

Detection of crossreactive determinants in grass pollen extracts using monoclonal antibodies against group IV and group V allergens

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Summary

Monoclonal antibodies (MoAb) to defined allergens of *P. pratense* were raised. Five of them were selected for detailed studies by means of immunoblotting after SDS-PAGE and IEF of extracts from *P. pratense* and *L. perenne*. Three antibodies (1D11, 3B2, 2D1) recognize structures with mol. wt of 29 and 34 kD and pI of 4.8–7.6, corresponding to group V allergens. Two other MoAbs (2D8, 3C4) are directed against strong basic structures with a mol. wt of 50 kD and pI of 7.8–9.5 according to group IV allergens. The specificity of antibodies was supported by direct ELISA with purified group V and IV allergens. The isolated allergens were characterized before by SDS-PAGE and CIE and that allergenicity was detected with sera of patients with allergic rhinitis. Using our selected MoAbs crossreactive epitopes on group V and IV allergens have been excluded. Our antibodies have been used to detect crossreactivity in 14 grass pollen extracts. The evaluation of the pollen extracts has been performed by enzyme immunoassay (EIA) inhibition. One MoAb (3C4) is able to recognize group IV allergens in all grass species analysed whereas the MoAb 2D8 seems to identify group IV structures in selected grasses only. Binding to conserved structures of group V has been proved for MoAb 1D11. Other group V specific MoAbs (2D1, 3B2) identify similar, however incomplete, spectra. These results have been confirmed also by the dot immunobinding assay.

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Introduction

The identification of grass pollen allergens, especially the major allergens, is of substantial importance in diagnosis, treatment and basic research of grass pollen allergy. The allergens from temperate grasses may be divided into at least five groups with different physicochemical characteristics [1–4]. Groups I and V are dominant [5] as seen from their high sensitization index (>90%).

Pollen allergens from different grass species are immunochemically similar when assayed by RAST inhibition experiments [6,7], by immunoelectrophoretic methods [8,9], or by techniques including MoAbs [10–12]. Whereas group I allergens are already analysed in detail and some of them are even cloned and sequenced, detailed information about other groups (outside *L. perenne* and *P. pratense*) are limited [review in 13].

Quite recently Lowenstein *et al.* characterized group V allergens in selected temperate grasses using immunoelectrophoretic techniques and monospecific rabbit antibodies raised against *Phl p V* [14,15]. The authors pointed out that CIE/CRIE precipitation patterns of different grass pollen extracts show many common features, indicating that precipitated antigens have similar immunological and physicochemical properties. *Phl p V* consists of at least two isoforms (Va and Vb) with similar immunological properties, but with different molecular size. Both isoforms bind IgE [14] and show an identical NH₂ terminal sequence of 25 amino acids. Apparently, there are no homologies with the other known four groups of allergens.

Another important allergen extensively analysed is a cathodic migrating allergen formerly designated as allergen 30 [16] or as high molecular weight basic allergen [3]. In the meantime this glycoprotein has been designated as group IV according to the recommendations of the

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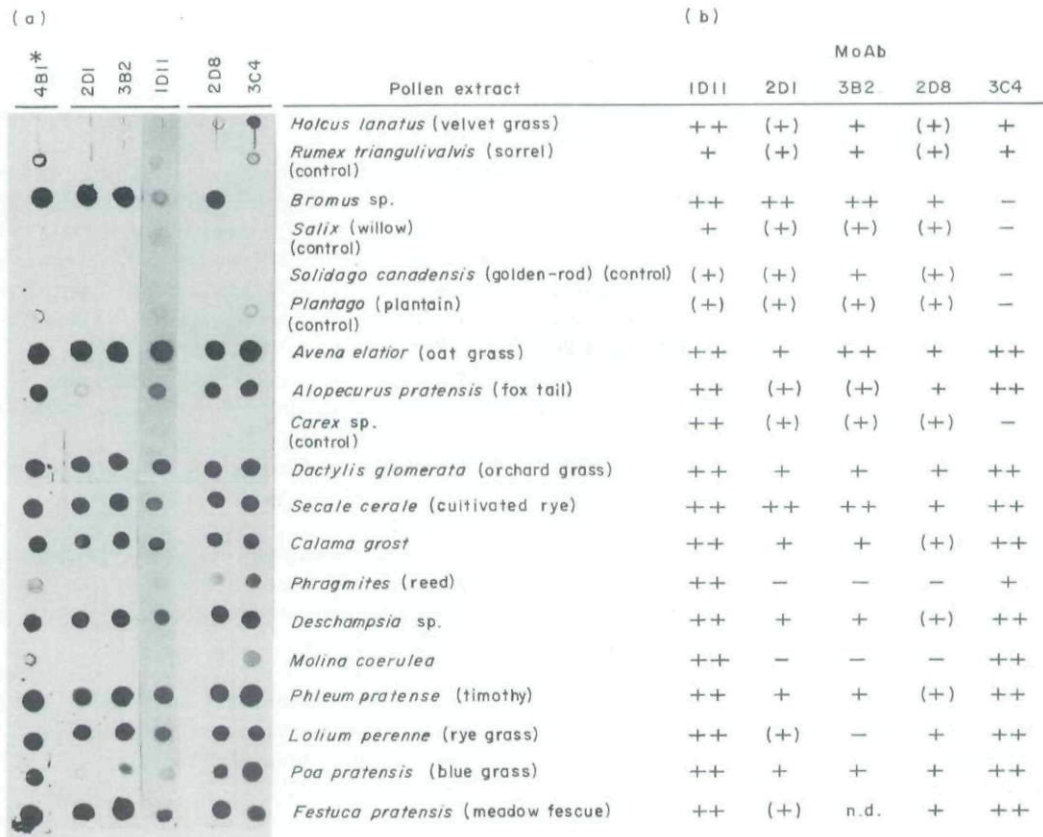


Fig. 9. Detection of crossreactivity among grass pollen extracts by the dot immunobinding assay (a) and EIA-inhibition (b) with anti-*Phl p* V (1D11, 3B2, 2D1) and anti-*Phl p* IV (2D8, 3C4). The EIA-inhibition results were defined as follows: binding of MoAbs was inhibited (50%) with extract dilutions of 1:100, ++, 1:10, +, 1:1, (—) non-specific or not inhibited, —. * anti-*Phl p* V (a gift from R. van Ree)

bind MoAbs specific for group V. Interestingly, Matthiesen *et al.* [15] could not find a reaction of rabbit anti *Phl p* V serum with *Phragmites* in CIE. Several reasons can be argued for this phenomenon. One possibility is that these grasses do not contain group V allergens. This seems unlikely, with regard to the EIA-inhibition results using 1D11. Another explanation is that the antigenic structure of allergen is altered by absorption to NC paper. What is questionable is, whether the studied MoAbs recognize sequential or conformational determinants? A restricted orientation or a conformation alteration of antigen could be the cause for weaker or even negative results in a few cases.

Using these two different methods, which differed obviously from the presentation of epitope, we could confirm a high antigenic crossreactivity between the grass pollens investigated.

In conclusion, these studies have clearly pointed out that both group V and group IV allergens are widespread in temperate grasses. Furthermore, the dot blotting

immunoassay with MoAbs can be used as a simple highly reliable assay for detection of crossreacting epitopes on groups of allergens with allergological relevance. As our group-specific MoAbs recognize obviously different epitopes, they are potential tools for standardization of diagnostic and therapeutic pollen extracts, e.g. by means of two-site binding assays.

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