

Original Article

Sequential IgE epitope analysis of a birch pollen allergen (Bet v1) and an apple allergen (Mal d1)

Motoharu Uehara,¹ Keisuke Sato,² Yusuke Abe³ and Makoto Katagiri²

¹Uehara Otolaryngology Clinic, ²Second Department of Pathology, Asahikawa Medical College, Hokkaido, Japan and ³Division of Infectious Disease, Children's Hospital of Buffalo, Buffalo, New York, USA

ABSTRACT

Cross-reactivity in IgE epitopes and T cell epitopes has been reported between major birch pollen allergen (Bet v1) and major apple allergen (Mal d1). To treat people with birch pollinosis complicated by apple hypersensitivity by peptide immunotherapy, a sequential IgE epitope analysis was performed to study IgE epitopes that recognize birch pollen and apple allergens at the level of peptides. Subjects in the present study were three patients who exhibited clinical symptoms indicative of birch pollinosis during the pollen season. Two of the three patients had apple hypersensitivity and the capsulated hydrophobic carrier polymer (CAP)-radioallergosorbent test (RAST) class for apple was at least 2. The IgE epitope assay was performed by peptide–CNBr gel binding assay. In two of the three patients, IgE bound strongly to Bet v1 51–70 peptide and also to 11–30, 21–40, 11–120, 111–130 and 141–159 peptides. In the other patient, IgE bound to 11–30, 61–80, 111–130 and 141–159 peptides in a comparable manner. The IgE epitopes of Bet v1 and Mal d1 were found in similar locations. The results of sequential IgE epitope analyses on Bet v1 and Mal d1 revealed epitopes near the proximal regions of these allergens. Thus, the three-dimensional structure of Mal d1 is likely similar to that of Bet v1, which suggests that peptide immunotherapy designed for birch pollen is more than likely to be effective against apple allergy.

Key words: apple, Bet v1, birch, immunotherapy, Mal d1, sequential IgE epitope.

INTRODUCTION

Birch pollinosis is a commonly occurring disease in northern Europe, North America and northern Japan (Hokkaido). In addition, it has long been recognized that people who are allergic to birch pollens are often allergic to fruits, such as apples. There are two types of birch pollen allergen: (i) the major allergen (Bet v1), which reacts in approximately 90% of patients with birch pollinosis; and (ii) the minor allergen (Bet v2). Mal d1 is the major apple allergen. Because the amino acid sequence of Bet v1 and Mal d1 share 64% similarity, some studies have identified this homology as the cause of common allergenicity.¹ Bet v2 is a type of protein called profilin, which is found in every eukaryote. Given that there is homology in its amino acid sequence among various species, some researchers have suggested that profilin may play a role in the common allergenicity for birch pollen and apples.² Cross-reactivity in IgE epitopes and T cell epitopes has been reported between Bet v1 and Mal d1.^{3,4} At present, it is generally accepted that both Bet v1 and Mal d1 play an important role in common allergenicity. In Europe and America, immunotherapy has been attempted using extracted birch pollen allergens. Although this type of immunotherapy has been shown to be somewhat effective, it has not been widely accepted as an established treatment for birch pollinosis due to associated undesirable adverse reactions.^{5,6} Therefore, to treat people with birch pollinosis complicated by apple hypersensitivity by peptide immunotherapy, a sequential IgE epitope analysis was performed to study IgE epitopes that recognize birch pollen and apple allergens at the level of peptides.

Correspondence: Motoharu Uehara, Uehara Otolaryngology Clinic, Southern Syowa 10-12, Kushiro, Hokkaido, Japan. Email: uehara@jeans.ocn.ne.jp

Received 1 May 2000. Accepted for publication 25 September 2000.

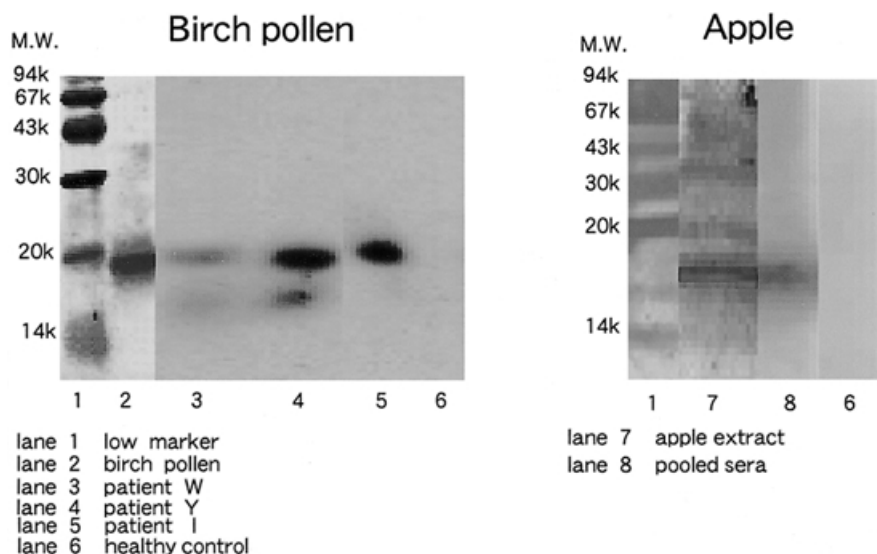


Fig. 1 Western blotting was performed as follows: crude allergens were extracted from birch pollens and apples and were subjected to 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Next, allergens were transferred to a polyvinylidene difluoride (PVDF) membrane by semidry blotting and the membrane was then treated with 3% bovine serum albumin, patient serum, [^{125} I]-antihuman IgE antibodies in this order, and analyzed by autoradiography. Lane 1, molecular weight marker stained with Coomassie brilliant blue R-250 staining; lane 2, birch pollen (*Betula platyphylla* var. *japonica*); lane 3, serum from patient W; lane 4, serum from patient Y; lane 5, serum from patient I; lane 6, serum from a healthy control; lane 7, apple extract; lane 8, pooled sera from three patients.

METHODS

Subjects were three patients who exhibited clinical symptoms indicative of birch pollinosis during the pollen season. In addition, eosinophils were detectable in the nasal discharge of these patients, and the capsulated hydrophobic carrier polymer–radioallergosorbent test (CAP-RAST; Pharmacia, Uppsala, Sweden) class for birch pollens was at least 4. Two of the three patients had apple hypersensitivity and the CAP-RAST class for apple was at least 2. The human leukocyte antigen (HLA) haplotype of the three patients was not examined.

Allergens were prepared as follows: ether-extracted *Betula platyphylla* var. *japonica* pollen was used as whole birch pollen extract and 1 g of this material was solubilized in 10 mL of 20 mmol/L phosphate buffer at 4°C for 3 h. After centrifugation (12 000 g, 10 min), the supernatant was ultrafiltrated with an ultrafiltration membrane (YM1; Amicon, Beverly, MA, USA). Extracts of apple were prepared by a low-temperature acetone powder method described previously.⁷

Western blotting was performed as follows: crude allergens were extracted from birch pollens and apples, and were subjected to 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Next,

allergens were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA) by semidry blotting and the membrane was then treated with 3% bovine serum albumin (BSA; Bio-Rad, Hercules, CA, USA), patient serum (primary antibodies: diluted five-fold), and [^{125} I]-antihuman IgE antibodies (Pharmacia) as secondary antibodies in this order and analyzed by autoradiography.

The CNBr-sepharose CL4B (Pharmacia) gel (1 mL) and synthesized peptides (10 mg) were bound and, after washing the resulting gel thoroughly, 0.2 mL of this gel and 0.2 mL serum were allowed to react overnight. After washing the gel–serum mixture, 0.2 mL [^{125} I]-anti-IgE antibody was added and the resulting gel–antibody mixture was allowed to react for 5 h. The radioactivity was then analyzed using a gamma counter.

The inhibition assay was performed as follows: serum IgE- and gel-bound crude allergens were bound in the presence or absence of 51–70 or 81–100 peptide. Each peptide was used at final concentrations of 0.04, 0.2 and 1 mg/mL.

Peptides were prepared as follows: on the basis of the amino acid sequence of Bet v1 and Mal d1, 20 amino acid-long peptides were synthesized (overlap of 10 amino acids). In addition, to bind peptides with gel, lysine was added to the C-terminus of each peptide. The molecular

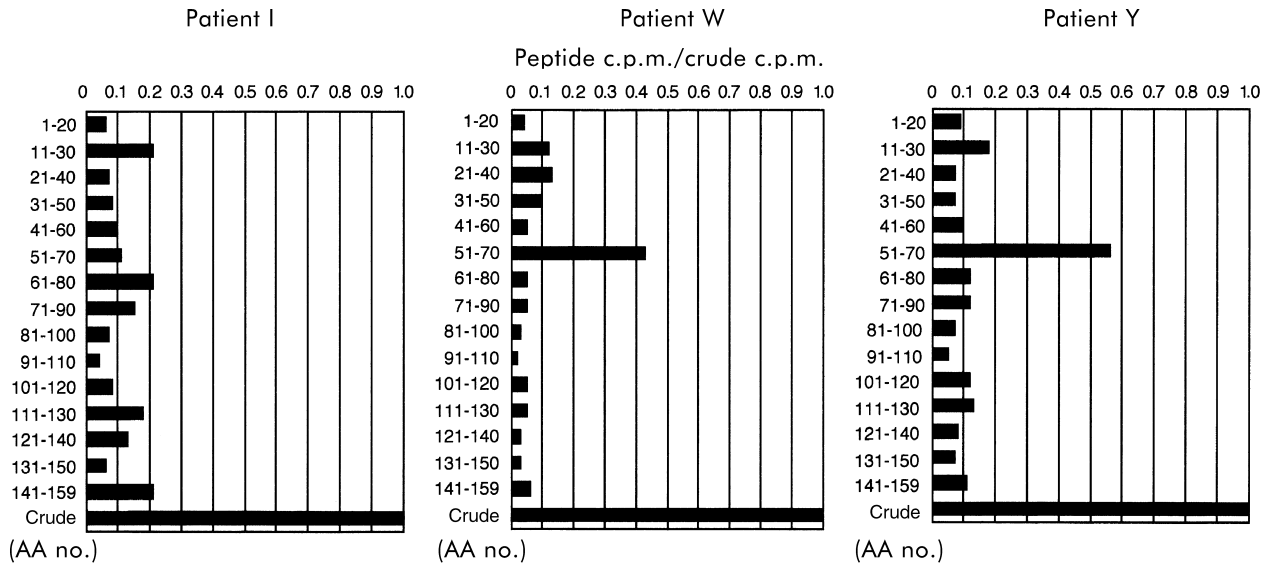


Fig. 2 CNBr-sepharose CL4B gel (1 mL) and synthesized peptides (10 mg) were bound and, after washing the resulting gel thoroughly, 0.2 mL of this gel and 0.2 mL serum were allowed to react overnight. After washing the gel-serum mixture, 0.2 mL [¹²⁵I]-antihuman IgE antibody was added and the resulting gel-antibody mixture was allowed to react for 5 h and radioactivity was then analyzed using a gamma counter. The reaction between patient serum and gel bound with rough allergens extracted from birch pollens was defined as '1' and the reaction of each peptide was expressed in relation to this reaction. AA no., amino acid number from the N-terminus of the protein.

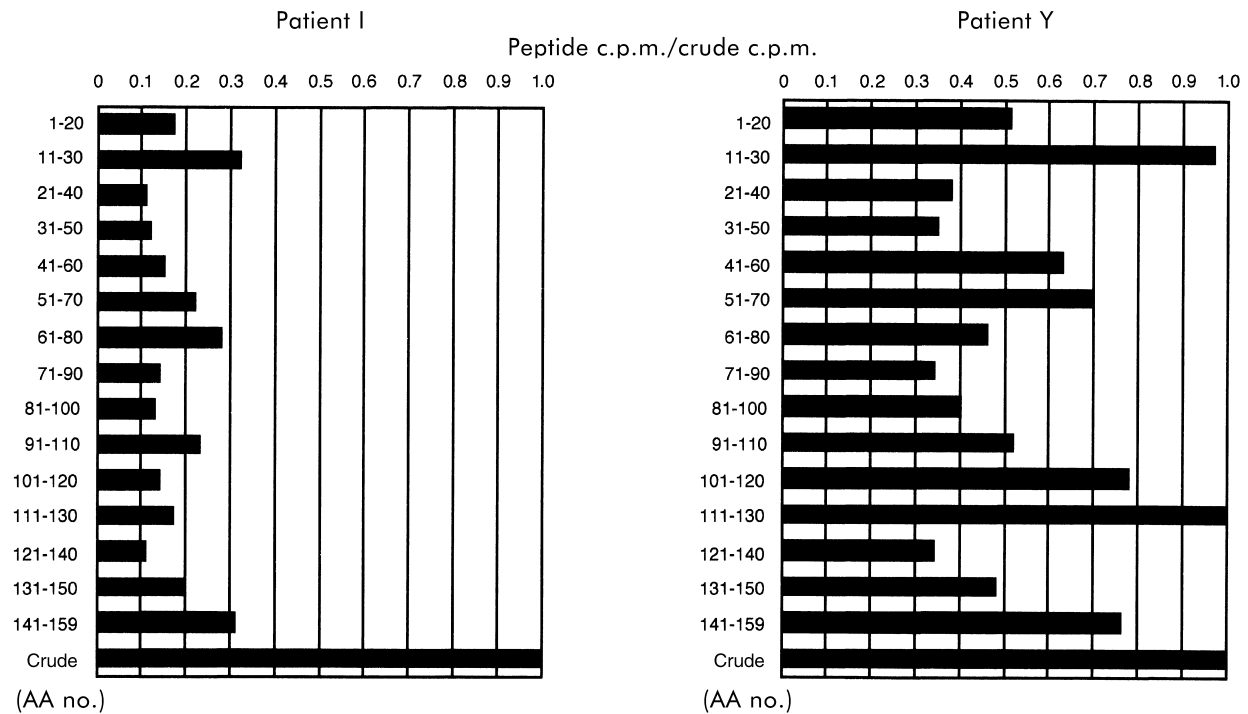


Fig. 3 The reaction between patient serum and gel bound with rough allergens extracted from apple extract was defined as '1' and the reaction of each peptide was expressed in relation to this reaction. AA no., amino acid number from the N-terminus of the protein.

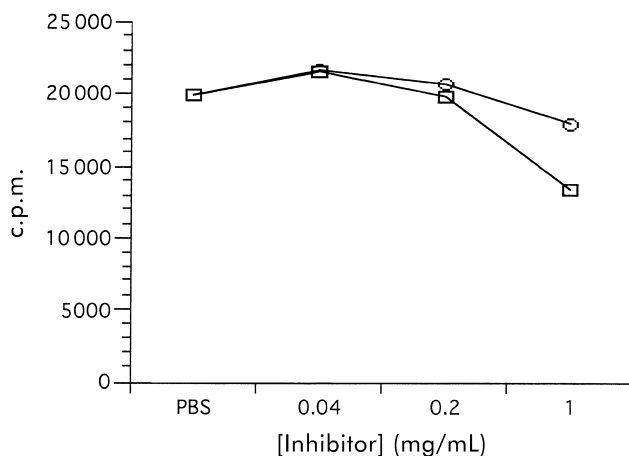


Fig. 4 51–70 peptide showed a high degree of reactivity in two patients, so we investigated whether the reaction between serum and rough allergen gel could be suppressed by 51–70 peptide. (□), preincubation in the presence of 51–70 peptide; (○), preincubation in the presence of 81–100 peptide. The results showed that, when compared with 81–100 peptide, the degree of suppression was stronger with 51–70 peptide. PBS, phosphate-buffered saline.

weight of the synthesized peptides was confirmed by mass spectrometry.

RESULTS

Western blotting

A band corresponding to Bet v1 was seen when the serum sample of any of the three patients was allowed to react with crude birch pollen allergens (Fig. 1). In addition, a band corresponding to Mal d1 was seen when the pooled sera of the three patients was allowed to react with crude apple allergens.

Bet v1-patient serum binding

The reaction between patient serum and gel bound with crude allergens extracted from birch pollens was defined as '1' and the reaction of each peptide was expressed in relation to this reaction. A reaction was defined as positive when it was greater than the mean $\pm$ 2.5 SD of the control serum or when the ratio of the reaction to control serum was greater than 0.1. In two of the three patients (patients W and Y), serum IgE bound strongly to 51–70 peptide and also to 11–30,

21–40, 111–130 and 141–159 peptides. In the other patient (patient I), serum IgE bound to 11–30, 61–80, 111–130 and 141–159 peptides in a comparable manner (Fig. 2).

Mal d1-patient serum binding

In one patient, serum IgE bound to 11–30, 41–60, 51–70, 101–120, 111–130 and 141–159 peptides, while in the other patient, serum IgE bound to 11–30, 51–70, 61–80, 91–110 and 141–159 peptides (Fig. 3).

51–70 peptide suppression test

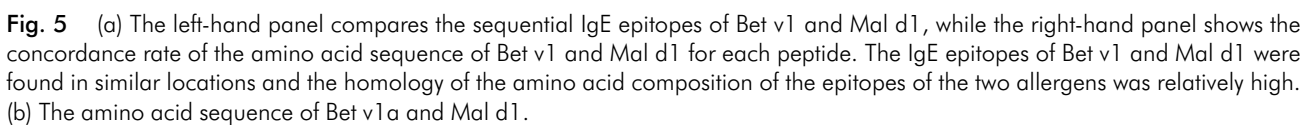
Because the 51–70 peptide showed a high degree of reactivity in two patients, we investigated whether the reaction between serum and crude allergen gel could be suppressed by 51–70 peptide (Fig. 4). Samples were preincubated in the presence of 51–70 peptide or 81–100 peptide. The results show that, when compared to 81–100 peptide, the degree of suppression was stronger with 51–70 peptide.

Comparison of the location of sequential IgE epitopes in Bet v1 and Mal d1

Figure 5a compares the sequential IgE epitopes of Bet v1 and Mal d1 (left-hand panel) and the concordance rate of the amino acid sequence of Bet v1 and Mal d1 for each peptide (right-hand panel). The IgE epitopes of Bet v1 and Mal d1 were found in similar locations and the homology of the amino acid composition of the epitopes of the two allergens was relatively high.

DISCUSSION

In currently available peptide immunotherapy, a peptide is designed by identifying the T cell epitope of an allergen. However, T cell epitopes vary from one patient to the next, depending on HLA type, and if it resembles the major histocompatibility complex binding motif, most areas of the protein can act as epitopes. Furthermore, preparing a peptide that is applicable to different races can be difficult. In contrast, in the case of an IgE epitope, a target epitope is present on the surface of allergens and is independent of HLA and, as such, it is easier to prepare more specific peptides. Chemical mediators are released when IgE on mast cells cross-links, but degranulation does not occur with single-valency allergens. Hence, if the entire sequence of Bet v1 is included with no more



over the $\beta 3$ and $\beta 4$ regions. Therefore, based on its three-dimensional structure, this area is likely to be an IgE epitope. Amino acid homology between Bet v1 and Mal d1 is relatively high at the 11–70 residues of Bet v1 and that region includes the IgE epitope. The results of sequential IgE epitope analyses on Bet v1 and Mal d1 revealed epitopes near the proximal regions of these allergens. Thus, the three-dimensional structure of Mal d1 is likely similar to that of Bet v1, which suggests that peptide immunotherapy designed for birch pollen is more than likely to be effective against apple allergy.

REFERENCES

- 1 Rohac M, Birkner T, Reimitzer I *et al.* The immunological relationship of epitope on major tree pollen allergens. *Mol. Immunol.* 1991; **28**: 897–906.
- 2 Valenta R, Duchene M, Pettenburger K *et al.* Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 1991; **253**: 557–60.
- 3 Ebner C, Birkner T, Valenta R *et al.* Common epitopes of birch pollen and apple. Studies by western and northern blot. *J. Allergy Clin. Immunol.* 1991; **88**: 588–94.
- 4 Fritsch R, Bohle B, Vollmann U *et al.* Bet v 1, the major birch pollen allergen, and Mal d1, the major apple allergen, cross-react at the level of allergen-specific T helper cells. *J. Allergy Clin. Immunol.* 1998; **102**: 679–86.
- 5 Herrmann D, Henzgen M, Frank E, Rudeschko O, Jäger L. Effect of hyposensitization for tree pollinosis on associated apple allergy. *J. Invest. Allergol. Clin. Immunol.* 1995; **5**: 259–67.sw
- 6 Asero R. Effects of birch pollen-specific immunotherapy on apple allergy in birch pollen hypersensitive patients. *Clin. Exp. Allergy* 1998; **28**: 1368–73.
- 7 Vieths S, Schöning B, Petersen A *et al.* Characterization of the 18 kDa apple allergen by two-dimensional immunoblotting and microsequencing. *Int. Arch. Allergy Immunol.* 1994; **104**: 399–404.
- 8 Gajhede M, Osmark P, Flemming M *et al.* X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. *Nat. Struct. Biol.* 1996; **3**: 1040–5.