

In situ localization of a high molecular weight cross-reactive allergen in pollen and plant-derived food by immunogold electron microscopy

Monika Grote, PhD,^a Sabine Fischer, MSc,^b Wolf-Dieter Müller, MD,^c and Rudolf Valenta, MD^b Münster and Jena, Germany, and Vienna, Austria

Background: A high molecular weight (60 kd) allergen has been recently identified as a cross-reactive moiety in pollen and plant-derived food. While the cross-reactive allergen has been characterized by immunochemical techniques, little is known concerning its biologic properties.

Objective: In this investigation we studied the in situ localization of the 60 kd cross-reactive allergen in tree, grass, and weed pollen, as well as in plant-derived food (apple and celery).

Methods: A monoclonal antibody (3A4) that was raised against the major mugwort pollen allergen, Art v 1, was used to demonstrate the presence of related allergens in nitrocellulose-blotted pollen and plant-food extracts. The tissue localization of the cross-reactive allergen was investigated by immunogold electron microscopy.

Results: Monoclonal antibody 3A4 recognized IgE epitopes of the 60 kd mugwort allergen and cross-reacted with moieties of comparable molecular weights in birch and timothy grass pollen, as well as in apple and celery extracts. In pollen and plant-derived food the allergen could be localized intracellularly in ribosome-rich areas in the mitochondria and the nucleus. No labeling was observed in the pollen or cell walls or in organelles that are engaged in storage (e.g., starch granules and lipid particles).

Conclusion: Tree, grass, and weed pollen, as well as plant-derived foods, contain a high molecular weight Art v 1-cross-reactive allergen that maps to similar cell compartments. (J Allergy Clin Immunol 1998;101:250-7.)

Key words: Cross-reactive allergen, birch pollen, grass pollen, mugwort pollen, apple, celery, Art v 1

Type I allergic reactions to plant-derived foods (fruits and vegetables) are mainly caused by the presence of IgE antibodies that cross-react with pollen allergens.¹ Although two of the cross-reactive allergens (the major birch pollen allergen Bet v 1 and profilin) have been characterized in detail,²⁻⁶ much less is known about a third cross-reactive allergen, representing a 60 kd moiety that occurs in weed, tree, and grass pollens.^{7,8} The 60 kd

Abbreviations used

BSA:	Bovine serum albumin
mAb:	monoclonal antibody
PBS:	Phosphate-buffered saline
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
TBST:	Tris-buffered saline containing Tween
Tris:	Tris(hydroxy)methylaminomethane

glycoprotein, which is presumably identical with the major mugwort pollen allergen Art v 1, has been found to cross-react with related allergens in tree and grass pollens and with allergens present in fruits and vegetables. It represents a glycoprotein with a remarkable resistance to proteolytic digestion.⁷ A possibly related moiety of comparable molecular weight that shares epitopes with the major ragweed allergen family Amb a 1 and Amb a 2 has been described in timothy grass pollen and is probably identical to grass group IV allergens.⁹ Very recently it has been demonstrated that IgE antibodies of patients allergic to latex who were sensitized to pollen and plant-derived food displayed cross-reactivity with a 60 kd latex pollen glycoprotein that contained periodate-sensitive IgE epitopes.¹⁰ Although a constantly increasing panel of functional recombinant plant allergens is becoming available,¹¹ to date no cDNA for the 60 kd cross-reactive allergen has been obtained and expressed as IgE binding protein. This may be due to difficulties in expressing the glycoprotein in a properly folded or glycosylated form, or it may even be due to the fact that posttranslational modifications themselves represent the cross-reactive IgE epitopes.

To gain more information on the properties and characteristics of the 60 kd cross-reactive allergen, we performed an immuno(cyto)chemical investigation. First, a monoclonal antibody (mAb 3A4) that was raised against the major mugwort allergen Art v 1⁷ was characterized regarding its epitopes and tested for cross-reactivity with related pollen, fruit, and vegetable allergens. Then mAb 3A4 was used to study the ultrastructural localization of the 60 kd cross-reactive allergen in weed, tree, grass pollen and in apples and celery by immunogold electron microscopy.

From ^aInstitute of Medical Physics and Biophysics, University of Münster; ^bInstitute of General and Experimental Pathology, University of Vienna, Vienna; and ^cInstitute of Clinical Immunology, University of Jena, Germany

Received for publication July 3, 1997; revised Sept. 25, 1997; accepted for publication Sept. 26, 1997.

Reprint requests: Monika Grote, PhD, Institute of Medical Physics and Biophysics, University of Münster, Robert-Koch-Str. 33, D-48 149 Münster, Germany.

Copyright © 1998 by Mosby, Inc.

0091-6749/98 \$5.00 + 0 1/1/86660

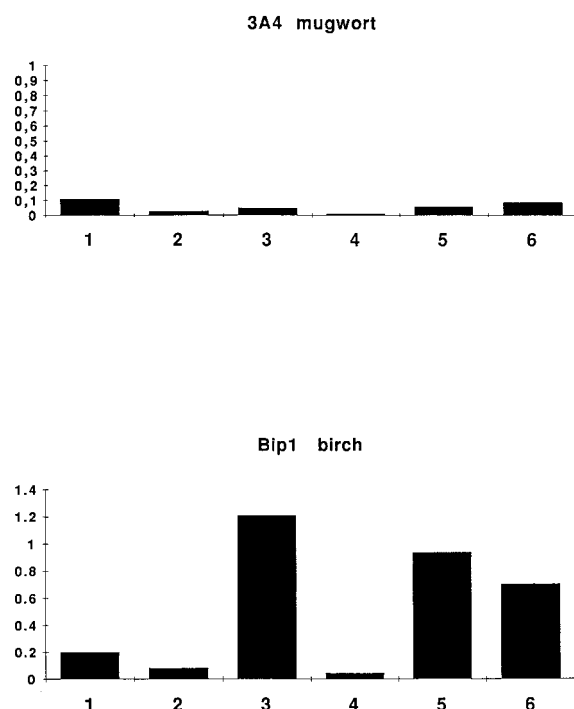


FIG. 1. ELISA-catching experiment. Monoclonal antibodies 3A4 and Bip 1 were used to catch Art v 1 and Bet v 1 from mugwort and birch pollen extracts, respectively. ELISA wells were then exposed to sera containing Art v 1-specific IgE (nos. 2, 3, and 4), to sera containing Bet v 1-specific IgE (nos. 3, 5, and 6), and to a serum from a nonallergic individual (no. 1). The optical densities reflecting the levels of bound IgE antibodies are displayed on the y-axis.

METHODS

Materials

Pollen from birch (*Betula verrucosa* L.), timothy grass (*Phleum pratense* L.), and mugwort (*Artemisia vulgaris* L.) were obtained from Allergen AB (Välinge, Sweden) and stored at 8° C until use. Apples and celery were obtained fresh from the local market.

Antibodies

Monoclonal antibody 3A4 was raised by immunization of female BALB/c mice with the major mugwort allergen Art v 1 purified as described by De La Hoz et al.¹² Mice were immunized intraperitoneally with complete Freund's adjuvant (Sigma, St. Louis, Mo.) together with 5 µg of purified allergen followed by booster injections with incomplete Freund's adjuvant. Four days before cell fusion, 10 µg of purified allergen was injected intravenously. Hybridomas were selected by ELISA testing with solid-phase-bound mugwort pollen extract and purified allergen. Monoclonal antibody 3A4 was identified as an IgM antibody by using a typing kit (Boehringer, Mannheim, Germany). The isotype of mAb 3A4 was confirmed by a dot blot experiment. Nitrocellulose-dotted mAb 3A4 and, for control purposes, an mAb of a different isotype, Bip 1 (IgG1),¹³ were probed with rat monoclonal anti-mouse IgM and IgG1 antibodies (Pharmingen, San Diego, Calif.). Mouse monoclonal IgG1 anti-Bet v 1 antibodies were used as control antibodies in the IgE competition experiments.¹⁴

For immunocytochemical control purposes, a mAb of irrelevant specificity (mAb 414) was obtained from BABCO (Berkeley,

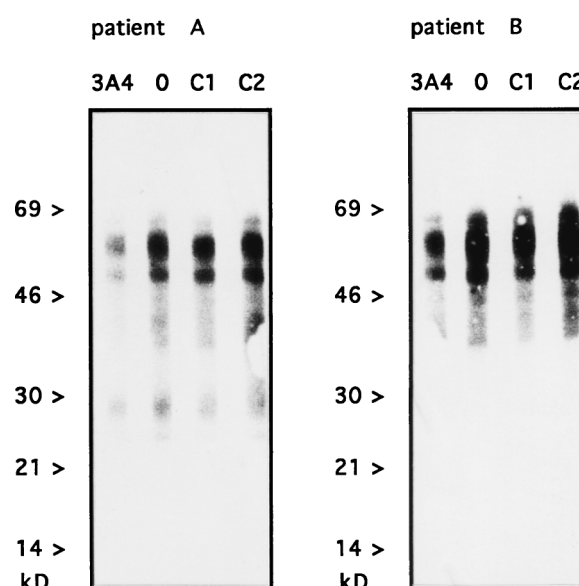


FIG. 2. Monoclonal antibody 3A4 inhibits patients' IgE from binding to the major mugwort allergen Art v 1. Nitrocellulose-blotted mugwort pollen extract was preincubated with mAb 3A4 (lane 3A4), buffer (lane 0), and two mouse mAbs without specificity for Art v 1 (lanes C1 and C2). Strips were then probed with serum IgE from two patients allergic to Art v 1 (patients A and B). Bound IgE antibodies were detected with ¹²⁵I-labeled anti-human IgE antibodies and visualized by autoradiography.

Calif.). Goat anti-mouse IgG plus IgM antibodies coupled to 10 nm colloidal gold particles were bought from British BioCell (Plano, Wetzlar, Germany).

Protein extracts

Two grams of pollen were extracted in 50 ml of distilled water for 2 hours at room temperature. The protein extracts were then centrifuged at 30,000 g for 30 minutes at 4° C. The supernatants were lyophilized and stored at -20° C until use.

Fresh celery and apple were cut into small pieces. Approximately 1 gm of tissue was homogenized under liquid nitrogen and the powder was transferred into falcon tubes containing 5 ml (celery) or 10 ml (apple) of sodium dodecylsulfate sample buffer.¹⁵ The samples were boiled for 5 minutes and centrifuged in a bench fuge at 10,000 rpm, and the supernatants were stored at -20° C until use.

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

Twelve percent SDS-PAGE was done according to the method of Laemmli.¹⁵ Blotting was performed as described by Towbin et al.¹⁶ Nitrocellulose strips were probed with mAb 3A4 against Art v 1 or with buffer A (50 mmol/L Naphosphate [pH 7.5], 0.5% wt/vol bovine serum albumin [BSA], 0.5% vol/vol Tween 20, and 0.05% NaN₃) only.

IgE inhibition experiment

Mugwort pollen extract (approximately 100 µg/cm preparative sodium dodecylsulfate-polyacrylamide gel) was separated by 12% SDS-PAGE and blotted onto nitrocellulose as described above. After blocking with buffer A, strips were preincubated with mAb 3A4 (culture supernatant diluted 1:2 in buffer A) and, for control purposes, in buffer A or two mouse

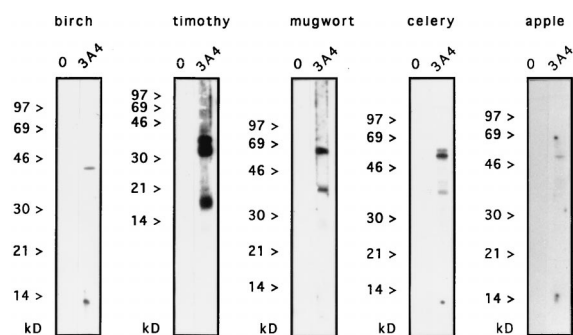


FIG. 3. Monoclonal antibody 3A4 cross-reacts with allergens present in pollen and plant-derived food. Nitrocellulose-blotted protein extracts from birch pollen, timothy grass pollen, mugwort pollen, celery roots, and apples were probed with mAb 3A4 (lane 3A4) and, for control purposes, with buffer alone (lane 0). Bound antibodies were detected with a ^{125}I -labeled sheep anti-mouse antiserum and visualized by autoradiography.

monoclonal anti-Bet v 1 antibodies without specificity for Art v 1 (C 1, C2)¹⁴ overnight at 4° C. Strips were then washed and incubated with sera from two patients allergic to mugwort pollen and diluted 1:5 in buffer A overnight at 4° C. After washing, bound IgE was detected with 1:10 diluted ^{125}I -labeled anti-human IgE antibodies (Pharmacia, Uppsala, Sweden).

ELISA catching experiment

To study whether mAb 3A4 binds to similar or different epitopes than human IgE antibodies, ELISA catching experiments were performed. ELISA plates were coated with rat anti-mouse IgM and rat anti-mouse IgG1, and both were diluted 1:1000 in phosphate-buffered saline (PBS; pH 6.7) overnight at 4° C. The plates were then washed with Tris-buffered saline containing Tween (TBST), blocked with TBST containing 0.5% BSA, and incubated with culture supernatants containing mAb 3A4 and Bip 1 for 4 hours. The plates were then washed and incubated with birch or mugwort pollen extract (diluted at 100 $\mu\text{g}/\text{ml}$ in TBST containing 0.5% BSA). The plates were then washed again and incubated 1:10 in TBST-diluted sera from patients allergic to mugwort and birch pollen and, for control purposes, with serum from a nonallergic donor.

Bound IgE antibodies were then detected with an alkaline phosphatase monoclonal anti-human IgE antibody (Pharmin-gen, San Diego, Calif.). After washing, the color reaction was started with alkaline phosphatase substrate (Sigma Diagnostics, St. Louis, Mo.). The optical density corresponding to the amount of bound IgE antibodies was determined in an ELISA reader (Dynatech, Denkendorf, Germany). Results represent means of duplicate determinations.

Preparation for electron microscopy

Vapor fixation of soluble allergens was carried out as follows. Birch pollen was fixed in p-formaldehyde vapor for 24 hours,^{17,18} timothy grass pollen was fixed in acrolein vapor for 7 days,¹⁹ and mugwort pollen was fixed in acrolein vapor for 11 days. The ensuing preparation steps were, in short, dehydration in dimethoxypropane and ethanol followed by embedding in Lowicryl K4M. Polymerization was done by ultraviolet light at -35° C.

Cubes of approximately 1 mm³ were dissected from the fruit flesh of the apple approximately 15 mm below the skin and from

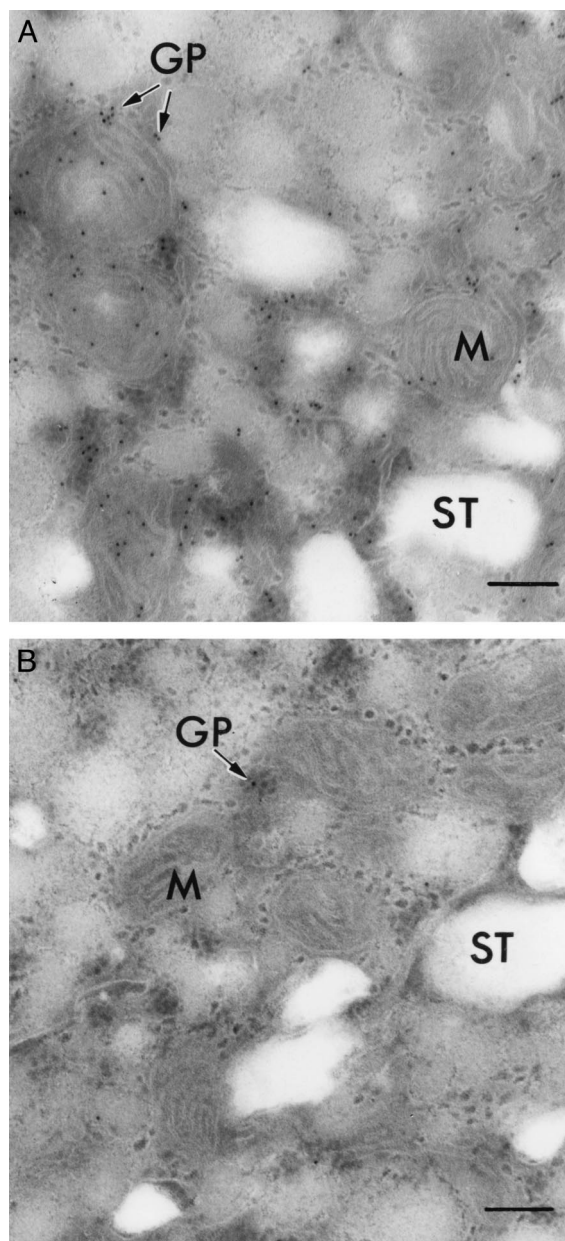


FIG. 4. Ultrathin sections of mugwort pollen after incubation with mAb 3A4 (A) or control antibody (B) followed by goat-anti mouse IgG coupled to 10 nm gold particles. Mitochondria and ribosome-rich areas between reserve materials are labeled with gold particles. No gold particles are observed in reserve materials (starch). Control is almost completely without gold particles. Magnifications: 44,940 $\times$ (A) and 46,900 $\times$ (B). Bar = 0.2 μm . GP, Gold particle; M, mitochondrion; ST, starch.

the celery approximately 30 mm below the skin. Tissue samples were fixed in 4% p-formaldehyde in 0.071 mol/L cacodylate buffer overnight at 4° C. Dehydration, embedding in Lowicryl K4M, and polymerization at -35° C were carried out as previously described.²⁰ After polymerization, ultrathin sections (silver color) were cut from all embedded specimens and placed on nickel grids for immunocytochemistry.

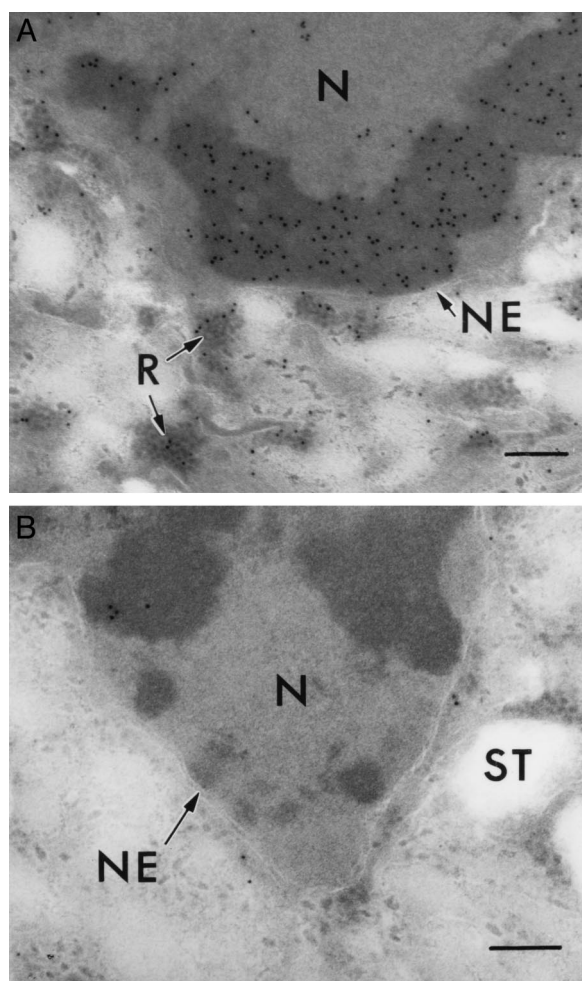


FIG. 5. Ultrathin section of mugwort pollen nucleus after incubation with mAb 3A4 (**A**) or control antibody (**B**) followed by goat anti-mouse IgG coupled to 10 nm colloidal gold particles. There is heavy labeling of the electron-dense (dark) areas of the nucleus and the ribosome-rich interspaces between the p-particles in the cytoplasm. The control experiment shows very few gold particles. Magnifications: 42,180 $\times$ (**A**) and 46,900 $\times$ (**B**). Bar = 0.2 μ m. *N*, Nucleus; *NE*, nuclear envelope; *R*, ribosome; *ST*, starch.

Immunocytochemistry

Grids with ultrathin sections were incubated (face down in a moist chamber) on drops of the following solution (PBS buffer at pH 7.4, Tris-BSA buffer at pH 8.2): (1) 5% BSA in PBS for 15 minutes, (2) 3A4 antibody diluted 1:100 in PBS for 2 hours, (3) PBS for 1 $\times$ 5 minutes and Tris-BSA for 2 $\times$ 5 minutes, (4) goat anti-mouse IgG plus IgM diluted 1:10 in Tris-BSA for 1 hour, and (5) Tris-BSA for 1 $\times$ 5 minutes and distilled water for 2 $\times$ 5 minutes.

The following controls were performed: (1) omission of the primary antibody (3A4), (2) substitution of primary specific antibody (3A4) by antibody of irrelevant specificity (mAb 414), and (3) incubation of sections with secondary antibody (gold-conjugated goat anti-mouse IgG plus IgM) alone.

Electron microscopy and documentation

Sections were stained with uranyl acetate (5 minutes) and lead citrate (10 seconds) and analyzed in a Hitachi H500

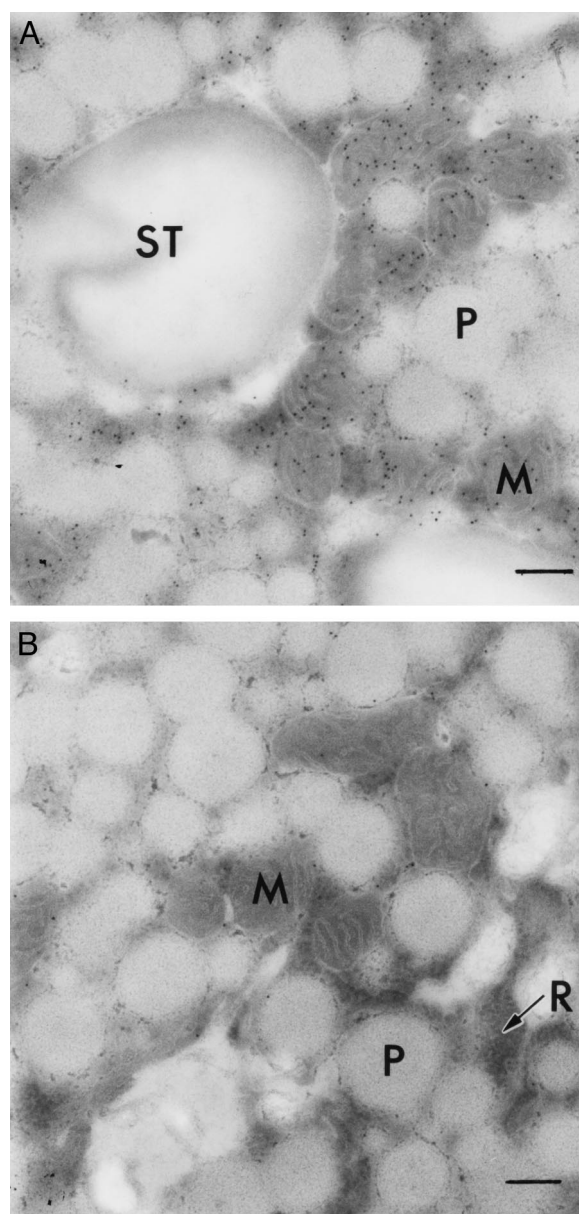


FIG. 6. Ultrathin section of timothy grass pollen after incubation with mAb 3A4 (**A**) or control antibody (**B**) followed by goat anti-mouse IgG coupled to 10 nm colloidal gold particles. Mitochondria and electron-dense areas of cytosol (ribosomes) are intensely labeled by the probe. Starch grains and polysaccharide inclusions (p-particles) are unlabeled. There is a very low degree of background labeling in the control. Magnifications: 33,400 $\times$ (**A**) and 34,460 $\times$ (**B**). Bar = 0.2 μ m. *M*, Mitochondrion; *P*, polysaccharide; *R*, ribosome; *ST*, starch.

transmission electron microscope operated at 75 kV. For documentation, Agfa-Gevaert 23D56 films were used.

RESULTS

Monoclonal antibody 3A4 recognizes an IgE epitope of the 60 kd mugwort pollen allergen

In an ELISA experiment we studied whether mAb 3A4 can catch Art v 1 from mugwort pollen extract and

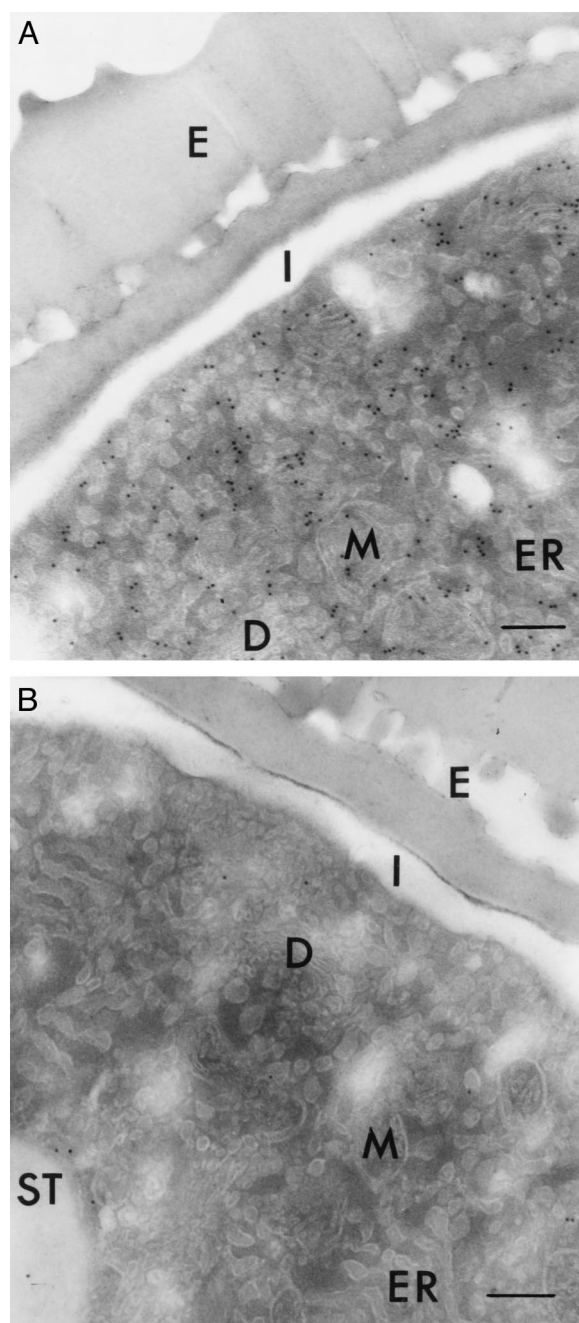


FIG. 7. Ultrathin section of birch pollen after incubation with mAb 3A4 (**A**) or control antibody (**B**) followed by goat anti-mouse IgG coupled to 10 nm gold particles. Whereas the pollen wall is unlabeled, many gold markers are observed in the cytoplasm distributed in the cytosol and inside mitochondria and dictyosomes. The control shows few nonspecific gold particles. Magnifications: 40,660 $\times$ (**A**) and 44,940 $\times$ (**B**). Bar 0.2 μ m. *D*, Dictyosome; *E*, Exine; *ER*, endoplasmic reticulum; *I*, intine; *M*, mitochondrion; *ST*, starch.

present the allergen to patients' IgE. Although Bip 1, an mAb raised against the major birch pollen allergen Bet v 1, was able to catch Bet v 1 from birch pollen extract and present the allergen to IgE antibodies of patients

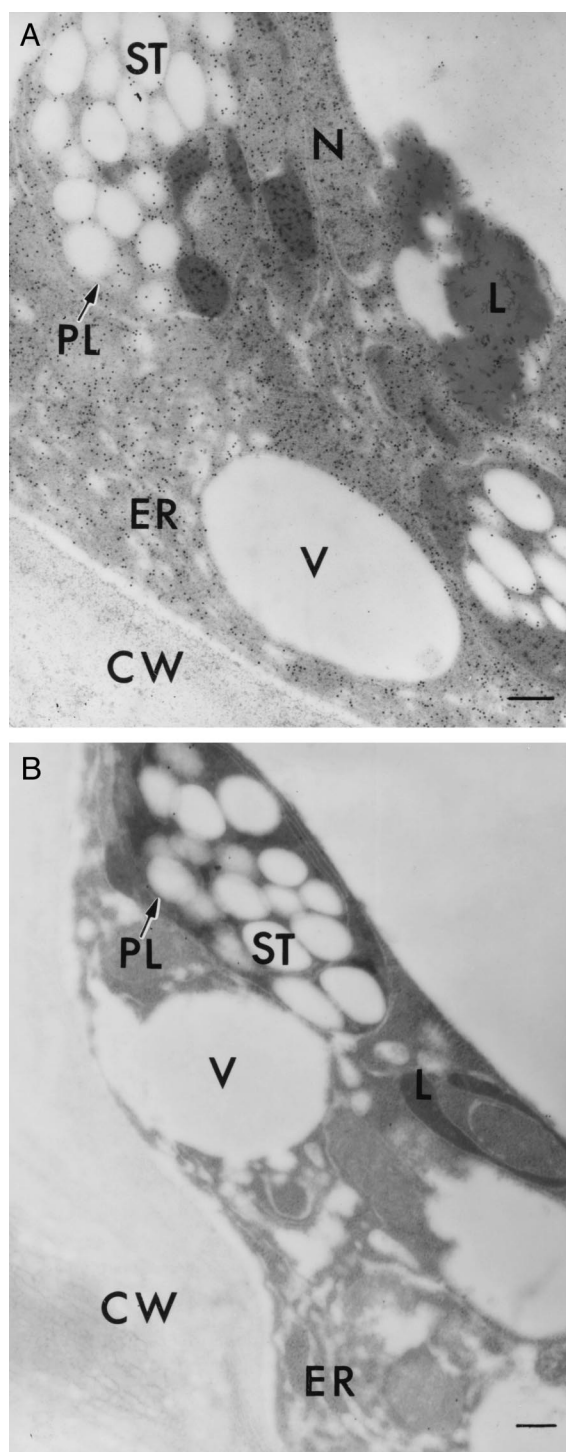


FIG. 8. Ultrathin section of a fruit-flesh cell from apple. Incubation with mAb 3A4 (**A**) and control antibody (**B**) followed by goat anti-mouse IgG coupled to 10 nm gold particles. Whereas there are no gold markers observed in the cell wall, the cytoplasm is heavily labeled, including the nucleus and plastids. There are no gold particles in vacuoles, starch granules, and lipidic materials. The control experiment shows very few nonspecifically adsorbed gold particles. Magnifications: 26,730 $\times$ (**A**) and 21,240 $\times$ (**B**). Bar = 0.2 μ m. *CW*, Cell wall; *ER*, endoplasmic reticulum; *L*, lipid; *N*, nucleus; *PL*, plastid; *ST*, starch; *V*, vacuole.

allergic to Bet v 1 (Fig. 1, sera 3, 5, and 6), mAb 3A4 failed to present Art v 1 (Fig. 1, sera 2, 3, and 4). In the Bip 1-mediated assay, no IgE binding was observed when sera without Bet v 1-specific IgE were used (Fig. 1, sera 2 and 4). Sera without Art v 1-specific IgE showed no IgE binding in the 3A4 catching experiment (Fig. 1, sera 4 and 5). In both experiments no IgE binding was observed when serum from a nonallergic individual was used (Fig. 1, serum 1). When ELISA plate-bound Bip 1 and 3A4 were incubated with mugwort and birch pollen extract, no allergen was caught and presented to serum IgE (data not shown). Together the results of these experiments indicated that mAb 3A4 is either unable to catch Art v 1 from a solution of mugwort pollen extract or binds to or close to Ig epitopes. To further investigate the 3A4 epitopes, inhibition experiments were performed. Nitrocellulose-blotted mugwort pollen extract was preincubated with mAb 3A4 (Fig. 2, lane 3A4) and, for control purposes, with buffer (Fig. 2, lane 0) or two mouse monoclonal antibodies without specificity for Art v 1 (Fig. 2, lane C1, C2) before exposure to serum IgE from two patients allergic to Art v 1. Preincubation with mAb 3A4 significantly reduced IgE binding to the 60 kd mugwort pollen allergen and bands of lower molecular weight, presumably representing Art v 1 variations. These data indicate that mAb 3A4 binds to or close to IgE epitopes and thus inhibits IgE binding to the allergen.

Monoclonal antibody 3A4 cross-reacts with allergens of comparable molecular weight in birch and timothy grass pollen and with apple and celery tissues

Nitrocellulose-blotted mugwort, birch, and timothy grass pollen and apple and celery extracts were probed with mAb 3A4 and, for control purposes, with buffer (Fig. 3). In mugwort pollen, apple, and celery extracts mAb 3A4 bound to a 60 kd moiety. In birch pollen, timothy grass pollen, mugwort pollen, and celery extracts a 40 kd band was detected and a 17 kd component was bound in timothy grass pollen extract (Fig. 3, lane 3A4). The secondary antiserum did not display any reactivity to the extracts (Fig. 3, lane O).

The cross-reactive allergen in mugwort, birch, and timothy grass pollens and in apple and celery is localized to similar compartments by postembedding immunogold electron microscopy

In mugwort, timothy grass, and birch pollens gold particles indicating the binding of mAb 3A4 are localized in the mitochondria and in the electron-dense, dark interspaces between the various storage organelles (mugwort and timothy grass: Fig. 4, A; 5, A; and 6, A) or between membrane-bound cell organelles and vesicles (birch: Fig. 7, A). These interspaces are characterized by the presence and accumulation of ribosomes that are clearly recognizable in mugwort pollen, less clearly recognized in timothy grass pollen, and only recognizable after a different type of fixation in birch pollen.¹⁸ The pollen nucleus is always heavily labeled in the three

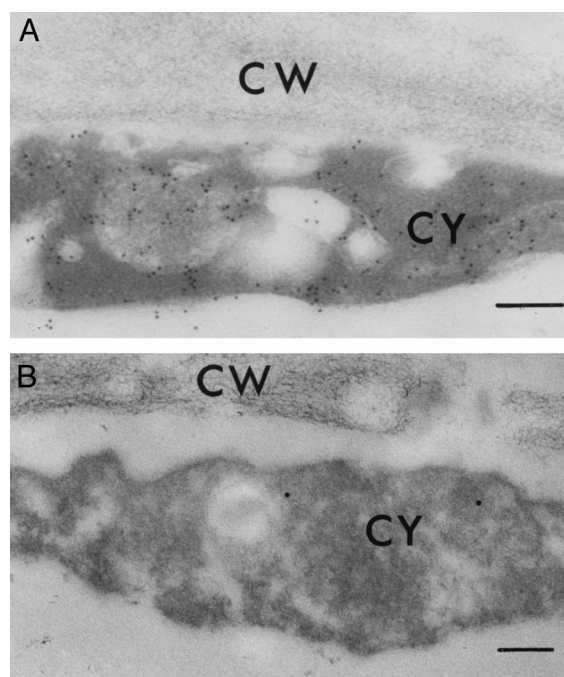


FIG. 9. Ultrathin section of celery root tissue after incubation with mAb 3A4 (A) or control antibody (B) followed by goat anti-mouse IgG coupled to 10 nm gold particles. Despite the low degree of morphologic preservation, it is clearly visible that gold particles are associated with the cytoplasm, whereas the wall is unlabeled. In the control experiment very few gold particles are observed. Magnifications: 41,500 $\times$ (A) and 53,300 $\times$ (B). Bar = 0.2 μ m. CW, Cell wall; CY, cytoplasm.

pollen species, especially in the electron-dense, dark areas (mugwort: Fig. 5, A). There is no labeling of pollen walls (birch: Fig. 7, A). All control experiments using a mAb of different specificity exhibited little nonspecific staining (Figs. 4, B; 5 B; 6 B; and 7, B). No gold particles were adsorbed to the sections after incubation with the gold-conjugated secondary antibody alone (micrographs not shown).

Figs. 8 and 9 show mapping of mAb 3A4 to apple fruit flesh and celery tissue. Because of aqueous p-formaldehyde fixation, which had to be used for preservation of antigenicity, ultrastructural preservation of the cells is only of medium (apple) or low (celery) quality. Nonetheless, it is clearly recognizable that gold particles are localized in the cytoplasm and not in the cell walls. In apple fruit flesh, with the exception of starch and lipids, there is labeling of the nucleus, mitochondria, endoplasmic reticulum, and plastids (Fig. 8, A). In celery tissue binding of mAb 3A4 throughout the cytoplasm is clearly recognizable (Fig. 9, A). Control experiments replacing mAb 3A4 with an mAb of irrelevant specificity show very few nonspecifically bound gold particles (Figs 8, B and 9, B).

DISCUSSION

In the first step of this investigation we performed immunochemical experiments for a further characterization of mAb 3A4. Monoclonal antibody 3A4 was raised

against the major mugwort pollen allergen, Art v 1, representing a glycoprotein of approximately 60 kd.¹² In addition to the 60 kd band, mAb 3A4 bound also to lower molecular weight mugwort pollen-derived species. Support for the contention that the lower molecular weight bands detected by mAb 3A4 indeed represent Art v 1-related moieties come from our observations that several other Art v 1-specific mAbs and serum IgE from patients allergic to Art v 1 displayed a similar reaction pattern and that the binding of Art v 1-specific IgE to the lower molecular weight species was inhibited by preincubation of sera with purified natural Art v 1.⁷ Because Art v 1 has been shown to represent an allergen that is highly resistant against tryptic digestion,⁷ it is assumed that the low molecular weight Art v 1 derivatives represent Art v 1 modifications that do not result from proteolytic digestion.

Preincubation of nitrocellulose-blotted mugwort pollen extract with mAb 3A4 significantly inhibited IgE binding to Art v 1 migrating at 60 kd, as well as to the presumed Art v 1-derived lower molecular weight moieties, indicating that 3A4 either binds to or close to an IgE epitope of Art v 1. Competition with IgE for one of the epitopes or steric hindrance of IgE binding have to be considered as possible mechanisms for the inhibition of IgE binding to Art v 1 by mAb 3A4. The incomplete inhibition of IgE binding is most likely due to the fact that the polyclonal anti-Art v 1 IgE response is directed also against epitopes that are not targets for mAb 3A4.

In summary, the results indicated that mAb 3A4 bound to or close to IgE epitopes of the major mugwort allergen, Art v 1, and cross-reacted with moieties of comparable but also different molecular weights in timothy grass and birch pollen and apple and celery extracts.

In a next step, the ultrastructural localization of the 3A4-defined allergen was studied in mugwort, birch, and timothy grass pollen and in apple and celery tissues by immunogold electron microscopy. The results of these morphologic studies show a clear labeling of all relevant pollen species and food tissues by mAb 3A4. There is a remarkable uniformity of the binding pattern. The antibody binds to sites present in ribosome-rich areas of the cytosol and to cell organelles like nucleus and mitochondria. There are no binding sites in the storage materials (polysaccharides and lipids). No binding sites were detected on the surface of the pollen or in pollen cell walls.

By comparing this broad localization pattern of the mAb 3A4-reactive 60 kd allergen with that of other pollen allergens previously studied, we recognized similarities but also differences. Bet v 1, the major birch pollen allergen, also maps to ribosome-rich areas and nuclei, but not to mitochondria.^{17, 18} Bet v 2, birch pollen profilin, was localized in the cytosol and the nucleus.²¹ In timothy grass pollen, group 1 and 5 allergens are localized in the pollen wall and in the cytosol (ribosome-rich areas). Group 5 allergen is additionally found inside the

amyloplasts (starch).¹⁹ Group 6 allergen in timothy grass pollen maps to the polysaccharide containing p-particles and to ribosome-rich areas of the cytosol (Grote et al., unpublished communication, 1997). In rye grass pollen, Lol p 1 is found in the exine, in electron-opaque areas of the cytoplasm (presumably ribosome-rich areas), in starch granules, in mitochondria, and in vegetative nuclei.²² The above survey shows that under appropriate fixation the ultrastructural localization of plant allergens performed to date indicates that allergenic moieties are localized primarily to all kinds of intracellular compartments but, surprisingly, are not detected on the surface of allergenic sources (pollen and plant-derived food).

Although the biochemical nature and the biologic role of the 3A4-defined 60 kd allergen is still unknown, its ubiquitous presence in pollens and plant-derived food provides an explanation for why it can act as a cross-reactive allergen for patients allergic to plants.

REFERENCES

- Valenta R, Kraft D. Type I allergic reactions to plant-derived food: a consequence of primary sensitization to pollen allergens. *J Allergy Clin Immunol* 1996;97:893-5.
- Breiteneder H, Pettenburger K, Bito A, Valenta R, Kraft D, Rumpold H, et al. The gene coding for the major birch pollen allergen Bet v 1, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989;8:1935-8.
- Valenta R, Duchêne M, Pettenburger K, Sillaber C, Valent P, Bettelheim P, et al. Identification of profilin as a novel pollen allergen: IgE autoreactivity in sensitized individuals. *Science* 1991; 253:557-60.
- Valenta R, Duchêne M, Ebner C, Valent P, Sillaber C, Deviller P, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377-85.
- Hirschwehr R, Valenta R, Ebner C, Ferreira F, Sperr W, Valent P, et al. Identification of common structures in hazel pollen and hazelnuts: a possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J Allergy Clin Immunol* 1992;90:927-36.
- Ebner C, Hirschwehr R, Bauer L, Breiteneder M, Valenta R, Ebner H, et al. Identifications of allergens in fruits and vegetables: IgE cross reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). *J Allergy Clin Immunol* 1995;95:962-9.
- Heiss S, Fischer S, Müller WD, Weber B, Hirschwehr R, Spitzauer S, et al. Identification of a 60 kd cross-reactive allergen in pollen and plant derived food. *J Allergy Clin Immunol* 1996;98:938-47.
- Bauer L, Ebner C, Hirschwehr R, Wüthrich B, Pichler C, Fritsch R, et al. IgE cross-reactivity between birch pollen, mugwort pollen and celery is due to at least three distinct cross-reacting allergens: immunoblot investigation of the birch-mugwort-celery syndrome. *Clin Exp Allergy* 1996;26:1161-70.
- Fischer S, Grote M, Fahlbusch B, Müller WD, Kraft D, Valenta R, et al. Characterization of Phi p 4, a major timothy grass (*Phleum pratense*) pollen allergen. *J Allergy Clin Immunol* 1996;98:189-98.
- Fuchs T, Spitzauer S, Vente C, Hevler J, Kapiotis S, Rumpold H, et al. Natural latex, grass pollen and weed pollen share IgE-epitopes. *J Allergy Clin Immunol* 1997, in press.
- Valenta R, Kraft D. Recombinant allergens for diagnosis and therapy of allergic diseases. *Curr Opin Immunol* 1995;7:751-6.
- De la Hoz F, Polo F, Moscoso del Prado J, Sellés JG, Lombardero M, Carreira J. Purification of Art v I, a relevant allergen of *Artemisia vulgaris* pollen. *Mol Immunol* 1990;27:651-7.
- Laffer S, Vangelista L, Steinberger P, Kraft D, Pastore A, Valenta R. Molecular characterization of Bip 1, a monoclonal antibody that modulates IgE binding to birch pollen allergen, Bet v 1. *J Immunol* 1996;157:4953-62.

14. Lebeque S, Dolecek C, Laffer S, Visco V, Denepoux S, Pin JJ, et al. Immunologic characterization of monoclonal antibodies that modulate human IgE binding to the major birch pollen allergen Bet v 1. *J Allergy Clin Immunol* 1997;99:374-84.
15. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
16. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some application. *Proc Natl Acad Sci USA* 1979;76:4350-4.
17. Grote M. Immunogold electron microscopy of soluble proteins: localization of Bet v I major allergen in ultra-thin sections of birch pollen after anhydrous fixation techniques. *J Histochem Cytochem* 1991;39:1395-401.
18. Grote M. Ultrastructural morphology and allergen detection in birch pollen after aqueous, anhydrous-liquid, and vapor fixation techniques. *Microsc Res Tech* 1992;21:242-8.
19. Grote M, Dolecek C, van Ree R, Valenta R. Immunogold electron microscopic localization of timothy grass (*Phleum pratense*) pollen major allergens Phi p I and Phi p V after anhydrous fixation in acrolein vapor. *J Histochem Cytochem* 1994;42:427-31.
20. Grote M, Fromme HG. Immunoelectron microscopic localization of diffusible birch pollen antigens in ultrathin sections using the protein A/gold technique. *Histochemistry* 1984;81:489-92.
21. Grote M. Ultrastructural detection of the allergen Bet v I in birch pollen and profilin in tobacco pollen by special techniques of immunogold electron microscopy. In: Kraft D, Sehon A, editors. *Molecular biology and immunology of allergens*. Boca Raton (FL): CRC Press; 1993. p. 201-3.
22. Staff IA, Taylor PE, Smith P, Singh MB, Knox RB. Cellular localization of water soluble, allergenic proteins in rye-grass (*Lolium perenne*) pollen using monoclonal and specific IgE antibodies with immunogold probes. *Histochem J* 1990;22:276-90.