

Food allergy to honey: Pollen or bee products?

Characterization of allergenic proteins in honey by means of immunoblotting

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Objective: To characterize the allergenic components of honey, 23 patients allergic to honey were investigated. All displayed allergic symptoms after ingestion of honey or honey-containing products, ranging from itching in the oral mucosa to severe systemic symptoms to anaphylactic shock.

Methods and results: Immunoblot analyses of the patients' sera revealed IgE binding to proteins at a molecular mass of 54 kd, 60 kd, 72 kd, or to a 30 kd/33 kd double band, or to both in sunflower honey extracts. The three bands corresponding to higher molecular mass proteins could also be detected in the three other kinds of honey (locust tree, European chestnut and forest honey) that were tested and represented bee products because IgE binding to these proteins was inhibited by extracts of honeybee heads and extracts of isolated bee venom sacs. The 30 kd/33 kd bands could be identified as sunflower honey-specific. When testing sera from patients allergic to bee venom with honey extracts, in seven of 10 cases IgE binding to bee-specific components could be observed.

Conclusion: Both proteins derived from secretions of pharyngeal and salivary glands of honeybee heads and pollen proteins contained in the honey cause allergic reactions to honey. (*J ALLERGY CLIN IMMUNOL* 1996;97:65-73.)

Key words: Allergy, honey, IgE inhibition, honeybee heads, bee venom

The natural sources of honey are nectar and honeydew. During collection, grains of pollen are admixed to this raw material. Ten grams of honey contains 20 to 100,000 grains of pollen,¹ which retain their allergenic properties during the honey-making process.² In the beehive, this raw material is further processed to mature honey by the bees, during which enzyme-rich secretions from salivary and pharyngeal glands, which are mainly located in

Abbreviations used

CH:	Chestnut honey
FO:	Forest honey
LO:	Locust tree honey
PLA ₂ :	Phospholipase A ₂
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
SF:	Sunflower honey

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the bees' heads, are added.¹ Propolis, a resinous beehive product, although the cause of an increasing number of cases of allergic contact dermatitis,³ apparently is of no importance for persons allergic to honey because caffeic acid esters, its main allergenic components, have not been identified in honey.⁴ In addition, honeydew, a product of sap-sucking insects, also contains enzymes that are derived from the salivary glands and bowels of these insects.¹ Bee body components, mold spores, algae, and other organic debris may be found in

TABLE I. Symptoms of patients allergic to honey (group I, $n = 23$) and patients allergic to bee venom (group II, $n = 10$)*

	Local reactions	Frequency	Systemic reactions	Frequency
Patients allergic to honey (group I)	Itching in the mouth	52%	Bronchial asthma	30%
	Gastrointestinal symptoms	17%	Generalized urticaria	9%
	Contact urticaria/angioedema	9%	Anaphylaxis	17%
Patients allergic to bee venom (group II)	Urticaria/angioedema	70%	Generalized pruritus	10%
			Bronchial asthma	20%
			Generalized urticaria	30%
			Anaphylaxis	30%

*Multisymptomatic cases are possible.

honey and could theoretically mediate allergic reactions in patients allergic to honey.⁵

According to Hofer et al., who performed a study on 173 patients with food allergy, the incidence of honey allergy was given as 2.3%.⁶ However, the information about food allergy caused by ingestion of honey is limited.⁵⁻¹⁰ Discussion of the various sources of protein in honey and their antigenicity was initiated in 1957.⁷ Birnbaum et al.⁸ described cross-reactivities between sunflower honey, sunflower pollen, and celery by RAST inhibition. It has been reported that allergy to honey could be attributed to the pollen content.⁹⁻¹¹ Helbling et al.⁵ aimed to identify the allergens of three different types of honey by analysis of diagnostic tests (skin tests and RASTs) and RAST inhibition. The authors showed that both bee-derived and pollen-related proteins are relevant for honey allergy. Moreover, they revealed cross-reactivities between honey and bee products.

The purpose of this study was to characterize the allergenic components of honey by the use of immunoblotting and IgE inhibition studies with particular regard to cross-reacting proteins between honey and bee venom.

METHODS

Sera

Group I. Sera from 23 patients allergic to honey (eight men and 15 women, aged 13 to 67 years; median age, 34 years) who had clinical symptoms ranging from itching in the mouth to severe anaphylactic reactions to shock (Table I), positive skin prick test results, positive RAST results (classes 2.0 to 3.1 to honey [Antigen rf 247; Pharmacia, Uppsala, Sweden]) were selected for this study.

Group II. Sera from 10 patients allergic to bee venom¹² (six men and four women, aged 6 to 62 years; median age, 22 years) who had clinical symptoms ranging from urticaria or angioedema to anaphylaxis (Table I) and positive RAST results (classes 4 and higher [Antigen ii, Pharmacia]).

Control group. Sera from three nonallergic persons (negative case history, negative skin prick test results, negative honey and bee venom RAST results) were pooled (normal human serum) and used for control experiments.

Protein extracts

Honeys. Four common types of honey (*Robinia pseudoacacia*, locust tree honey [LO]; *Helianthus annuus*, sunflower honey [SF]; *Castanea sativa*, European chestnut honey [CH]; and forest honey [FO]) supplied by the Institute of Bee Research, Bad Vöslau, Austria, were used. Chemical analyses, including estimation of water content, pH, electrical conductivity, and hydroxymethylfurfural concentration were performed.

Ten grams of honey was suspended in 10 ml of distilled water. Proteins were extracted by overnight shaking at 4° C. After centrifugation at 40,000 g for 60 minutes at 4° C, the supernatant was filtered and subsequently dialyzed (Spectra/Por 1, Molecularporous Dialysis Membrane, molecular weight cutoff: 6 to 8,000; Spectrum, Houston, Texas) against distilled water for 48 hours with two changes of distilled water. The dialysates were lyophilized and subsequently stored at -20° C. Protein concentrations were determined according to the method of Bradford.¹³

Honeybee heads and bee venom sacs were obtained from the Institute of Bee Research. Fifteen honeybee heads were introduced into 5 ml of Laemmli¹⁴ buffer, and seven bee venom sacs were introduced into 3 ml of Laemmli buffer, homogenized in an Ultra-Turrax T25 (IKA Labortechnik, Staufen, Germany), for 10 minutes in the 95° C water bath, and centrifuged (10 minutes $\times$ 11,000 rpm). The supernatants were directly used for sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Honeybee head and bee venom sac extracts for IgE inhibition experiments were prepared as follows: 15 honeybee heads and eight bee venom sacs were added to test buffer (50 mmol/L sodium phosphate buffer [pH 7.5] containing 0.5% [vol/vol] bovine serum albumin, 0.05% [wt/vol] sodium azide) and 5 mmol phenylmethylsulfonylfluoride, 5 mmol benzamidine (Sigma Chemical, St. Louis, Mo.), homogenized in an Ultra-Turrax

T25, and centrifuged (10 minutes $\times$ 11,000 rpm). The supernatants were stored at -20°C . Protein concentrations were determined according to the Bradford method.¹³

SDS-PAGE, protein transfer, and immunoblotting

SDS-PAGE (12% homogeneous gels, $140 \times 115 \times 1$ mm, 5% stacking gels) was performed according to the method of Laemmli¹⁴ in a vertical slab gel apparatus (Hoefer, San Francisco, Calif.) with modifications as previously described.¹⁵ The honey samples were diluted (4:1) in Laemmli buffer before heating at 95°C for 10 minutes and subsequently loaded onto gels (40 μg of extract per centimeter). Bee venom sac and honeybee head samples were loaded onto gels in a concentration of 40 $\mu\text{l}/\text{cm}$ and 90 $\mu\text{l}/\text{cm}$, respectively. Transfer of separated proteins to nitrocellulose sheets was done according to the method of Towbin et al.¹⁶ IgE immunoblots were performed as previously described.¹⁵ To exclude immunoreactivity against proteins that are absent after the procedure, experiments with extracts of honeybee heads were repeated with nondenaturing conditions for electrophoresis.^{17, 18}

Immunoblots with monoclonal and rabbit antibodies

BIP 1, a monoclonal antibody directed against Bet v 1,¹⁹ was used to identify antigens homologous to Bet v 1²⁰ in honey or in bee-derived products. Immunoblots were performed as previously described.¹⁵

MUP 4, MUP 8, and MUP 20, monoclonal antibodies directed against allergens of mugwort pollen, were introduced to characterize mugwort pollen homologous antigens in honey or in bee-derived products. Blots were performed as described.¹⁵

A rabbit anti-profilin antibody²¹ was used to identify the plant panallergen profilin^{22, 23} in honey or in bee-derived products. Nitrocellulose strips containing honey proteins, honeybee head proteins, and bee venom sac proteins were submerged with a 1:500 dilution of anti-profilin antibody. Bound rabbit antibody was detected with iodine 125-labeled donkey anti-rabbit antibody (Amersham, Amersham, U.K.).

IgE inhibition experiments

Inhibition with honeybee head extracts. Sera from five patients displaying IgE binding to three higher molecular mass bands and a double band in SF immunoblots (Fig. 2; lanes 1, 2, 3, 4, and 5) were pooled (pool A), diluted 1:10 in test buffer, and preincubated with 300 μg of honeybee head inhibition extract under continuous agitation overnight at 4°C . Thereafter, nitrocellulose-blotted SF proteins were incubated with this dilution (1 ml/nitrocellulose strip), and bound IgE was detected as described.¹⁵ The same procedure was performed with serum pools from patients who displayed IgE binding to three higher molecular mass bands

(pool B; Fig. 2, lanes 11, 12, 13, 14, 15, 16, 17) exclusively, or to a double band (pool C; Fig. 2, lanes 9, 10) in SF immunoblots.

Inhibition with SF extracts. Sera from seven patients allergic to honey (pool B) displaying IgE binding to three bands at molecular masses of 57 kd, 66 kd, and 80 kd in honeybee head immunoblots were used, diluted 1:10 in test buffer, and preincubated with 40 μg and 80 μg of SF extract. Thereafter, nitrocellulose-blotted honeybee-head proteins were incubated with this dilution (1 ml/nitrocellulose strip), and bound IgE was detected as described.¹⁵

Sera from six patients allergic to bee venom (group II) exhibiting IgE reactivity to three bands at molecular masses of 46 kd, 50 kd, and 65 kd in addition to the 19 kd band (Fig. 5, bee venom sac; lanes 1, 2, 3, 7, 8, and 10) were pooled (pool X), diluted 1:10 in test buffer, and preincubated with 5 μg , 25 μg , and 125 μg , respectively, of SF extract. Thereafter, nitrocellulose-blotted bee venom sac proteins were incubated with this dilution (1 ml/nitrocellulose strip), and bound IgE was detected as described.¹⁵

RESULTS

Characterization of honey extracts

Coomassie brilliant blue R-stained 12% SDS-polyacrylamide gel containing the separated proteins of the four types of honey used in this study (locust, sunflower, chestnut, forest) exhibited multiple protein bands ranging from 10.5 kd to 72 kd. The honey extracts seemed to contain shared and individual proteins (Fig. 1). SF was chosen for detailed investigation.

Immunoblot experiments

Sera from group I (patients allergic to honey). All group I patients' sera displayed IgE binding to either bands of a three-band complex (54 kd, 60 kd, and 72 kd) or a double band (30 kd, 33 kd), or both in SF strips (Fig. 2). Serum pools A and B also exhibited IgE reactivity to the three bands corresponding to higher molecular mass of the other types of honey (Fig. 3; lanes 1 and 2). Pool A and pool C, showing IgE binding to a 30 kd/33 kd double band in the SF immunoblot (Fig. 3; SF, lanes 1, 3), did not react with corresponding proteins in the other honey extracts. In forest honey, an additional IgE-binding protein with 11 kd could be identified (Fig. 3; FO, lanes 1, 2).

Forty eight percent (11 of 23) of patients in group I displayed IgE binding to three bands at molecular mass of 57 kd, 66 kd, and 80 kd with honeybee head extract (Fig. 4, A; lanes 1, 2, 3, 11, 12, 13, 14, 15, 16, 17, 19). Thirty percent (7 of 23) of group I patients' sera reacted with bee venom

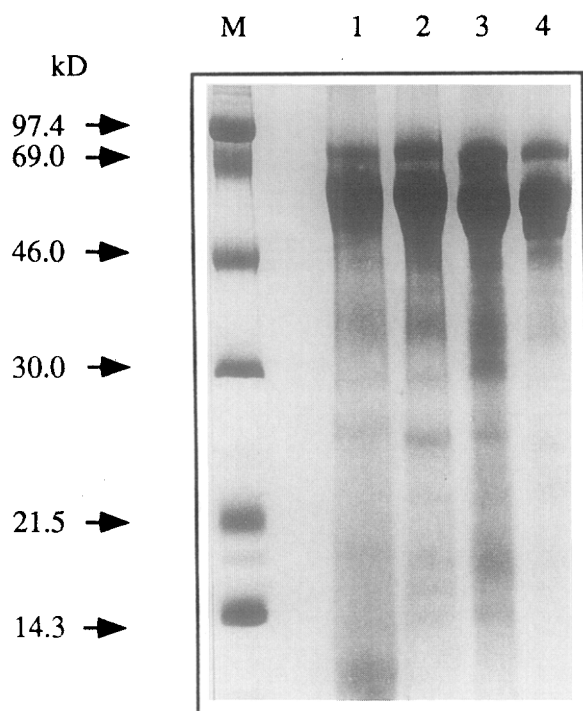


FIG. 1. Honey extracts: Coomassie brilliant blue R-stained 12% SDS-polyacrylamide gel shows the separation of four different types of honey: lane 1, FO; lane 2, CH; lane 3, SF; lane 4, LO; lane M, molecular weight marker (Amersham International, U.K.).

extracts at a molecular mass of 19 kd (Fig. 4, B; lanes 1, 2, 3, 14, 16, 19, 23).

IgE reactivity of sera from group II (patients allergic to bee venom). In the bee venom sac immunoblot, eight of 10 sera revealed a 33 kd band (Fig. 5; bee venom sac, lanes 1, 2, 3, 5, 7, 8, 9, 10). All sera showed IgE binding to a 19 kd protein, which according to literature represents phospholipase A₂ (PLA₂).²⁴ In addition, three blots exhibited a three-band pattern (44 kd, 50 kd, and 65 kd) (Fig. 5; lanes 7, 8, 10), three displayed IgE binding with a 44 kd/50 kd double band (Fig. 5; lanes 1, 2, 3), and one serum reacted with a 44 kd protein (Fig. 5; lane 5).

Seven of 10 sera bound IgE to three bands (46 kd, 54 kd, and 69 kd) of the SF extract (Fig. 5; SF, lanes 1, 2, 3, 5, 7, 8, 10). IgE reactivity to the same three bands was exhibited by pooled sera of group II (pool X) with the three other types of honey (Fig. 5; LO, CH, FO). Performing electrophoresis and immunoblotting with pool X under nondenaturing conditions revealed identical binding patterns (not shown).

Sera from the control group (three nonallergic patients) displayed IgE reactivity neither with SF

extracts nor with extracts of honeybee heads or bee venom sacs (Fig. 2; Fig. 4, A and B; and Fig. 5, lane N).

Immunoblots with antibodies to pollen allergens

Immunoblots were performed with BIP 1, MUP 4, MUP 8, MUP 20, and a rabbit anti-profilin antibody. Binding of these antibodies could not be observed with different honey extracts or with extracts of honeybee heads or bee venom sacs (data not shown).

IgE inhibition experiments

IgE binding to the three bands in SF was significantly inhibited by preincubation of serum pool A with 300 µg of honeybee head extract (Fig. 6; SF, lane 2) compared with control (Fig. 6; SF, lane 1). Similarly, preincubation of serum pool B with 300 µg of honeybee head extract abolished IgE binding to the structure in the SF immunoblot (Fig. 6; SF, lane 5). In contrast, preincubation of serum pool C with 300 µg of honeybee head extract had no effect on the IgE-binding properties to SF of this pool (Fig. 6; SF, lane 8). Sunflower extract inhibited, in all cases, IgE binding to SF extracts (Fig. 6, lanes 3, 6, 9).

Preincubation of serum pool B with 40 µg, as well as with 80 µg of SF extract, abolished IgE binding to the three bands in the honeybee head immunoblot (Fig. 6; CA, lanes 2, 3) compared with control (Fig. 6, lane 1). Preincubation with honeybee head extract inhibited IgE binding in the honeybee-head immunoblot (Fig. 6, lane 4).

Preincubation of serum pool X with 5 µg, 25 µg, and 125 µg, respectively, of SF extract also inhibited the IgE binding properties to the three bands correlative in the bee venom sac immunoblot (Fig. 6; lanes 2, 3, 4). IgE binding was blocked by preincubation with bee venom sac extract (Fig. 6; lane 5).

DISCUSSION

Allergy to honey can present a severe problem because systemic allergic reactions are not rare in persons allergic to honey.^{5, 7-10} Forty eight percent (11 of 23) of our patients exhibited anaphylactic symptoms after ingestion of small amounts of honey. And, insidiously, honey may be contained in hidden forms in various foods, such as chocolate bars, candies, and gingerbread.⁵

In the literature, allergy to honey is often attributed to the pollen content.⁸⁻¹¹ Various pollen proteins in different honey extracts would make

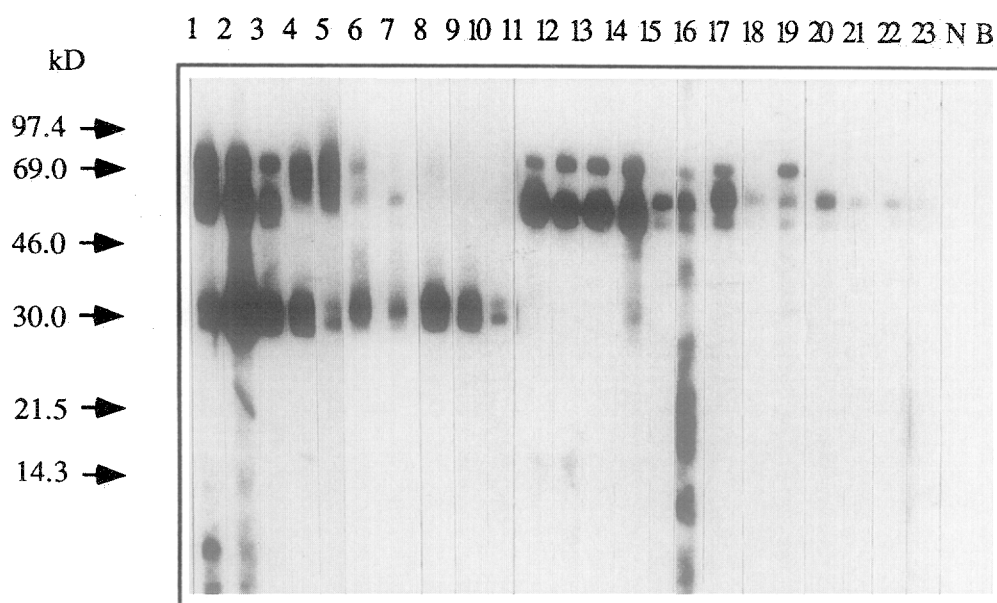


FIG. 2. Autoradiograph of IgE-immunoblot analysis of SF extract with sera from patients of group I. Lane N, Normal human serum pool; lane B, buffer control.

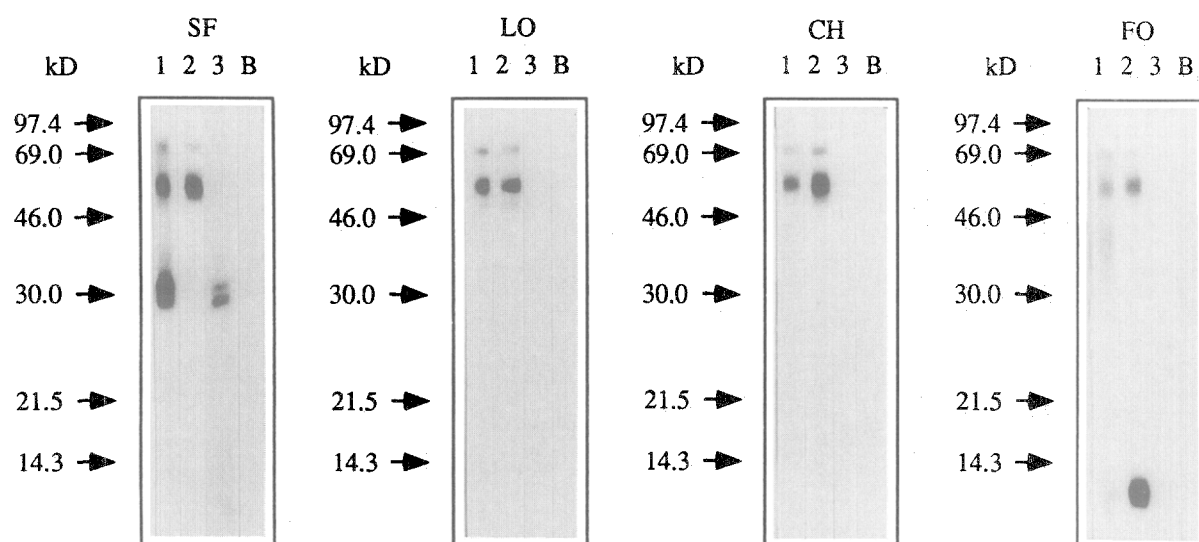


FIG. 3. Autoradiograph of IgE-immunoblot analysis of extracts of SF, LO, CH, and FO with serum pools from patients of group I. Lane 1, pool A; lane 2, pool B; lane 3, pool C; lane B, buffer control.

one expect a heterogeneous protein pattern. However, a Coomassie-stained SDS-polyacrylamide gel of separated proteins of different honeys (Fig. 1) revealed three bands at molecular masses of 54 kD, 60 kD, and 72 kD in all the types of honey tested, which strongly suggests them to be of bee origin.

The salivary and pharyngeal glands participating in honey production are mainly located in the honeybee heads.¹ To analyze the allergenic properties of their enzyme-rich secretions, we investi-

gated the IgE-binding properties of honeybee heads using sera from patients allergic to honey (Fig. 4, A). It was possible to show that all sera reacting with the three bands in the SF immunoblot (Fig. 2) exhibited IgE binding to proteins with molecular masses of 57 kD, 66 kD, and 80 kD in the honeybee head extract (Fig. 4, A). Cross-reactivity was confirmed in IgE inhibition experiments, inasmuch as IgE binding of patients allergic to honey to these three bands of sunflower extracts was

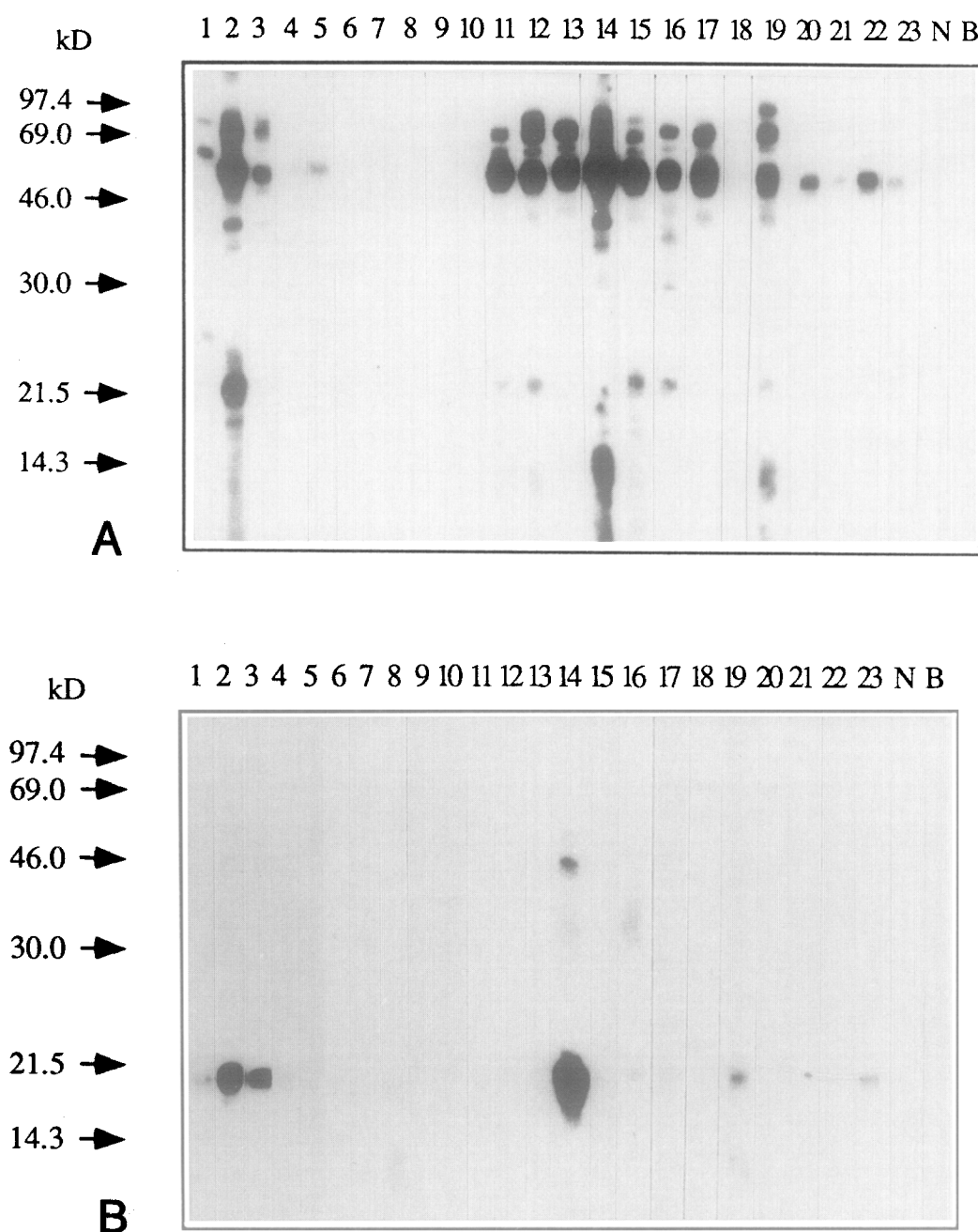


FIG. 4. **A,** Autoradiograph of IgE immunoblot analysis of honeybee head (CA) extract with sera from group I. **B,** Autoradiograph of IgE immunoblot analysis of bee venom sac (BV) extract with sera from group I. *Lane N*, Normal human serum pool; *lane B*, buffer control.

abolished after preincubation with honeybee head extract (Fig. 6). Eighty seven percent (20 of 23) of group I patients' sera displayed IgE reactivity to bands of the three-band complex (Fig. 2), which allocates a major role to bee-derived proteins.

Coomassie blue-stained SDS-PAGE-separated honey proteins exhibited a number of protein bands in addition to the three bands (Fig. 1).

Immunoblots of the four types of honey selected for this study, however, revealed only two additional IgE binding proteins: a 30 kd/33 kd double band was recognized by sera from pool A and pool C in the SF extract (Fig. 3; SF, lanes 1, 3). IgE binding to this double band was not reduced in IgE-inhibition experiments with CA extract (Fig. 6; SF, lane 8). Moreover, no corresponding allergen

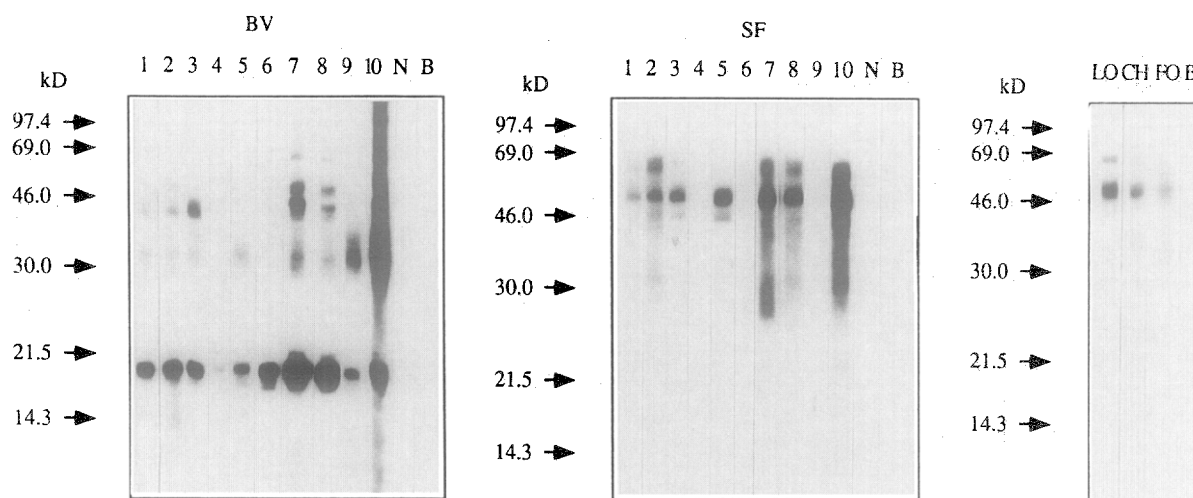


FIG. 5. Autoradiograph of IgE immunoblot analysis of extracts of bee venom sacs (BV), SF, and LO, CH, and FO with sera from group II. Lane N, Normal human serum pool; lane B, buffer control.

could be observed in the three other types of honey. This structure is therefore very likely to be of plant origin and sunflower-specific. In addition to the three-band structure, FO contained an 11 kd IgE-binding protein, which could represent an undefined pollen protein.

Forty-four percent (10 of 23) of the patients allergic to honey displayed IgE reactivity with the 30 kd/33 kd double band, and 9% (2 of 23) of the patients reacted with this structure exclusively. One could speculate that the latter was a collective of patients allergic to SF exclusively; that is, they could possibly eat other types of honey without problems. This was also suggested by Bousquet et al.⁹ in a case report on a beekeeper who had adverse reactions to one sort of honey exclusively.

Sensitization to common pollen-related allergens were of minor importance in our experiments. Four honey extracts (SF, LO, CH, FO) were tested in immunoblots with a monoclonal antibody (BIP 1) specific to the major allergen of birch pollen (Bet v 1)¹⁹ and with a serum pool with IgE antibodies to Bet v 1; SF and FO nitrocellulose strips were submerged with monoclonal antibodies (MUP 4, MUP 8, and MUP 20) specific for mugwort pollen allergens and a rabbit antibody reacting with profilin,^{22, 23} a plant pan-allergen. We found no binding of these antibodies to honey extracts, honeybee heads, or bee venom (data not shown). The particular grains of pollen admixed to the honey tested are generally rare candidates for allergic sensitization because they represent pollen of insect-pollinated plants. The portion of common pollen of wind-pollinated plants (mainly grasses,

weeds, and birch) was below 5% of the total pollen content, according to analysis of the honeys used in our study (data not shown). Surprisingly, 78% of our patients showed positive skin prick tests and RASTs to pollens from trees, grasses, and weeds, which seems to be of minor importance for the development of immediate-type reactions in patients allergic to honey but characterizes most of our patients as real "atopics." Fernández et al. described cross-reactivity between sunflower and mugwort pollen.¹¹ Indeed, in our experiments, all patients possessing IgE to mugwort pollen corresponding to RAST classes 3 and higher (data not shown) displayed IgE binding to sunflower-specific proteins in the SF immunoblot.

When looking at the IgE-binding pattern of patients allergic to bee venom in the SF immunoblot (Fig. 5), a relation between allergy to honey and allergy to bee venom became evident. This pattern was similar to the picture seen in the SF immunoblot with patients allergic to honey in pool B (Fig. 2). Seven out of 10 patients allergic to bee venom showing IgE binding to three bands of higher molecular mass in addition to the obligatory 19 kd band (PLA₂)²⁴ in the bee venom-sac immunoblot (Fig. 5) displayed a similar three-band pattern in the SF immunoblot (Fig. 5) that could be inhibited with SF extract (Fig. 6). Two of 23 patients allergic to honey reported type I allergic reactions after bee stings (patients 2 and 16). Indeed, all these patients displayed IgE binding to bee venom extracts (Fig. 4, B) and recognized PLA₂.

Aalberse et al.²⁵ have demonstrated that certain

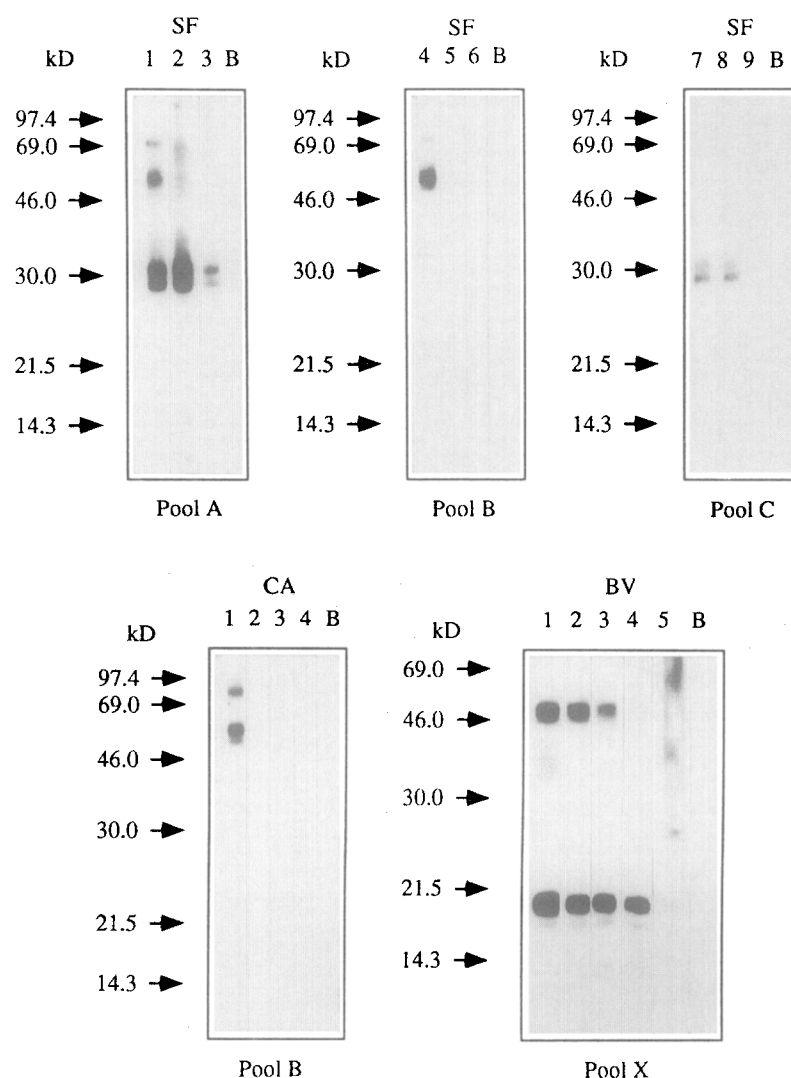


FIG. 6. Autoradiograph of IgE inhibition experiments performed with SF and CA extracts, respectively. Serum pool A, pool B, and pool C were preincubated with 0 μ g and 300 μ g of CA extract (SF; lanes 1, 2, 4, 5, 7, 8), and 80 μ g of SF extract (SF; lanes 3, 6, 9). Nitrocellulose-blotted sunflower honey proteins were incubated with this solution, and immunoblotting was performed. Serum pool B was preincubated with 0 μ g, 40 μ g, and 80 μ g of SF extract (CA; lanes 1 to 3) and 300 μ g of CA extract (CA; lane 4). Thereafter nitrocellulose-blotted honeybee head proteins were incubated with this solution, and immunoblotting was performed. Serum pool X was preincubated with 0 μ g, 5 μ g, 25 μ g, and 125 μ g of SF extract (BV; lanes 1 through 4) and 100 μ g of BV extract (BV; lane 5). Nitrocellulose-blotted honeybee sac proteins were incubated with this solution, and immunoblotting was performed. Lane B, buffer control.

cross-reactions involving bee venom were a result of carbohydrate determinants. In our study, however, cross-reacting structures detected by patients allergic to bee venom in extracts of bee venom, as well as of honey, were not due to carbohydrates because deglycosylation of bee head extract did not change IgE binding to the structure in the bee head immunoblot (data not shown). Moreover, in control experiments, we performed PAGE under nondenaturing conditions so we would not miss

any proteins in the bee head extracts that could possibly be undetected under denaturing conditions. The immunoblot picture obtained under nondenaturing conditions did not differ from the results in the SDS-PAGE system.

When put together, these results provide the following picture: bee products, as well as plant-derived proteins, play an important role in sensitization of patients allergic to honey. The risk of developing a bee venom allergy is very low consid-

ering the IgE reactivity to bee-derived proteins in honey. On the other hand, we could detect a high prevalence for specific IgE to honey in patients allergic to bee venom, although no adverse reactions after ingestion of honey have been reported in our group of patients allergic to bee venom.

REFERENCES

1. Zander E, Maurizio A. Der Honig. Herkunft, Gewinnung, Eigenschaften und Untersuchung des Honigs. 2nd ed. Stuttgart; Verlag Eugen Ulmer, 1975.
2. Stanley RG, Linskens HF. Pollen. Biology, biochemistry, management. Berlin: Springer Verlag, 1974.
3. Hausen BM, Wollenweber E, Senff H, Post B. Propolis allergy (I) origin, properties, usage and new literature review. *Contact Dermatitis* 1987;17:163-70.
4. Stüwe HT, Bruhn G, König WA, Hansen BM. The synthesis of caffeic acid esters, a new group of naturally occurring allergens. *Naturwissenschaften* 1989;76:426-7.
5. Helbling A, Peter C, Berchtold E, Bogdanov S, Müller U. Allergy to honey: Relation to pollen and honey bee allergy. *Allergy* 1992;47:41-9.
6. Hofer T, Wüthrich B. Nahrungsmittelallergien II. Häufigkeit der Organ-manifestationen und der Allergie-auslösenden Nahrungsmittel. *Schweiz Med Wochenschr* 1985;115:1437-42.
7. Strem E, Stoesser A. Does honey in infant feeding cause allergy? *Ann Allergy* 1958;375-9.
8. Birnbaum J, Tafforeau M, Vervloet D, Charpin J, Charpin D. Allergy to sunflower honey associated with allergy to celery. *Clin Exp Allergy* 1989;19:229-30.
9. Bousquet J, Campos J, Michel FB. Food intolerance to honey. *Allergy* 1984;39:73-5.
10. Helbling A, Wüthrich B. Ein ungewöhnlicher Fall von Honigallergie. *Allergologie* 1987;10:252-5.
11. Fernández C, Martín-Esteban M, Fiandor A et al. Analysis of cross-reactivity between sunflower pollen and other pollens of the Compositae family. *J ALLERGY CLIN IMMUNOL* 1993;92:660-7.
12. Reisman R. Insect allergy. In: Middleton E Jr, Reid CE, Ellis EF, Adkinson NF Jr, Yunginger JW, eds. *Allergy: principals and practice*. 3rd ed. St. Louis: CV Mosby, 1988;1345-58.
13. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
14. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
15. Jarolim E, Rumpold H, Endler AT, et al. IgE and IgG antibodies of patients with allergy to birch pollen as tools to define the allergen profile of *Betula verrucosa*. *Allergy* 1989;44:385-95.
16. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci* 1979;76:4350-4.
17. Ornstein L. Disc electrophoresis I. Background and theory. *Ann NY Acad Sci* 1964;121:321.
18. Davis BJ. Disc electrophoresis II. Method and applications to human serum proteins. *Ann NY Acad Sci* 1964;121:404.
19. Jarolim E, Tejkl M, Rohac M, et al. Monoclonal antibodies against birch-pollen allergens: characterisation by immunoblotting and use for single-step affinity purification of the major allergen Bet v 1. *Int Arch Allergy Appl Immunol* 1989;90:54-60.
20. Ebner C, Birkner T, Valenta R, et al. Common epitopes of birch pollen and apples: studies by Western and Northern blot. *J ALLERGY CLIN IMMUNOL* 1991;88:588-94.
21. Vallier P, Dechamp C, Valenta R, Vial O, Deviller P. Purification and characterization of an allergen from celery immunochemically related to an allergen present in several other plant species. Identification as a profilin. *Clin Exp Allergy* 1992;22:774-82.
22. Valenta R, Duchêne M, Ebner C, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;2:377-85.
23. Valenta R, Duchêne, Pettenburger K, et al. Identification of profilin as a novel pollen allergen: IgE autoreactivity in sensitized individuals. *Science* 1991;253:557-60.
24. Kemeny DM, Harries MG, Youlten LJ, Mackenzie-Mills M, Lessof MH. Antibodies to purified bee venom proteins and peptides I. Development of a highly specific RAST for bee venom antigens and its application to bee sting allergy. *J ALLERGY CLIN IMMUNOL* 1983;71:505-14.
25. Aalberse RC, Koshte V, Clemens JGJ. Immunoglobulin E antibodies that cross-react with vegetable foods, pollen and Hymenoptera venom. *J ALLERGY CLIN IMMUNOL* 1981;68:356-65.