgG antibodies to group V allergen from different grass species

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Sera from two groups of patients receiving grass pollen immunotherapy were tested on IgG reactivity with group V allergen from six different grass species. One group of patients was treated with a mixture of 10 grass species, and the other with a mixture of five. Only *Lolium perenne*, *Dactylis glomerata*, and *Phleum pratense* were present in both mixtures. Although *Anthoxanthum odoratum* and *Secale cereale* were absent from the mixture of five, IgG responses to *Ant o* V and *Sec c* V were comparable in both patient groups. This reactivity was inhibited for 92–99% with *L. perenne* extract, illustrating the cross-reactive nature of the IgG antibodies. The presence of *A. odoratum* and *S. cereale* in the mixture resulted in only minor amounts of species-specific anti-group V IgG. These results indicate that application of just one grass species in immunotherapy might be sufficient to induce an IgG response that covers other relevant Gramineae species as well.

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Grass pollen immunotherapy is generally accepted as an efficient treatment of hay fever (1, 3). Treatment is usually performed with mixtures of different grass species. The rationale behind this approach is to cover the spectrum of grass pollen allergens as completely as possible. There are, however, some disadvantages attached to the application of complex extracts of mixed grass pollen species. This complexity, for example, makes quantitation of single allergens by means of monoclonal/polyclonal antibody-based immunoassays difficult, because such antibodies often have different affinities for related allergens from distinct grass species. The pressure of regulatory authorities with respect to the characterization of pollen extracts used for the manufacture of diagnostic and therapeutic products is steadily increasing. Simplification of these products is therefore desirable.

In a previous study, it was demonstrated that IgE responses to the major grass pollen allergens *Lol p* I and *Lol p* V consisted predominantly of cross-reactive antibodies (6). In all patients, binding of IgE to these allergens was inhibited for > 95% with several different grass pollen extracts, including extracts of *Anthoxanthum odoratum* and *Secale cereale*. These two Gramineae species belong to other tribes than *Lolium perenne* within the subfamily of Festucoideae (9).

In the present study, we investigated whether IgG responses, induced by grass pollen immunotherapy, were also highly cross-reactive. An affirmative answer could justify the application of an extract from a single grass pollen species in immunotherapy.

Two groups of patients were studied, one treated with a mixture of five grass species, and the other with a mixture of 10, including *A. odoratum* and

S. cereale, which were not present in the mixture of five. By measuring IgG antibodies against six purified group V allergens (including *Ant o* V and *Sec c* V) and by performing inhibition assays, we investigated whether the presence of *A. odoratum* and *S. cereale* had significant influence on the strength and specificity of the response against their group V allergens.

Material and methods

Pollen extracts

Pollen (*L. perenne*, *Dactylis glomerata*, *Festuca rubra*, *Phleum pratense*, *A. odoratum*, and *S. cereale*) were purchased from Diephuis Laboratories (Groningen, The Netherlands). Extracts were made as described previously (7).

Purification of group V allergens

Group V allergens were affinity-purified from crude pollen extracts (*L. perenne*, *D. glomerata*, *F. rubra*, *P. pratense*, *A. odoratum*, and *S. cereale*) with a Sepharose-coupled monoclonal antibody against group V allergen (4B1), as described earlier (5, 7).

Patients

Two groups of patients were studied. The first group consisted of 10 patients from Poland involved in a double-blind, placebo-controlled immunotherapy trial (2) with Purethal®-grasses (HAL Allergen Laboratories, Haarlem, The Netherlands). Treatment was given preseasonally, starting in October/November, and ending by the end of April (total dose 120 000 AU). The placebo group ($n = 10$) of this clinical trial served as negative control. Purethal-grasses is an allergoid preparation, obtained by modification with glutaraldehyde and containing 10 different grass species: *L. perenne*, *D. glomerata*, *P. pratense*, *F. rubra*, *Arrhenatherum elatius*, *Poa pratensis*, *Agrostis stolonifera*, *Holcus lanatus*, *A. odoratum*, and *S. cereale*. Serum samples were taken pre-seasonally in the first year of therapy.

The second group consisted of 10 patients from The Netherlands receiving grass pollen immunotherapy, whose sera were submitted for routine allergy diagnostics. They were all treated with Alutard Grassmix-5 (ALK, Horsholm, Denmark), containing the following grass species: *L. perenne*, *D. glomerata*, *P. pratense*, *F. pratensis*, and *Alopecurus pratensis*. Treatment was performed throughout the year, according to the manufacturer's dosage scheme. Serum samples were taken 1–3 years after the start of the immunotherapy. Both Purethal and Alutard extracts were adsorbed onto aluminum hydroxide.

Allergen-binding RIA/competitive RIA

IgG antibodies against six group V allergens were measured in an RIA with radiolabeled allergen and Sepharose-coupled protein A. Cross-reactivity of IgG antibodies was determined in a competitive version of this RIA. Both procedures were described earlier (8).

Results

Sera from two groups of 10 patients receiving grass pollen immunotherapy were tested on specific IgG against six grass pollen group V allergens. One group was treated with Purethal (10 grass species), and the other with Alutard (five grass species). In both groups, IgG antibodies against all six group V allergens were demonstrated (Fig. 1). The mean IgG titers found in the group treated with Alutard tended to be 2–3 times higher than in the Purethal-treated group also for group V allergens from *F. rubra*, *A. odoratum*, and *S. cereale*, which were not present in the Alutard mix. This probably reflects the fact that most of the patients of the Alutard group were treated for longer periods. A placebo group ($n = 10$) was tested as negative control. The mean of the IgG results was below 7 units/ml for all group V allergens, which is at least a factor of 25 lower than in both treated groups (Fig. 1). Binding of IgG to *Ant o* V and *Sec c* V was inhibited for 92–99% with *L. perenne* extract, for both groups of patients (Fig. 2). The amounts of *L. perenne* extract needed

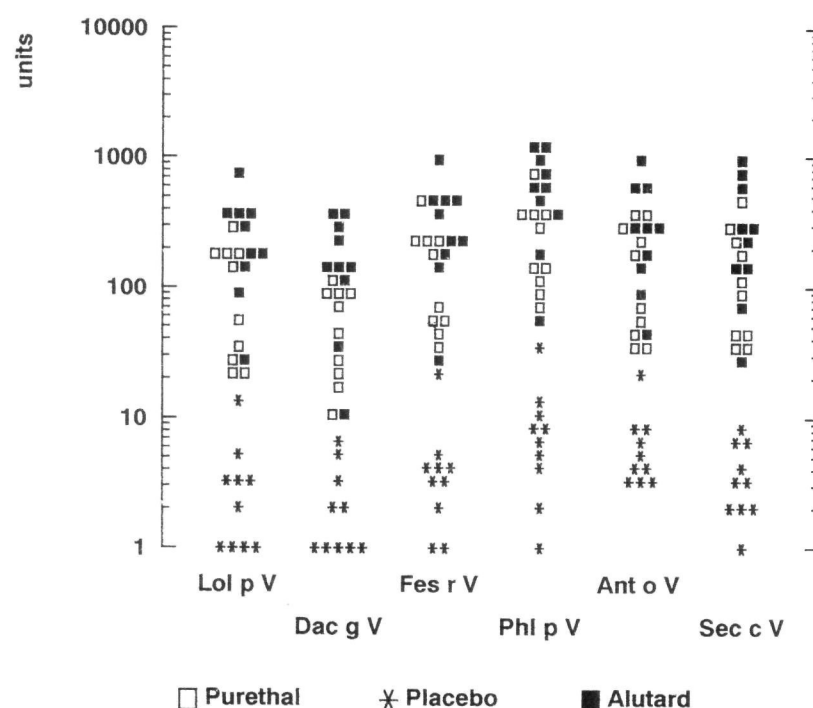


Fig. 1. IgG reactivity with group V allergens. Specific IgG against six purified group V allergens was measured in allergen-binding RIA. Group of patients treated with mixture of five grasses showed normal reactivity with *Ant o* V and *Sec c* V, group V allergens from grass species not included in mixture of five. Placebo group was in all cases below two treated groups. All results are expressed in arbitrary units.

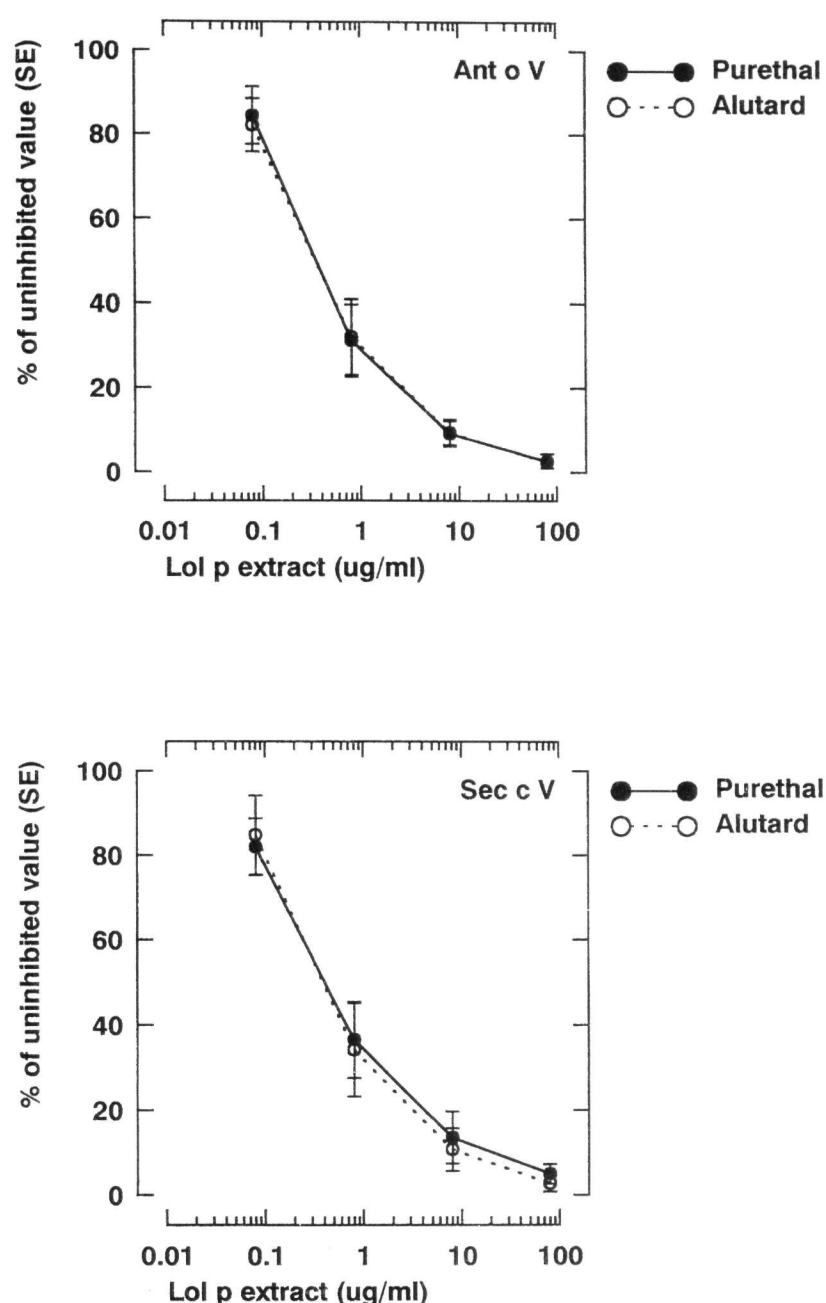


Fig. 2. Inhibition of IgG binding to *Ant o V* and *Sec c V*. Binding of IgG was inhibited with *Lolium perenne* extract. Amounts of pollen extract are expressed in μg dry weight per ml. Inhibition curves shown are mean results found for seven patients in case of patients treated with Purethal, and for 10 patients treated with Alutard. Results are expressed as percentages of uninhibited value. Error bars show standard error of mean.

for 50% inhibition (0.5 and 0.6 $\mu\text{g}/\text{ml}$ for *Ant o V* and *Sec c V*, respectively) did not differ significantly from the amount needed to inhibit *Lol p V* binding (0.4 $\mu\text{g}/\text{ml}$).

Discussion

In this study, it was demonstrated that IgG antibodies, induced by immunotherapy, against the major group V grass pollen allergen, are predominantly cross-reactive. IgG binding to *Ant o V* and *Sec c V* was for all sera inhibited for $\geq 92\%$ with *L. perenne* extract. Species-specific antibodies were probably not completely absent. This was suggested by the

finding that anti-*Ant o V*/anti-*Lol p V* and anti-*Sec c V*/anti-*Lol p V* IgG ratios were lower in patients treated with the five-grass mixture (lacking *A. odoratum* and *S. cereale*) than in patients treated with the 10-grass mixture (not shown). The difference was significant only for the anti-*Ant o V*/anti-*Lol p V* IgG ratios ($P = 0.03$).

When we keep in mind that IgE responses against the major grass pollen allergens *Lol p I* and *V* are highly cross-reactive with other grass species (6), it may be anticipated that the small share of species-specific IgG antibodies are of minor or no relevance to a good clinical outcome of immunotherapy. Of course, immunotherapy affects not only B cells, but also T cells. Data on cross-reactivity of T-cell epitopes from different grass pollen species are not yet available.

In conclusion, for the sake of simplification of immunotherapeutics, application of extracts from single grass species might be desirable. Clinical investigations should be designed to validate this hypothesis for grasses, as previously done for birch, hazel, and alder (4).

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