

Specificities of IgE and IgG Antibodies in Patients with Birch Pollen Allergy

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Abstract. 58 sera from patients with established birch pollen allergy showed characteristic antibody-binding patterns in immunoblotting experiments. Regarding IgE, 56/58 patients recognized a protein of molecular weight (MW) 17 kilodaltons (kD), previously defined as Bet v I. 23/58 patients in addition reacted with a variety of 11 minor allergens with MWs ranging from 13 to 68 kD. A 13-kD protein was proved to represent an independent minor allergen. IgG binding in patients and healthy individuals was more pronounced on the minor allergens than on Bet v I. 3 different allergens were not detected by IgG of healthy individuals. In two-dimensional electrophoresis/immunoblot, a monoclonal antibody and human IgE (in both cases directed against Bet v I) detected a very similar cluster of spots, probably representing isoallergens of Bet v I.

Introduction

Standardization of allergen extracts is a necessary step to develop well-defined extracts for hyposensitization therapy. Commercially available birch pollen (BP) extracts are standardized according to their content of Bet v I, the major BP allergen [1]. It is known that there exists a number of minor allergens [2-9], some of which have been recently characterized by immunoblotting (IB) [10]. The importance of those allergens for the individual patient suffering from BP pollinosis is not known yet. This study presents an approach to further characterization of Bet v I and minor allergen molecules by means of IB experiments. This technique facilitates the direct comparison of reactivity patterns of different human immunoglobulin isotypes and mouse monoclonal antibodies (MoAb) directed against BP proteins.

Results

IB Experiments

BP proteins were extracted at pH 7.1 in the presence of protease inhibitors, separated according to molecular weight (MW) in 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-

PAGE) and transferred to nitrocellulose. Sera from 58 untreated patients suffering from established BP allergy (typical case history, positive skin prick test, positive radioallergosorbent test, RAST) [11] were investigated for their IgE and IgG antibodies directed against BP proteins in IB experiments.

Regarding IgE binding 12 proteins were found. Bet v I was detected by IgE binding in 56/58 (96.6%) patients. 25/58 (43.1%) patients showed IgE binding to minor allergens (13, 15, 18, 27, 29, 32, 36, 39, 44, 57 and 68 kilodaltons, kD). 2/58 (3.4%) patients showed IgE binding to the 13-kD allergen revealing no IgE binding to Bet v I.

All patients' IgE- and IgG-binding patterns corresponded closely: to all proteins detected via IgE, also IgG responses could be observed, but to a different extent. IgG binding to Bet v I was rather weak, but it was more pronounced to the 11 minor allergens mentioned above. 18 healthy individuals were selected according to negative case history concerning type I reactions, negative skin prick test and negative RAST to BP extracts. IgG binding to BP proteins was compared to that of allergic patients. IgG binding in both groups seemed to be rather similar, but in healthy individuals IgG was not directed against 15-, 27-, and 29-kD proteins.

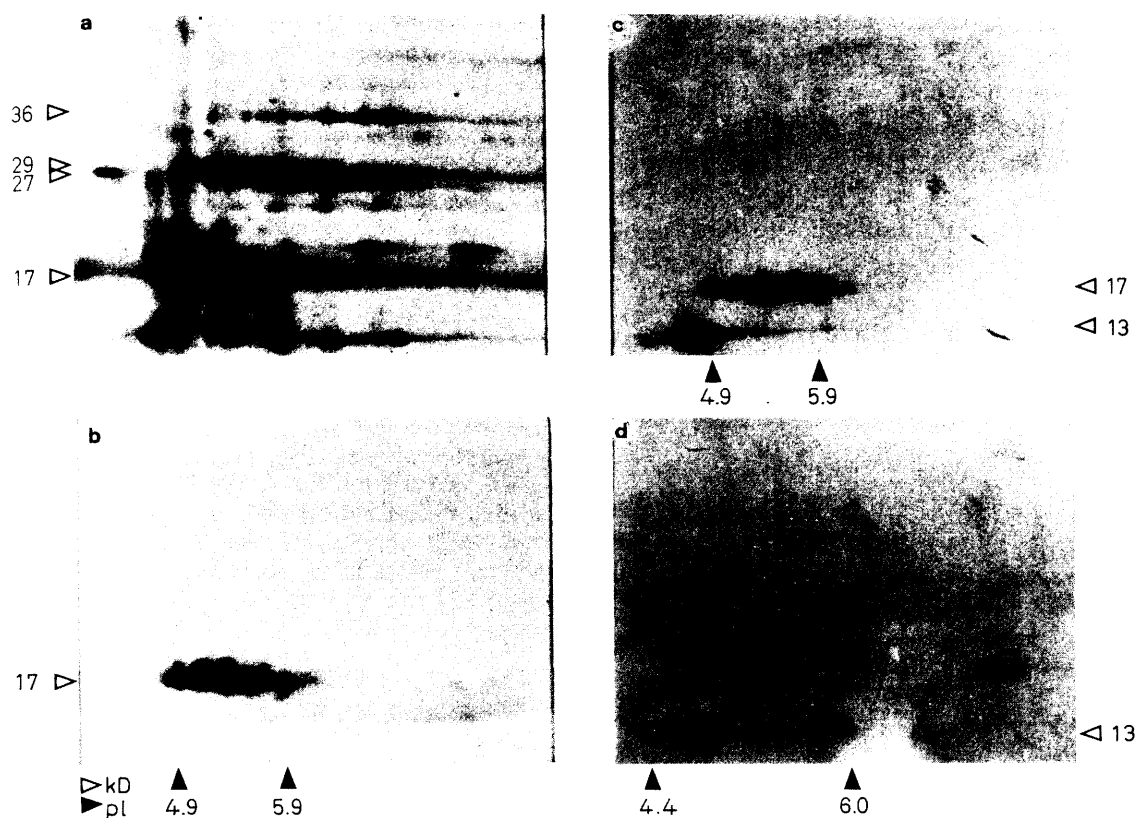


Fig. 1. Two-dimensional electrophoresis (pH 3.5–9.5, 10–23% gradient SDS-PAGE) was combined with IB. Four nitrocellulose sheets were probed with different reagents: sheet **a** was tested with a polyclonal rabbit anti-birch antibody recognizing approximately 92 different BP antigens, sheet **b** with a MoAb (BIP 1), reacting with Bet v I (17 kD), sheet **c** with a patient's serum reacting via IgE with Bet v I and a 13-kD protein, and sheet **d** with a patient's serum containing IgE antibodies directed exclusively against the 13-kD minor allergen. Black arrows indicate isoelectric points, white arrows MWs of antigens.

Monoclonal Antibodies

BALB/c mice were immunized with BP extracts and selected for fusion procedures according to IB patterns. This study presents MoAb BIP 1 reacting with Bet v I (fig. 1b).

Two-Dimensional Electrophoresis/IB

BP extracts were separated in a pH gradient (3.5–9.5) in the second dimension in a 10–23% SDS-PAGE and subsequently transferred to nitrocellulose membranes. Polyclonal rabbit anti-birch antibody bound to approximately 92 protein spots (fig. 1a). The MoAb BIP 1 detected Bet v I as a cluster of at least 10 protein spots in the pH range 4.9–5.9 (fig. 1b). Another sheet was probed with a serum from a BP-allergic patient, containing IgE against Bet v I and a 13-kD protein (fig. 1c). Regarding IgE binding to Bet v I, a very similar spot pattern as detected by BIP 1

was observed. A fourth sheet was incubated with patient's serum and showed IgE directed exclusively against the 13-kD protein (4 protein spots, pH 4.4–6.0), thus exhibiting antigenic independence of the 13-kD minor allergen (fig. 1d).

Conclusion

The IB results indicate that a number of 11 different minor allergens from BP are relevant as IgE-binding components for 43.1% of BP-allergic patients. Those proteins should be included in extracts used for hyposensitization therapy. In 2/58 patients, IgE did not react with Bet v I but with a 13-kD protein. In that case, hyposensitization treatment with extracts adjusted to their content of Bet v I, but not to the content of minor allergens, might not be successful.

Suggesting a protective function of IgG regulating IgE-allergen contacts, the very weak IgG binding to Bet v I in allergic patients who have not been undergoing hyposensitization therapy might indicate too low amounts of anti-Bet v I IgG which may facilitate an allergic breakthrough.

In healthy individuals, IgG antibodies to BP proteins may come into existence due to the normal response to BP proteins.

The MoAb BIP 1 reacting in a similar fashion with Bet v I as human IgE promises to be a useful tool for further characterization of this allergen molecule and its antibody-binding sites.

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