

13. Brindley LL, Sweet JM, Goetzl EJ. Stimulation of histamine release from human basophils by platelet factor 4. *J Clin Invest* 1983;72:1218.
14. Shore PA, Burkhalter A, Cohn VH. A method of fluorometric assay of histamine in tissue. *J Pharmacol Exp Ther* 1959; 127:182.
15. Bergendorff A, Uvnas B. Storage of 5-HT in rat mast cell: evidence for anionic binding to carboxyl groups in a granule heparin-protein complex. *Acta Physiol Scand* 1972;84:320.
16. Schulman E, Lin MC, Proud D, McGlashan DW, Lichtenstein LM, Plaut M. Human lung macrophages induce histamine release from basophils and mast cells. *Am Rev Respir Dis* 1985;131:230.
17. Thueson DO, Speck LS, Lett-Brown M, Grant JA. Histamine-releasing activity (HRA). II. Interaction with basophils and physicochemical characterization. *J Immunol* 1979;123:633.
18. Lett-Brown MA, Thueson DO, Plank DE, Langford MP, Grant JA. Histamine-releasing activity (HRA). IV. Molecular heterogeneity of the activity from stimulated human thoracic duct lymphocytes. *Cell Immunol* 1984;87:434.
19. Alam R, Wyczolkowska J. Action of the human lymphokine histamine-releasing factor on mouse peritoneal mast cells. *Immunol Lett* 1985;11:111.
20. Findley SR, Lichtenstein LM. Basophil releasability in patients with asthma. *Am Rev Respir Dis* 1980;122:53.
21. Tung R, Lichtenstein LM. In vitro histamine release from basophils of asthmatic and atopic individuals in D₂O. *J Immunol* 1982;128:2067.
22. Kazimierczak W, Plaut M, Knauer KA, Meier HL, Lichtenstein LM. Deuterium oxide-induced histamine release from basophils of allergic subjects. I. Responsiveness to deuterium oxide requires an activation step. *Am Rev Respir Dis* 1984; 129:592.
23. Neijens HJ, Raatgep RE, Degenhart HJ, Duiverman EJ, Kerrebijn KF. Altered leukocyte response in relation to the basic abnormality in children with asthma and bronchial hyperresponsiveness. *Am Rev Respir Dis* 1984;130:744.
24. Arvilommi H, Rasanen L. Spontaneous lymphokine synthesis by human blood mononuclear cells. *Nature* 1975;257:144.
25. Wardley RC, Rouse BT, Babiuk LA. Lymphocyte activation by cell separation procedures. *Immunol Commun* 1976;5:637.
26. Parish WE. T-lymphocyte substances controlling eosinophilia. *Clin Allergy* 1982;12(suppl):47.
27. Hsieh K-H. Altered interleukin-2 production and responsiveness after hyposensitization to house dust. *J ALLERGY CLIN IMMUNOL* 1985;76:188.
28. Kus J, Tse KS, Vedal S, Chang-Yeung M. Lymphocyte subpopulation in patients with allergic and nonallergic asthma. *Clin Allergy* 1985;15:523.
29. Dorsch W, Hintschlich C, Neuhauser J, Weber J. Sequential histamine inhalations cause increased bronchial histamine reactivity in guinea pigs: role of platelets, thromboxanes, and prostacyclin. *Naunyn Schmiedeberg Arch Pharmacol* 1984; 327:148.
30. Creese BR, Bach MK. Hyperreactivity of airway smooth muscle produced in vitro with leukotrienes. *Prostaglandins Leukotrienes Med* 1983;11:161.
31. Lee TH, Austen KF, Corey EJ, Drazen JM. Leukotriene E₄-induced airway hyperresponsiveness of guinea pig smooth muscle to histamine and evidence for three separate sulfido-peptide leukotriene receptors. *Proc Natl Acad Sci USA* 1984; 81:4922.
32. Morley J, Page CP, Sanjar S. Pharmacology of the late response to allergens and its relevance to asthma prophylaxis. *Int Arch Allergy Appl Immunol* 1985;77:73.

Demonstration of spice-specific IgE in patients with suspected food allergies

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IgE against the spices coriander and/or curry, mace, celery, and white pepper was measured in 150 sera from patients with suspected food allergies. Fifteen percent of the 288 spice RASTs resulted in $\geq 10\%$ binding of ^{125}I -labeled anti-IgE. Twelve sera were studied in detail. In most sera IgE against various spices was present; all 12 sera contained IgE against mugwort-pollen extract. In RAST-inhibition experiments with three different sera, a cross-reactivity between mugwort pollen and coriander could be demonstrated. Leukocytes from two patients released histamine on incubation with spice extracts. This study suggests that IgE against various spices may play a role in some adverse reactions to food. (J ALLERGY CLIN IMMUNOL 1987;79:108-13.)

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Abbreviations used

PACM: Buffer containing piperazine-*N,N'*-bis (2-ethanesulfonic acid), albumin, calcium, and magnesium

CCD: Cross-reacting carbohydrate determinant

The ingestion of aromatic plants like celery can cause severe attacks of angioedema and urticaria in atopic patients.¹ Various authors¹⁻³ have observed an association between a positive skin test to mugwort-pollen extract and clinical reactions to celery. In sera from most patients with a positive skin test to celery, IgE against mugwort pollen can be demonstrated.⁴ With a new type of celery disc, IgE against celery could recently be demonstrated in 17 of 20 sera from patients with celery allergy.⁴ Peltre et al.⁵ demonstrated by immunoblotting techniques that common structures exist between IgE-binding antigens in celery and mugwort pollen.

We recently described a patient who developed occupational asthma on inhalation of dust from spices.⁶ In serum from this patient, high levels of specific IgE against coriander, curry, and other spices could be demonstrated.⁶ In this study we demonstrate that IgE against various spices can also be found in a substantial number of sera from patients with a suspected food allergy.

PATIENTS

Sera were obtained from patients who were referred to the Dijkzigt hospital for the allergologic evaluation of their symptoms of urticaria, angioedema, or eczema. Control sera from patients with pollinosis with positive RASTs for mugwort pollen and control sera with various total IgE levels were selected from sera and submitted for diagnostic IgE antibody determinations.

MATERIAL AND METHODS

Allergens

Powdered spices were obtained from a local grocery. The spices used were coriander (*Coriandrum sativum*), mace (*Myristica fragrans*), white pepper (*Piper nigrum*), and curry. Curry is a mixture of various spices. Its most important components are coriander, *Capsicum* peppers, and black pepper.⁷ Whole celery seed (*Apium graveolens*) was ground before use. Mugwort pollen (*Artemisia vulgaris*) was obtained from Pharmacia Diephuis, Groningen, Netherlands.

Estimation of total IgE and allergen-specific IgE

Total IgE was determined by a competitive binding assay (IgE radioimmunoassay, Pharmacia, Uppsala, Sweden).

Specific IgE against spices and mugwort pollen was determined by RAST as described previously⁶ with agarose beads as allergen support.⁸ In brief, 100 mg of spices or mugwort pollen was extracted with 2 ml of coupling buffer (0.1 mol/L NaHCO₃ and 0.5 mol/L NaCl, pH 8.5) for 1 hour at room temperature. The protein content of these allergen extracts, estimated according to the method of Schacterle and Pollack⁹ ranged from 1.3 to 1.9 gm/L. Protein from the complete supernatant was coupled to 100 mg of CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's instructions. Two milligrams per test of the various spice-Sepharose preparations and 4 mg per test of mugwort-pollen Sepharose, suspended in 0.5 ml of RAST buffer (0.82% NaCl, 10 mmol/L of phosphate, 0.1% NaN₃, 0.2% Tween 20, and 0.3% bovine serum albumin [Sigma Chemical Co., St. Louis, Mo.], pH 7.4), were incubated for at least 4 hours with 0.05 ml of patient serum. After four washes, radioiodinated rabbit antihuman IgE antibodies (Pharmacia) were added. After an overnight incubation and four washes, the bound radioactivity was counted in a Wallac (LKB Instruments, Bromma, Sweden) gamma counter. The results of the RAST were expressed as percentage binding of ¹²⁵I-anti-IgE relative to the total amount (= 100%) of ¹²⁵I-anti-IgE added. IgE against other food components was estimated with the use of Phadebas RAST discs (Pharmacia). RAST-inhibition experiments were performed as described previously.⁶

Histamine release from whole blood

Twenty milligrams of spices or pollen for use in the histamine release test was extracted for 1 hour in PACM buffer (25 mmol/L of piperazine-*N,N'*-bis[2-ethanesulfonic acid], 110 mmol/L NaCl, 5 mmol/L of potassium chloride, 1 mmol CaCl₂, 0.5 mmol MgCl₂, and 0.03% human serum albumin, pH 7.4). After centrifugation, tenfold dilutions of the supernatant in PACM buffer were incubated with heparin-anticoagulated blood according to the method of Siraganian and Hook.¹⁰ Histamine, released into the supernatant, was measured by an automated fluorometric technique.¹⁰ The amount of histamine, released into the supernatant, was expressed as percentage of the total amount of histamine present in the cell suspension.

RESULTS

IgE against coriander, curry, mace, celery, and white pepper

Since it became possible to measure specific IgE against various spices,⁶ sera from about 150 patients with a suspected food allergy have now been tested in one or more of the various spice RASTs. Table I illustrates that 15% of the 288 spice RASTs resulted in $\geq 10\%$ binding of ¹²⁵I-anti-IgE. A number of sera with $\geq 10\%$ binding of ¹²⁵I-anti-IgE in one or more of the spice RASTs was also tested for IgE against other spices. Table II illustrates that most sera contained comparable levels of IgE against the various spices. In sera from patients B, I, and L, however,

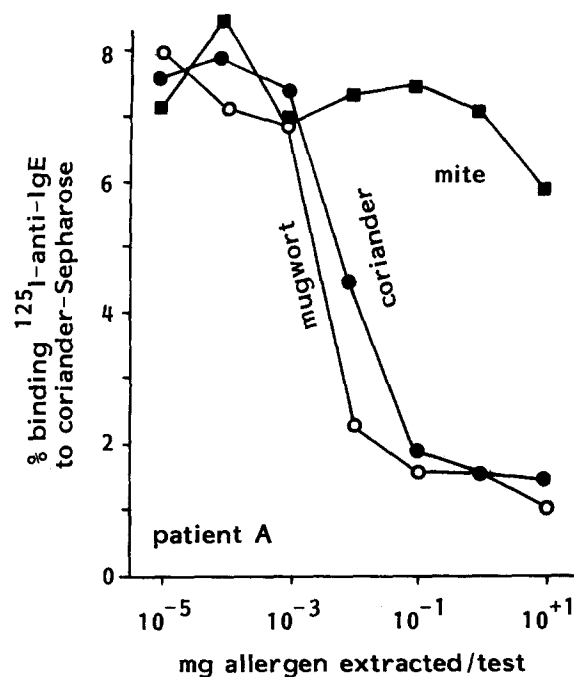


FIG. 1. Estimation of the cross-reactivity between coriander and mugwort-pollen extract by RAST inhibition; 50 μ l of serum per test from patient A was used.

IgE was preferentially directed against white pepper, mace, and coriander, respectively. In nine of the 12 sera from patients A through L (Table II), substantial ($\geq 10\%$ binding of ^{125}I -anti-IgE) levels of IgE against peanut, wheat, or hazelnut were present (data not presented). Control sera with high total IgE levels demonstrated negligible IgE binding to the solid phase-coupled spice or mugwort-pollen extracts (Table III).

The cross-reactivity between coriander and mugwort pollen

In all sera from patients A through L from Table II, IgE against mugwort pollen was found. In RAST-inhibition experiments with sera from patients A, D, and K, both mugwort-pollen extract and coriander extract were able to inhibit the coriander IgE RAST, whereas an extract of house dust mite had no such activity. Fig. 1 illustrates the results of the experiment with serum from patient A; similar results were obtained with sera from patients D and K. When 11 sera from patients with pollinosis with positive mugwort-pollen RASTs were tested in the coriander IgE RAST, only two sera were positive (Table IV). The case histories of these two patients revealed that patient 2 had eczema and patient 8 reported itching in nose and throat while salads or apples were eaten.

TABLE I. Results of 288 IgE antibody measurements arranged according to binding percentage of ^{125}I -anti-IgE

Binding ^{125}I -anti-IgE (%)	No. of specific IgE antibody measurements			
	Coriander	Mace	Curry	Celery
0 to 4	76	61	46	31
5 to 9	8	8	8	7
10 to 19	11	9	11	4
20 to 29	1	3	1	3

Histamine release from basophil leukocytes induced by spices

When whole blood from patients A and K, anti-coagulated with heparin, was incubated with extracts of various spices, $>50\%$ of the cellular histamine was released (Figs. 2 and 3). Extracts of white pepper and mugwort pollen did not release histamine from leukocytes of two normal donors. We have demonstrated before⁶ that extracts of coriander, curry, and mace do not release histamine from leukocytes of normal, non-allergic donors. Extracts, made by extracting 10 mg of spices or pollen in 1 ml PACM, contained histamine or histamine-like fluorescent material up to an amount of 20 ng of histamine per milliliter. The histamine-release data depicted in Figs. 2 and 3 were corrected for the histamine-like material, which was present in the allergen extracts. At 1 mg of spices or pollen extracted per milliliter in PACM, the amount of histamine-like material in the allergen extracts (~ 2 ng of histamine per milliliter) was negligible when the amount was compared with the total amount of histamine in the cell suspension (51 ng of histamine per milliliter for patient A in Fig. 2 and 94 ng of histamine per milliliter for patient K in Fig. 3).

DISCUSSION

Although many authors^{1-4,11} have described patients with an allergy to celery, it has been difficult to demonstrate specific IgE against celery by RAST.^{3,4} In these studies^{3,4} an extract of celery tuber was coupled to a solid phase; the present study demonstrates that celery seed is another suitable source of allergen. This investigation demonstrates that IgE against other spices can often be found in sera that contain IgE against celery. Two sera from Table II, however (sera from patients I and L), were negative in the celery RAST. Although most sera from patients in Table II contained comparable levels of IgE against the various spices, IgE in sera from patients B, I, and L was

TABLE II. Food RAST percent binding of ^{125}I -anti-IgE

Patient	Coriander	Curry	Mace	Celery	White pepper	Mugwort	Total IgE (IU/ml)	Symptoms
A	9	11	6	4	8	12	25	A(e), U(e)
B	8	5	3	8	18	21	100	E
C	15	17	12	16	12	19	580	E
D	17	15	14	9	ND	20	185	A, U
E	12	19	19	21	ND	13	250	A(e), U(e)
F	13	14	8	15	ND	20	2000	E
G	23	25	22	25	ND	25	1650	E
H	14	14	13	13	ND	27	1450	U(e)
I	1	5	18	2	ND	12	180	A, U
J	12	15	11	7	ND	10	190	A, U
K	14	18	19	20	ND	21	1450	E
L	11	5	1	2	ND	21	750	A

ND = not done; A = angioedema; (e) = exercise induced; E = eczema; U = urticaria. Twelve sera in which IgE against one or more spices were found were also tested for IgE against other spices.

TABLE III. Percent binding of ^{125}I -anti-IgE to agarose-coupled spice and pollen extracts without serum (blanc) and with control sera with high total IgE levels (between 1000 and 2500 IE/ml)

	Blanc	n	Control sera high total IgE	n
Coriander	0.8 (0.2)	12	1.7 (0.9)	9
Curry	0.9 (0.3)	12	2.0 (0.6)	6
Mace	0.3 (0.2)	8	1.4 (1.0)	7
Celery	0.6 (0.1)	6	2.2 (1.0)	6
White pepper	0.2 (0.1)	5	0.8 (0.5)	4
Mugwort pollen	1.6 (0.6)	13	2.2 (0.6)	10

The standard deviations are presented in parentheses.

preferentially directed against white pepper, mace, and coriander, respectively. This indicates that the various spices contain different IgE-binding antigens. The presence of another structure, common to all these spices and mugwort pollen, is suggested by the observation that all sera from patients A through L (Table II) contained IgE against mugwort pollen. Indeed, with all three sera tested, mugwort-pollen extract could inhibit the coriander IgE RAST. In contrast, IgE from a patient with occupational asthma, induced by inhalation of dust from spices, was directed against structures in coriander extract that did not cross-react with mugwort-pollen extract.⁶ This suggests that the IgE response to inhaled spices differs from the IgE response to ingested spices.

Thiel et al.¹⁴ reported that 14% of the patients with an allergy to spices was nonallergic to mugwort or wormwood (*Artemisia absinthium*) pollen.

TABLE IV

RAST % binding ^{125}I -anti-IgE			
Patient	Mugwort pollen	Coriander	Total IgE (IU/ml)
1	12	4	285
2	20	13	1100
3	12	5	185
4	17	1	303
5	23	4	620
6	13	3	255
7	12	1	185
8	13	10	305
9	12	2	55
10	19	5	901
11	29	1	160

Sera from patients with pollinosis, in which IgE against mugwort pollen was found, were also tested for IgE against coriander.

All sera of patients from Table II with IgE against one or more spices also contained IgE against mugwort pollen; the reverse, however, was not true (Table IV). A similar pattern of cross-reactivity between IgE-binding antigens from birch pollen and antigens from fruits and vegetables was recently described by Halmeppuro et al.¹² These authors concluded that all immunologic determinants in fruits and vegetables appeared to be present also in birch pollen, but all birch pollen determinants were not present in fruits and vegetables. Aalberse et al.¹³ described cross-reactivity between vegetable food and grass pollen caused by a CCD. Aalberse et al.¹³ suggested that IgE against CCD was clinically insignificant. Seven serum donors with

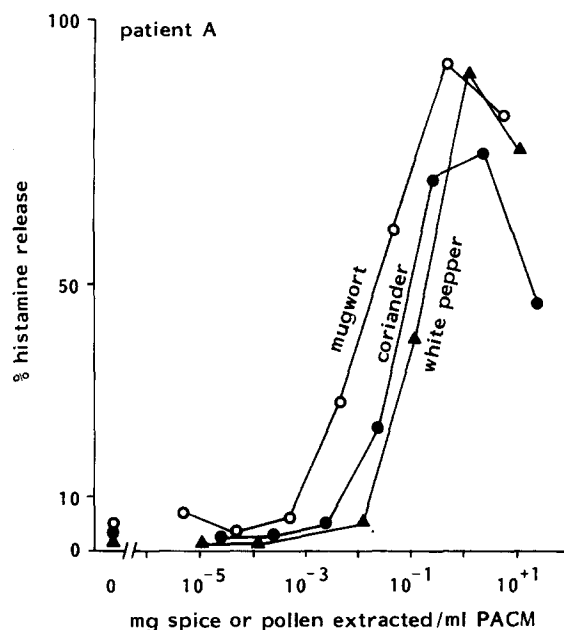


FIG. 2. Incubation of whole blood from patient A with extracts of mugwort pollen, coriander, and white pepper.

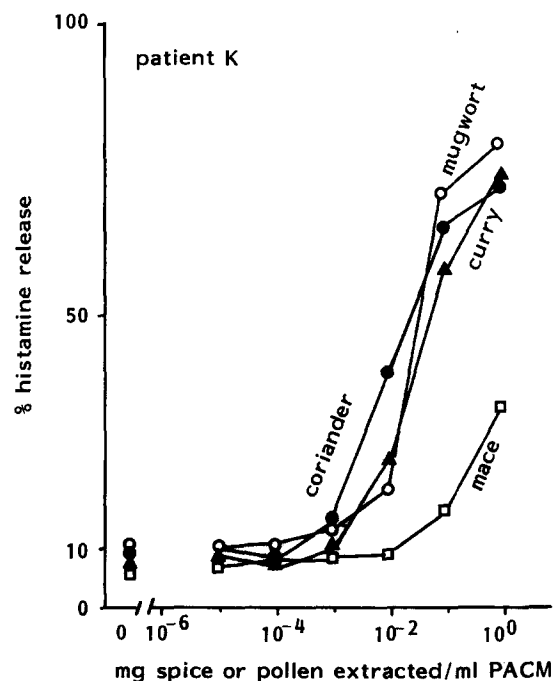


FIG. 3. Incubation of whole blood from patient K with extracts of mugwort pollen, coriander, curry, and mace.

anti-CCD IgE denied symptoms when they were eating vegetables or fruits. The results of the histamine release test (Figs. 2 and 3), however, demonstrated that IgE-binding antigens from spices are biologically active and able to cross-link basophil-bound IgE molecules.

Most patients with IgE against one or more spices also had IgE against other food allergens like peanut, hazelnut, or wheat. This makes it difficult to evaluate the clinical significance of spice-specific IgE in these patients.

Three patients from Table II (patients A, E, and H) only experienced symptoms during exercise. Similarly, Kidd et al.¹⁵ described two patients with a history of generalized urticaria, weakness, nausea, and throat tightness associated with exercise only if it occurred 2 hours after the ingestion of celery. Ingestion of celery without subsequent exercise elicited no symptoms.¹⁵

Spices like mace and coriander are only minor components of food and may be easily overlooked in histories of adverse reactions to food. Our study demonstrates that IgE against spices can be elicited in many sera from patients with a suspected food allergy.

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REFERENCES

1. Kauppinen K, Kousa M, Reunala I. Aromatic plants—a cause of severe attacks of angioedema and urticaria. *Contact Dermatitis* 1980;6:251-4.
2. Thiel CI, Fuchs E. Über korrelative Beziehungen bei Kräuterpollen und Gewürzallergenen. In: Steigleder GK, ed. *Köln RAST symposium 3*. Berlin: Grosse Verlag, 1981:161-5.
3. Wüthrich B, Hofer T. Food allergy: the celery-mugwort-spice syndrome. *Dtsch Med Wochenschr* 1984;109:981-6.
4. Pauli G, Bessot JC, Dietemann-Malarid A, Braun PA, Thierry R. Celery sensitivity: clinical and immunological correlations with pollen allergy. *Clin Allergy* 1985;15:273-9.
5. Peltre P, Pauli G, Ville G, David B. Simultaneous occurrence of IgE antibodies to ragweed pollen and to celery root in patients' sera [Abstract]. *Folia Allergol Immunol Clin* 1983; 30(suppl 4):84.
6. van Toorenenbergen AW, Dieges PH. Immunoglobulin E antibodies against coriander and other spices. *J ALLERGY CLIN IMMUNOL* 1985;76:477-81.
7. Stobart T. *Herbs, spices, and flavorings*. London: Penguin Books, 1977:98-101.
8. Adkinson NF. Measurement of total serum immunoglobulin E and allergen-specific immunoglobulin E antibody. In: Rose NF, Friedman H, eds. *Manual of clinical immunology*, 2nd ed. Washington, D.C.: American Society for Microbiology, 1980:794-807.
9. Schacterle GR, Pollack RL. A simplified method for the quantitative assay of small amounts of protein in biological material. *Anal Biochem* 1973;51:654-5.
10. Siraganian RP, Hook WA. Histamine release and assay methods for the study of human allergy. In: Rose NF, Friedman H, eds. *Manual of Clinical Immunology*, 2nd ed. Washington, D.C.: American Society for Microbiology, 1980:808-21.

11. Forsbeck M, Ros AM. Anaphylactoid reaction to celery. *Contact Dermatitis* 1979;5:191.
12. Halmepuro L, Vuontela K, Kalimo K, Björkstén F. Cross-reactivity of IgE antibodies with allergens in birch pollen, fruits, and vegetables. *Int Archs Allergy Appl Immunol* 1984;74:235-40.
13. Aalberse RC, Koshte V, Clemens JGJ. Immunoglobulin E antibodies that cross-react with vegetable foods, pollen, and Hymenoptera venom. *J ALLERGY CLIN IMMUNOL* 1981;68:356-64.
14. Thiel CL, Fuchs E, Kalveram HJ, Foreck G. Über korrelative Beziehungen bei Kräuterpollen und Gewürzallegenen. Zweite Mitteilung. In: Steigleder GK, ed. *Kölner RAST symposium*. Karlsruhe: Braun Verlag, 1984:118-27.
15. Kidd JM, Cohen S, Sosman A, Fink J. Food-dependent exercise-induced anaphylaxis. *J ALLERGY CLIN IMMUNOL* 1983; 71:407.

Sensitivity of immunoassays for detecting cross-reactivity of homologous venom proteins of yellow jackets

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The venoms of yellow jackets of Vespula flavopilosa, V. pennsylvanica, V. squamosa, and V. vulgaris have similar proteins. Their major components comprise antigen 5, hyaluronidase, and phospholipase A₁. The homologous venom proteins share very similar biochemical properties. With the exception of antigen 5 and phospholipase of V. squamosa, they also have very similar antigenic properties. The venom proteins of V. squamosa and V. vulgaris were equally effective in inducing secondary antibody responses in mice that were primed with V. squamosa proteins. No cross-reaction of V. squamosa and V. vulgaris venom proteins was detected on immunodiffusion with specific mouse antisera. A very weak cross-reaction was detected by inhibition of ELISA, and a moderate cross-reaction was detected by direct ELISA. The varying cross-reactivity is a consequence of the different sensitivities of the assays. The sensitivity of the direct ELISA is apparently caused by a greater enhancement of the binding of low-affinity antibodies for the solid-phase antigen than that of high-affinity antibodies. Most untreated yellow jacket-sensitive patients tested had about tenfold higher levels of IgG specific for V. vulgaris proteins than those specific for V. squamosa proteins. This pattern of antibody specificity can be accounted for on the basis of cross-reactivity of these yellow jackets as suggested by the results with the mouse system. (J ALLERGY CLIN IMMUNOL 1987;79:113-20.)

For the diagnosis and treatment of insect allergy to hornets, yellow jackets, and wasps, it is useful to know the extent of immunologic cross-reactivity of their venom proteins. These insect venoms have similar protein compositions and are known to be allergens in man. Three of the venom proteins are hyaluronidase, phospholipase A₁, and antigen 5 of as yet unknown biochemical function. The homologous

proteins of these insects are found to have varying extents of cross-reactivity.¹⁻⁴

I will describe in this article the findings on the cross-reactivity of venom proteins of four species of yellow jackets, *Vespula flavopilosa*, *V. pennsylvanica*, *V. squamosa*, and *V. vulgaris*. They differ widely in their geographic distribution in North America.⁵ Other investigators have reported conflicting results on the cross-reactivity of these species of yellow jackets. One group reported that the venom of *V. squamosa* is distinct from the venoms of other species of yellow jackets when the venom is tested by immunodiffusion with rabbit antisera specific for *V. maculifrons* venom,⁶ but another group reported that the venom proteins of *V. squamosa* are antigenically re-

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