

THE IMMUNOLOGICAL RELATIONSHIP OF EPITOPES ON MAJOR TREE POLLEN ALLERGENS

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Abstract—The major allergens of birch (*Bet v* I), alder (*Aln g* I), hazel (*Cor a* I) and hornbeam (*Car b* I) were investigated by means of high-resolution two-dimensional electrophoresis combined with immunoblotting. Eleven sera derived from patients allergic to birch pollen as well as mouse monoclonal antibodies BIP 1 and BIP 4, raised against *Bet v* I, were used as probes. Human IgE antibodies detected 10 spots in birch (M, 17 kDa, pI 4.9–5.9); four spots in alder (M, 18.5 kDa, pI 4.7–5.3); four spots in hazel (M, 17 kDa, pI 5.0–5.8); and 12 + 7 spots in hornbeam (M, 16.5 kDa, pI 4.9–6.6 and M, 18 kDa, pI 5.2–6.7), respectively, representing major allergens. Each patient tested reacted in a similar fashion with the spot cluster(s) of a certain allergen. BIP 1 detected the same spot clusters as patients' IgE. BIP 4 reacted with the 17-, 18.5- and 18-kDa spots of birch, alder and hornbeam, but did not react with the 17-kDa spots of hazel and the 16.5-kDa spots of hornbeam. In inhibition experiments with birch pollen extract as inhibitor, IgE binding to *Bet v* I, as well as to *Aln g* I, *Cor a* I and *Car b* I was abolished, thus suggesting that IgE binding to major tree pollen allergens is confined to shared epitopes. These findings indicate that it might be sufficient to use only *Bet v* I for diagnostic procedures as well as for immunotherapy in patients with tree pollen allergy.

INTRODUCTION

Type I allergy to birch pollen is a common disease in Northern and Central Europe and in the northern and eastern parts of North America. Using sodium dodecyl sulphate–polyacrylamide electrophoresis (SDS–PAGE) and immunoblotting (IB) it has been demonstrated that most patients suffering from birch pollinosis have IgE antibodies directed against a major protein with a molecular weight (M_r) of 17 kDa (Jarolim *et al.*, 1989a). This major allergen has recently been defined by immunochemical methods as an acidic glycoprotein with isoelectric points (pI) varying between 4.9 and 5.9 (Ipsen and Loewenstein, 1983). According to World Health Organization nomenclature it has been designated *Bet v* I (Marsh *et al.*, 1986). Further characterization of the *Bet v* I molecule by high-resolution two-dimensional electrophoresis (2DE) performed in our laboratory revealed a heterogeneity of the birch pollen major allergen, comprising 10 different spots with M_r of 17 kDa and pI values between 4.9 and 5.9 (Jarolim *et al.*, 1989b). Furthermore, Breiteneder *et al.* (1989) were able to clone and sequence a cDNA coding for the complete *Bet v* I molecule by screening a cDNA library of the phage lambda gt 11 with a

serum derived from a patient with type I allergy to birch pollen. Though *Bet v* I is well characterized, much less is known about major allergens derived from other tree pollen. However, clinical symptoms as a result of exposure to other tree pollen often occur among patients with birch pollinosis (Eriksson *et al.*, 1987). This cross-sensitization has been shown to have an immunological basis by radioallergosorbent test (RAST), crossed radioimmuno-electrophoresis (CRIE) and SDS–PAGE/IB and to be most pronounced within botanically related trees such as birch, alder, hazel and hornbeam (Eriksson *et al.*, 1987; Hemmens *et al.*, 1988). There is evidence that the major allergens of alder, *Aln g* I, and hazel, *Cor a* I, also represent glycoproteins with M_r between 17 and 20 kDa (Florvaag *et al.*, 1982, 1986, 1988). Partial immunochemical identity has been shown between the major allergens of birch, alder and hazel (Ipsen *et al.*, 1985). No data on the protein level are available regarding the major allergen of hornbeam, *Car b* I.

As cross-reactions among various related tree pollens are of major clinical importance, but detailed data on the molecular level are missing, it would be interesting to gain more information about cross-reacting structures among the four major tree pollen allergens on the protein level. Such knowledge is essential if we are to decide whether birch pollen extract alone or even purified or recombinant *Bet v* I would be sufficient for the diagnosis as well as for treatment of tree pollen allergy in general.

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As we were able to reveal a heterogeneity of the *Bet v* I molecule by means of high-resolution 2 DE we employed the same powerful technique to investigate whether a similar heterogeneity could be found within the major allergens of alder, hazel and hornbeam pollen.

The second important question is whether a heterogeneity of allergenic proteins also reflects a heterogeneity of IgE binding epitopes, thus being of clinical relevance. We therefore probed extracts of pollen derived from birch, alder, hazel and hornbeam with sera of 11 different patients with type I allergy to birch pollen in 2 DE/IB experiments.

Recently, the high homology of the N-terminal 45 amino acids of the major allergens of birch, alder, hazel and hornbeam has been identified (Ipsen and Hansen, 1990). To investigate whether conservation of epitopes can be found among these four major allergens, we also used mouse monoclonal antibodies against *Bet v* I, recently characterized as BIP 1 and BIP 4 in our laboratory (Jarolim *et al.*, 1989b) for immunoblotting.

MATERIALS AND METHODS

Pollen extraction procedure

Pollen derived from *Betula verrucosa* (birch) (BP), *Alnus glutinosa* (alder), *Corylus avellana* (hazel) and *Carpinus betulus* (hornbeam) were purchased from Allergon AB (Engelhom, Sweden). All pollen material was extracted according essentially to Ipsen and Loewenstein (1983), with slight modifications as previously described (Jarolim *et al.*, 1989a).

Sera

Sera from eleven patients (five males, six females, age 18–51 years) with established allergy to birch pollen (typical case history, positive skin-prick tests and positive RAST to BP extract) were selected according to positive reaction of IgE antibodies with *Bet v* I in SDS–PAGE/IB screening (data not shown). All 11 patients also demonstrated positive skin reactions to alder and hazel pollen extracts. According to positive skin-prick tests, four patients (Nos 2, 3, 6 and 11) additionally suffered from allergy to cock's foot and rye. Three patients (Nos 2, 3 and 11) were allergic to wheat, two patients (Nos 2 and 3) to maize and one patient (No. 3) to mugwort. None of the patients had received any hyposensitization treatment.

After collection, sera were stored at -20°C until use.

Monoclonal antibodies

Monoclonal antibodies (moabs) BIP 1 and BIP 4 against *Bet v* I, the major birch pollen allergen, were produced by immunizing BALB/c mice with BP-extract, as previously described in detail (Jarolim *et al.*, 1989b).

High-resolution two-dimensional electrophoresis (2 DE)

The 2 DE was carried out on a modification of the ISO-DALT system of Anderson and Anderson (1977) (Electro Nucleonics, Oak Ridge, TN (U.S.A.), as previously described (Endler *et al.*, 1987).

Specimen preparation

Pollen extract (5 mg) was dissolved in 100 μl of distilled water and 300 μl of sample preparation buffer (pH 9.5), containing 20 g/l sodium dodecyl sulfate, 50 g/l 2-mercaptoethanol, 50 mmol/l cyclohexylaminosulphonic acid (CHES, Calbiochem, U.S.A.) and 10 g/l glycerol. After heating for 5 min at 95°C , 17 μl of this mixture were loaded onto the iso gels.

First (ISO) dimension

Isoelectric focusing was performed in polyacrylamide (35 g/l) tube gels under denaturing conditions: 9 mol urea, 20 g surfactant NP-40, 2.5 g Servalyt 2–11, 4.2 g Servalyt 5–9 (Serva, Germany) and 14.3 g 2D Pharmalyte 3–10 (Pharmacia, Sweden) were added to 1 l of acrylamide solution. Isoelectric focusing was performed at 17,600 volt-hours (Vh) after prefocusing for 75 min at 200 V with 0.85 g/l phosphoric acid as anolyte and 1.0 mol/l sodium hydroxide as catholyte. Iso gels were immediately fused onto second-dimension slab gels without equilibration.

Second (DALT) dimension

SDS–PAGE in a 90–180 g/l polyacrylamide slab gel was run in DALT tanks with a buffer containing 3.5 mmol/l SDS, 24 mmol/l Tris(hydroxymethyl)aminomethane (Tris) and 0.2 mol/l glycine.

Proteins were either visualized by silver staining [according to P. Jungblut (personal communication)] or transferred to nitrocellulose sheets.

Prestained molecular weight markers (Rainbow Markers, Amersham, U.K.) were run simultaneously on the SDS–PAGE for determination of molecular weight.

The pH gradient of isoelectric focusing gels was determined by cutting a pair from each batch into 20 equal parts. Each part was put into 2 ml of degassed distilled water. After incubation for at least 2 hr on a shaker, the pH was measured.

Immunoblot

Transfer. Proteins separated by 2 DE were transferred according to Towbin *et al.* (1979) onto nitrocellulose (NC) (Schleicher and Schuell, Dassel, Germany; pore size 0.2 μm) in a blotting apparatus (Hoefel Scientific Instr., San Francisco, CA, U.S.A.) at a constant voltage for 420 Vh. The transfer buffer consisted of 25 mmol/l Tris, 192 mmol/l glycine and 200 ml/l methanol at pH 8.3.

Detection of antibodies

IgE. NC sheets were saturated by incubation in phosphate buffer [50 mM sodium phosphate buffer (PB; pH 7.5)–0.5% bovine serum albumin (BSA)–0.5% Tween-20 (TW)–0.05% sodium azide] for 30 min at room temperature (RT). Then they were incubated overnight with patients' serum, diluted 1:10 with PB–BSA–TW, at 5°C . After incubation sheets were washed with PB–BSA–TW for 1 hr at RT.

Bound IgE was detected by incubation with ^{125}I -rabbit-anti-human IgE (Pharmacia, Uppsala, Sweden) (6.6×10^6 cpm per sheet) with 0.1% gelatine (Janssen, Olen, Belgium) in PB–BSA–TW overnight at RT.

BIP 1 and BIP 4. NC sheets were saturated with Tris-buffered saline (TBS: 50 mmol/l Tris–150 mmol/l NaCl; pH 7.4), 0.5% Tween-20 (TW) and 3% nonfat milk powder (MP) for 30 min at RT and afterwards incubated with undiluted hybridoma supernatants (20 ml/sheet) overnight at 4°C . Sheets were washed for 1 hr in TBS–TW and bound IgG was incubated with rabbit-anti-mouse IgG (Fc) (Jackson, MD, U.S.A.), diluted 1:1000 in TBS–TW–MP for 1 hr at RT. Sheets were washed for 1 hr with TBS–TW at RT and incubated with ^{125}I -donkey-anti-rabbit IgG (Amersham, U.K.) diluted in TBS–TW–MP (5.5×10^6 cpm/sheet) for 1 hr at RT.

Autoradiography was performed at -70°C for 12–96 hr, using Kodak Ortho G films in exposure cassettes (Kodak X-o-matic) with intensifying screens (Lanex fast screens).

SDS–PAGE/IB inhibition experiments

Six patients' sera (Nos 1, 4, 8, 9, 10 and 11 of the 2 DE experiments) as well as a pool serum consisting of equal parts of 35 patients' sera reacting with *Bet v* I in SDS–PAGE/IB were used for inhibition experiments. IgE antibodies of the pool serum recognized the same spot pattern of *Bet v* I, *Aln g* I, *Cor a* I and *Car b* I as individual patients' sera in 2 DE/IB experiments.

Extracts of birch, alder, hazel and hornbeam pollen were separated by SDS–PAGE (12.5% polyacrylamide; 2.5 mg pollen extract per gel) according to Laemmli (1970), as previously described (Jarolim *et al.*, 1989b). The proteins were transferred to NC at 159 mA for 6 hr in the same transfer buffer as was used for 2 DE experiments. NC strips were cut into 5-mm strips, saturated for 30 min in PB–BSA–TW and treated with 1 ml of serum diluted 1:4 in PB–BSA–TW with or without preincubation of 1 mg/ml birch pollen extract. Incubation and washing conditions were the same as described for the 2 DE experiments. IgE binding was detected with ^{125}I -rabbit-anti-human IgE (Pharmacia, Sweden) [300,000 cpm/ml PB–BSA–TW–0.1% (w/v) gelatine]. Negative control strips were processed in buffer and ^{125}I -rabbit-anti-human IgE.

ELISA inhibition experiments

Inhibition of BIP 1 and BIP 4 binding to BP. ELISA plates (Nunc, Copenhagen, Denmark) were coated with BP-extract at a final concentration of 5 μg /well in borate-buffered saline for 6 hr at RT. Remaining binding sites were blocked by incubation with borate-buffered saline containing 1% BSA and 0.05% Tween at RT for at least 30 min. Inhibition experiments were performed by preincubation of ELISA plates with either BIP 1 or BIP 4 at concentrations of 1, 3, 10, 30, 100 and 300 μg , respectively, in phosphate-buffered saline (PBS) with 0.5% BSA and 0.05% Tween, overnight at 4°C . An anti-rat-macrophage antibody, VEP 6 (Rumpold *et al.*, 1982) was used for control experiments. After washing five times with PBS–0.05% Tween, mouse monoclonal antibodies (moabs) BIP 1 and BIP 4, labelled with horseradish peroxidase (POX), were added in concentrations of 0.2 and 50 μg /ml PBS–1% BSA–0.05% Tween, respectively. Incubation was performed for 5 hr at 4°C . After washing another five times, colour development was achieved by addition of 2,2'-azino-bis(3-ethylbenzthiazoline) sulfonic acid (ABTS) as substrate (Sigma). Development was stopped after 30 min (RT) with 0.32% sodium fluoride (100 μl /well). Optical density was measured at 490/405 nm with a Dynatech microplate reader.

RESULTS

Reactivity of human IgE with major tree pollen allergens

Eleven sera derived from patients with type I allergy to birch pollen were used for probing in 2 DE/IB experiments with extracts from birch, alder, hazel and hornbeam pollen. The reaction patterns are shown in Figs 1a, 1b and 2 (patient No. 11) and were as follows:

Birch (Bet v I). In the case of birch, IgE antibodies of each patient tested recognized at least 10 spots with M_r 17 kDa and pI s ranging from 4.9 to 5.9 in 2 DE/IB experiments.

Alder (Aln g I). In the case of alder, all patients' sera contained IgE reactivity with a cluster of four spots with M_r 18.5 kDa and pI s between 4.7 and 5.3.

Hazel (Cor a I). In the case of hazel, five spots were detected by patients' IgE with M_r 17 kDa and pI s from 5.0 to 5.8.

Hornbeam (Car b I). In the case of hornbeam, two protein clusters with M_r 16.5 kDa (12 spots, pI 4.9–6.6) and 18.0 kDa (seven spots, pI 5.2–6.7), respectively, were detected by human IgE. All patients tested had IgE antibodies reacting with all four tree species tested. The major allergens of birch, alder, hazel and hornbeam pollen, as recognized by human IgE, all could be resolved into several spots as described above, and therefore were found to be heterogeneous molecules with regard to pI . Though the spot patterns varied from one species to another,

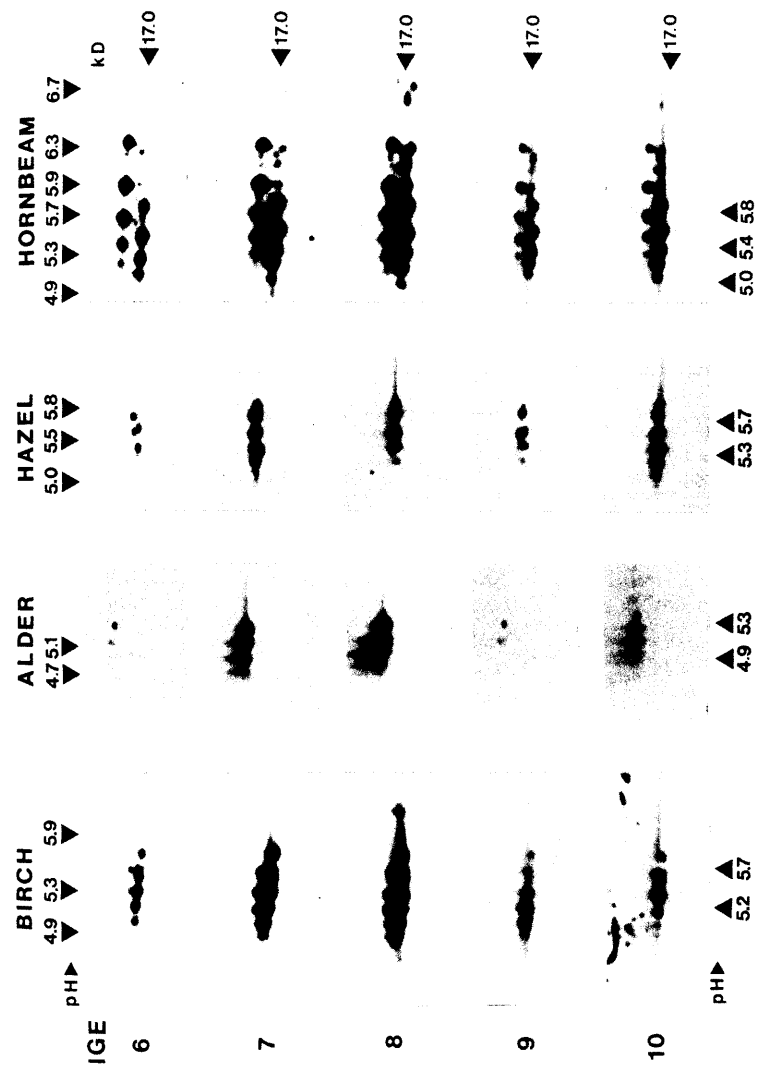
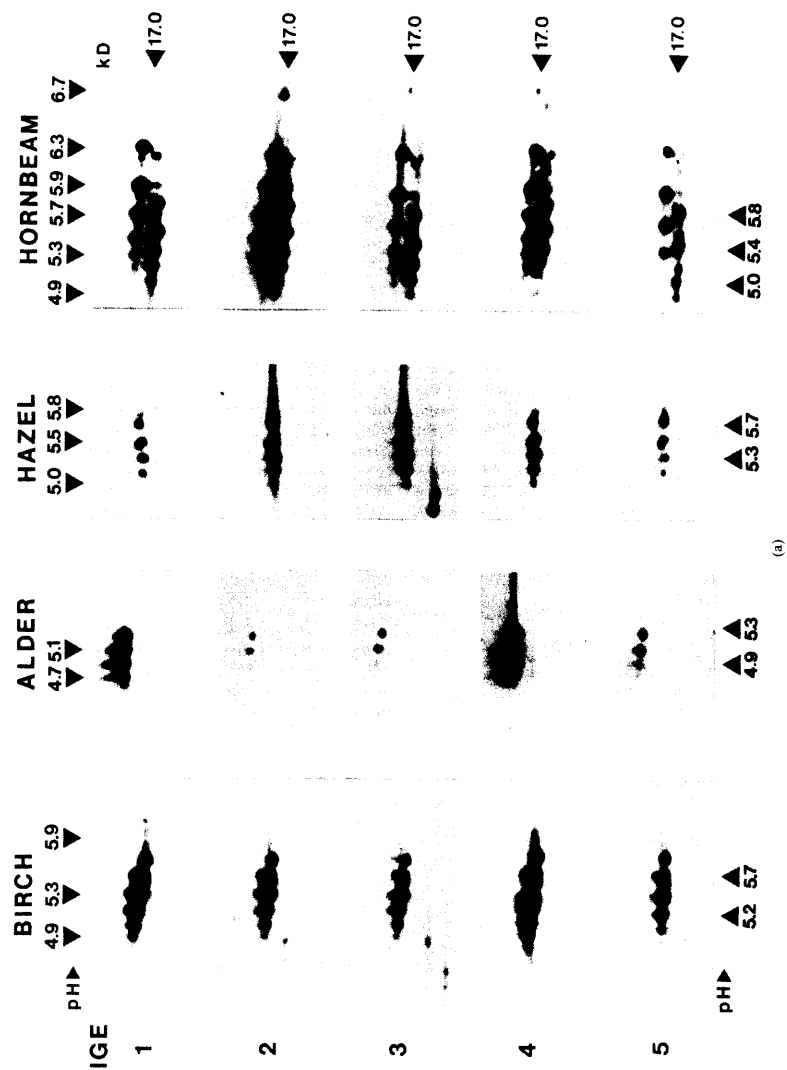


Fig. 1. 2 DE/IB analysis: binding pattern of IgE antibodies from patients [(a), Nos 1-5; (b) Nos 6-10] to the major allergens of pollen derived from birch (*Bet r* 1), alder (*Aln g* 1), hazel (*Cor a* 1) and hornbeam (*Car b* 1).

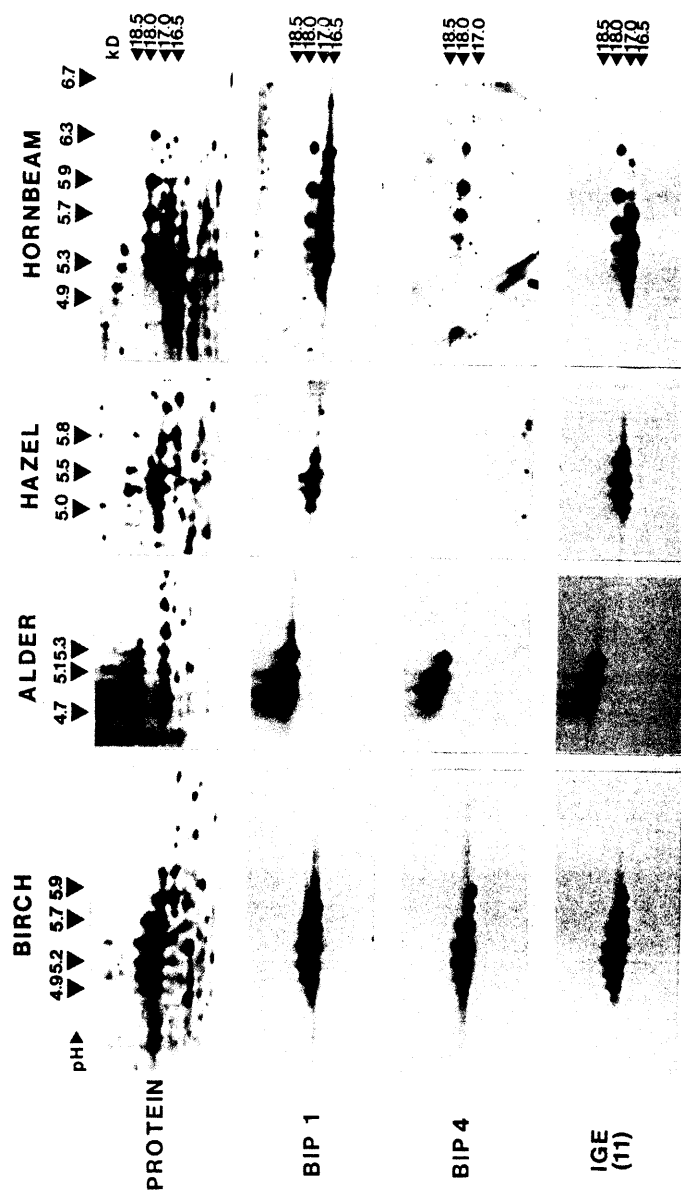


Fig. 2 2 DE protein pattern of major allergens of pollen derived from birch, alder, hazel and hornbeam as demonstrated by silver staining (protein), and 2 DE/IB analysis of moabs BIP 1, BIP 4 and human IgE antibodies of patient No. 11 (IgE).

each patient serum tested recognized the same spot cluster within the same species.

However, differences were found in the intensity of IgE antibody binding to the various tree pollen major allergens between the different patients. Patient 7 in Fig. 1b, for example, demonstrated strong reactivity with *Bet v* I, *Aln g* I, *Cor a* I and *Car b* I, whereas patient 6 showed weak reactivity with these four tree pollen major allergens.

The identified variations in the intensity of IgE reactivity always concerned the whole spot cluster of a certain major allergen. No dominant spots could be identified within the pollen proteins with any of the patients' sera tested.

Reactivity of monoclonal antibodies BIP 1 and BIP 4 with tree pollen major allergens

BIP 1 and BIP 4, two moabs directed against *Bet v* I, the major allergen of birch pollen, were used as probes in 2 DE/IB experiments with extracts of birch, alder, hazel and hornbeam pollen.

BIP 1 detected the same spot clusters in birch, alder, hazel and hornbeam pollen extracts as patients' IgE (Fig. 2).

BIP 4, however, recognized the spot clusters of *Bet v* I and *Aln g* I, and only the 18-kDa protein spots of hornbeam pollen proteins (Fig. 2). BIP 4 did not react with the hazel major allergen, *Cor a* I, and the 16.5-kDa protein of *Car b* I, which was detected by patients' IgE as well as by moab BIP 1.

SDS-PAGE/IB inhibition experiments

Six patients' sera (Nos 1, 4, 8, 9, 10 and 11 of the 2 DE experiments) as well as a serum pool ($n = 35$) of birch pollen allergic patients were probed for IgE reactivity to extracts of birch, alder, hazel and hornbeam pollen by means of SDS-PAGE/IB experiments. Preincubation of patients' sera with 1 mg/ml BP-extract abolished the reactivity with *Bet v* I as well as with *Aln g* I, *Cor a* I and *Car b* I (Fig. 3).

ELISA inhibition experiments

ELISA experiments were performed in which it was attempted to inhibit the binding of POX-labelled BIP 1 antibody to BP by preincubating BP-extract-coated ELISA plates with BIP 4 and vice versa. Each of the antibodies inhibited the binding of the other in

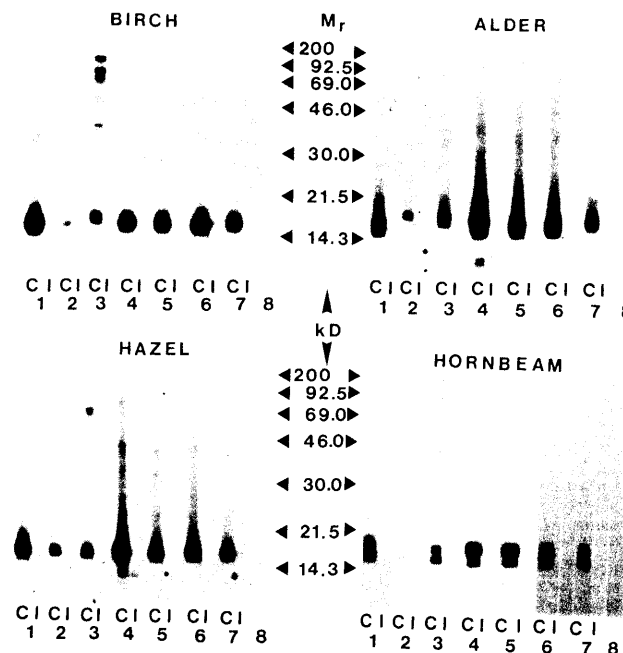


Fig. 3. IgE binding of six patients' sera (lanes 1 = No. 8, lanes 2 = No. 10, lanes 3 = No. 9, lanes 4 = No. 1, lanes 5 = No. 4, lanes 6 = No. 11) and of a pool serum (lanes 7) to extracts of pollen derived from birch, alder, hazel and hornbeam as demonstrated by SDS-PAGE/IB. Sera were probed without (lanes "C") and with preincubation with birch pollen extract (1 mg/ml) (lanes "I"). Lane 8 = buffer control.

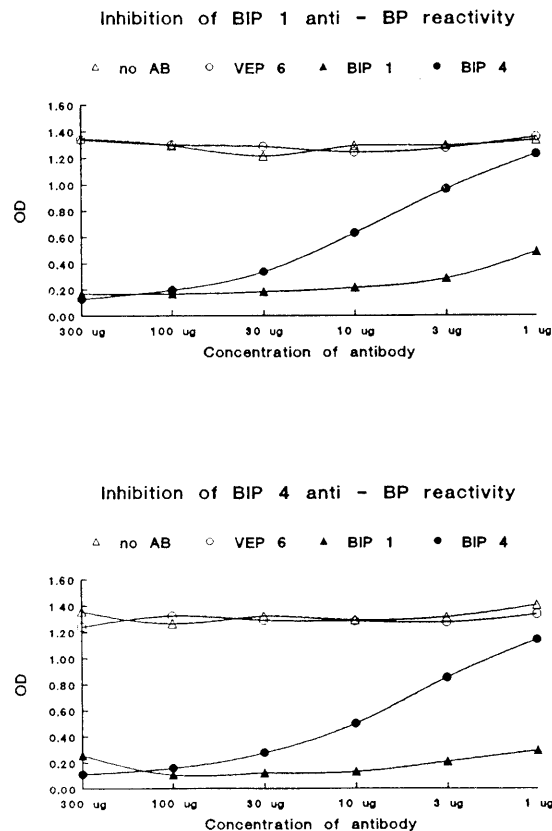


Fig. 4. The binding of POX-labelled BIP 1 and BIP 4 antibodies to ELISA plates coated with birch pollen extract and preincubated with various concentrations of unlabelled BIP 1 (upper panel) or BIP 4 (lower panel). VEP 6 was used as a control antibody. OD—optical density at 490/405 nm.

a dose-dependent manner (Fig. 4). BIP 1 inhibited the binding of BIP 4 more effectively than BIP 4 itself.

DISCUSSION

High-resolution 2 DE allows utmost resolution on the protein level. Heterogeneity of allergenic proteins has been shown previously by us in the case of *Bet v* I (Jarolim *et al.*, 1989b) and by others in the case of birch, alder and hazel (Florvaag *et al.*, 1986, 1988). It also has been demonstrated that *Bet v* I shares common epitopes with alder pollen (Hemmings *et al.*, 1988) and some fruits and vegetables (Calkhoven *et al.*, 1987; Halmepuro *et al.*, 1984).

To study this heterogeneity of tree pollen major allergens further and to gain more information about

their clinical relevance we investigated the reactivity of 11 individual patients' sera with the major allergens of birch, alder, hazel and hornbeam by means of 2 DE/IB experiments. By this approach, the major allergens of birch, alder, hazel and hornbeam could be resolved into 10, 4, 5 and 12 + 7 spots, respectively. IgE antibodies of 11 patients' sera reacted in a similar fashion with the clusters of the four tree pollen major allergens (Fig. 1a and b).

As the patients investigated showed allergy to different trees by means of skin tests, inhibition experiments were performed probing six selected patients' sera (Nos 1, 4, 8, 9, 10 and 11) as well as a pool serum ($n = 35$) of patients with type I allergy to tree pollen in SDS-PAGE/IB experiments. In all individual sera as well as in the pool serum, IgE

binding to *Bet v* I, *Aln g* I, *Cor a* I and *Car b* I was abolished by preincubation with birch pollen extract (Fig. 3). These results clearly indicate the presence of common, well-conserved IgE binding epitopes among tree pollen major allergens. This is in accordance with previous findings about high homology/identity of the 40–45 N-terminal amino acids among the major allergens of birch, alder, hazel and hornbeam (Ipsen and Hansen, 1990). Furthermore, most recent northern blot analysis of these allergens with a recently characterized cDNA coding for *Bet v* I as a probe revealed that the mRNA sequences of these allergenic molecules are well conserved (Valenta *et al.*, 1991).

The nature of the heterogeneity of major tree pollen allergens as revealed by 2 DE/IB experiments is speculative. Post-translational protein modification, like glycosylation, may contribute to the heterogeneity observed in our experiments. The fact that individual patients' sera recognize identical spot patterns implies that IgE antibodies are directed against the protein moiety of allergen molecules. These data are supported by experiments in which *in vitro* translated *Bet v* I and a recombinant *Bet v* I fusion protein, which both lack glycosylation, have been shown to be fully reactive with patients' IgE (Breiteneder *et al.*, 1988, 1989). Another possibility is that the isoallergens of *Bet v* I, *Aln g* I, *Cor a* I and *Car b* I, as identified by 2 DE/IB, represent products of a small gene family. This view is strongly supported by Southern blot experiments (Valenta *et al.*, 1990) as well as by the differences in the reactivity of BIP 1 and BIP 4 in our 2 DE/IB experiments. The first monoclonal antibody, BIP 1, exhibited an identical binding pattern to the tree pollen major allergens, as was observed with human IgE. In contrast, BIP 4 reacted exclusively with *Bet v* I, *Aln g* I and the 18-kDa cluster of *Car b* I, but not with *Cor a* I and the 16.5-kDa protein cluster of *Car b* I. These findings suggest that BIP 1 and BIP 4 are possibly directed against different epitopes. To further investigate the relationship of these epitopes, ELISA experiments were performed in which it was attempted to inhibit the binding of POX-labelled BIP 1 antibody to BP by preincubating ELISA plates with BIP 4 and vice versa. As each of the antibodies inhibited the binding of the other in a dose-dependent fashion, it can be concluded that the BIP 1 and BIP 4 epitopes are closely related. The two epitopes could represent two structural entities in close proximity, and in the case of *Cor a* I and the 16.5-kDa hornbeam allergen the BIP 4 epitope is deleted. Another possibility could be that there are only slight differences between the two epitopes such as substitution of one or a few amino acids.

Defining the allergenic epitopes on tree pollen proteins is a pivotal step in the development of recombinant allergens, which would represent better tools for diagnostic purposes as well as for hyposensitization therapy than the currently used crude extracts (Breiteneder *et al.*, 1989). The presence of

well-conserved IgE binding epitopes among the major allergens of birch, alder, hazel and hornbeam as revealed in the present study suggests that it might be sufficient to use solely *Bet v* I for diagnostic procedures and for immunotherapy in patients with tree pollen allergy.

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THE INDUCTION OF NEUTRALIZING ANTIBODIES BY SYNTHETIC PEPTIDES OF THE ENVELOPE PROTEIN OF TYPE D SIMIAN RETROVIRUS-1 (SRV-1)

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Abstract—It has been recently demonstrated that two serotypes of type D simian retroviruses, namely SRV-1 and SRV-2, exhibit extensive immunological cross-reactivity but do not exhibit cross-reactivity at the level of neutralizing antibodies. We have also shown recently that an area which includes residues 147–162 of the envelope protein of SRV-1 constitutes an epitope to which neutralizing antibodies against SRV-1 but not against SRV-2 are directed. However, in spite of the capacity of various immunogenic preparations to induce antibodies which react with SRV-1 these antibodies were incapable of neutralizing *in vitro* viral infectivity.

Work reported herein demonstrates that various immunogens consisting of a larger peptide, namely 142–167 of the envelope protein of SRV-1, induce antibodies capable of binding with the envelope protein of SRV-1 and with the whole virus. Moreover, these antibodies exhibit the capacity to neutralize *in vitro* the infectivity of SRV-1 but not of SRV-2.

INTRODUCTION

Several distinct serotypes of simian type D retroviruses (SRV) exhibit extensive serologic cross-reactivity but no cross-reactivity exists at the level of serum neutralizing antibodies (Bryant *et al.*, 1986; Marx *et al.*, 1985; Werner *et al.*, 1990). Thus, serum from SRV-1 infected rhesus monkeys or from mice immunized with recombinant envelope protein (rEP) of SRV-1 exhibit the capacity to inhibit *in vitro* the infectivity of Raji cells by SRV-1 but not by SRV-2 (Werner *et al.*, 1990). Similarly, a mouse monoclonal antibody has been shown to possess the capacity of strain-specific neutralization of viral infectivity (Werner *et al.*, 1990).

We have recently reported that the antibodies which neutralize SRV-1 infectivity are directed to an area encompassing residues 147–162 of the envelope protein (Werner *et al.*, 1990). This has been demonstrated by the ability of the synthetic peptide 147–162 to inhibit the neutralizing activity of the antibodies *in vitro* and by the ability of the peptide-KLH conjugate to serve as an immunoadsorbent agent for the recovery of SRV-1 neutralizing antibodies. Immunization of mice with various preparations of peptide 147–162-KLH conjugates induced antibodies which exhibited binding to SRV-1 and to SRV-1 recombinant envelope protein. However, these antibodies consistently failed to neutralize the *in vitro* infectivity of Raji cells by SRV-1. To explain this

observation we considered the possibility that the peptide indeed represents an area on the envelope viral protein to which neutralizing antibodies are directed, and that the failure of the peptide or its conjugate to induce neutralizing antibodies is because the peptide represents only a portion of the neutralizable epitope (Werner *et al.*, 1990). To test this hypothesis we have synthesized a larger peptide comprising residues 142–167 of the viral envelope protein and tested its immunogenic properties.

Data presented in this paper indicate that antibodies induced by various preparations of peptide 142–167 exhibit the capacity to neutralize *in vitro* Raji cell infectivity by SRV-1 and, as expected, they were unable to neutralize infectivity by SRV-2.

MATERIALS AND METHODS

Viruses

SRV-1 and SRV-2 were obtained from the California Regional Primate Research Center, Davis, CA. Cultivation, identification and purification of the viruses have been described (Daniel *et al.*, 1984; Marx *et al.*, 1984, 1985; Heidecker *et al.*, 1987; Kwang *et al.*, 1987).

Recombinant SRV-1 envelope protein

The expression, isolation, purification and characterization of SRV-1 recombinant envelope protein (rEP) have been reported (Kwang *et al.*, 1988). The expressed protein is a somewhat truncated form of native envelope protein with a molecular weight of

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