

lower degree of identity was found between mugwort and chrysanthemum to ragweed, sea startwort, goldenrod and camomile (Table 1). Using RAST inhibition Leiferman et al. (2) have obtained similar results.

No absence of the reaction of immunochemical identity or partial identity between any antigens extracted from different grass pollens and investigated by these methods was observed, but considerable quantitative variations occurred. Thus, antigens of whole pollen extract from the following grasses: red top, meadow foxtail, sweet vernal grass, false oat grass, orchard grass, meadow fescue, velvet grass, rye-grass, reed, timothy, blue grass, were all partially identical to antigens of false oat grass, orchard grass, rye-grass and timothy, as shown by CLIE. Extracts and antibodies against these last antigenic preparations were all prepared as described in (4). Similar results have been obtained by Tangen & Nilsson (6) and Leiferman and Gleich (3) using RAST inhibition.

In a recent study (5) crossed immunoelectrophoresis in various modifications has directly revealed the immunological partial identity between isolated major allergens of timothy pollen (antigens 19 and 25) and the corresponding pollen allergens of false oat, orchard and rye grass, using rabbit antibodies against all four grasses. The inhibitory effects of antigens 19 and 25 relative to those of whole pollen extracts have been determined by means of RAST, inhibiting serum pools from selected grass-pollen-allergic patients and using insolubilized whole pollen extracts from the four grasses as allergosorbents. The inhibitory effects of antigens 19 and 25 were determined to be 97, 91, 92 and 73% of those of whole extracts of timothy, false oat, orchard and rye grass, respectively.

These results are in general agreement with botanical knowledge of relationship between different plants and with clinical experience. The significance of such investigations is not only of academic interest but of practical value for the evaluation of test results. A high degree of partial identity means that only a few extracts should be necessary; a low degree (less than 90%) that several extracts are necessary to cover all patients' individual responses. If a patient reacts to say birch, hazel

and alder, it should preferably be checked if this is due to the same or different allergens before starting a desensitization. For this purpose CRIE or RAST inhibition could be used.

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Immunochemical Studies of Hymenoptera Venom Allergens

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The most common insect sting allergies in the United States are those caused by bees and vespids. The vespids include hornets, yellowjackets and wasps. Some reports claim that 0.4-0.8% of the population of the United

States is sensitive to these insects. In the past diagnosis and treatment of insect allergy have been commonly carried out with whole-body insect extracts. In recent years there has been a renewed interest in the use of

appropriate insect venoms for diagnosis and treatment of such allergies (4).

Undoubtedly the best studied hymenoptera venom is honey bee venom which has been exhaustively characterized for its peptide and protein components by Habermann and his co-workers (1). Over 75% of the dry weight of bee venom can be accounted for as histamine, peptides and proteins. The most abundant component, melittin, is a hemolytic peptide of 2840 daltons, accounting for 50% of venom weight. The most abundant protein component is phospholipase of 15 800 daltons, accounting for 12% of venom weight. The other three known components include one protein, hyaluronidase of 50 000 daltons, one neurotoxic peptide, apamin of 2038 daltons, and one mast cell degranulating peptide; each of these three components accounts for 2% of venom weight. With the exception of hyaluronidase, the amino acid sequences of these peptides and proteins have been determined.

We have studied the allergenic activities of four of these venom components, apamin, melittin, hyaluronidase and phospholipase, by the method of histamine release from leukocytes of seven sensitive persons (2). Apamin was completely inactive. The most abundant protein, phospholipase, is clearly the major allergen since it was about 3–12 times more active than the venom in six out of seven patients; only in one patient was phospholipase half as active as the venom. The less abundant hyaluronidase was 4–90 times more active than the venom in three of the seven tested, and it was 0.6–1 times as active as the venom in the remaining patients. Although melittin is the most abundant component, it is a weak minor allergen, probably because of its small size. The average allergenic activities of phospholipase, hyaluronidase and melittin are, respectively, about 5, 3 and 0.05 times that of venom. The data also show the variable responses of different individuals to different antigens. For example, one patient was highly reactive to phospholipase but poorly reactive to hyaluronidase, while the reverse was true for another patient, and a third patient was highly reactive to both of these components.

Individuals not only exhibit variable responses to different antigens, but they can probably also exhibit variable responses to different antigenic determinants of the same allergen molecule. This is suggested by studies of chemically modified derivatives of bee venom phospholipase (2). Succinylation of eight of the 11 ϵ -amino groups of phospholipase gave a derivative which was about half as active as the native antigen in two of the five patients tested, but about 1/100 as active in the remaining three patients. A similar finding was made with a derivative obtained on CNBr cleavage of the three methionyl residues of phospholipase. If the five patients tested had identical IgE antibody responses to the different determinants of phospholipase, one would have expected uniform decreases of the allergenic activity of the modified phospholipase in these patients.

Compared to bee venom, relatively fewer bio-

chemical studies have been carried out with vespid venoms. Our own studies (3) on the protein components of white-faced hornet (*V. maculata*), yellow hornet (*V. arenaaria*) and yellowjacket (*V. maculiformis*, *V. vulgaris* and *V. germanica*) venoms showed that these three vespid venoms have very similar protein compositions. The yellowjacket venom we used was a mixture from three species, as indicated. We did this because that was the material available at the time of our studies.

All three vespid venoms contain hyaluronidase and phospholipase, which can be detected by their enzymic activities. In addition, all three vespid venoms contain a major component, antigen 5, which is a basic protein of unknown biochemical function and which can be detected with rabbit specific anti-sera. These three components are separable by a combination of Sephadex gel and cellulose ion exchange chromatographies.

The molecular weights of vespid venom hyaluronidase, phospholipase and antigen 5 are, respectively, about 45 000, 35 000 and 25 000, and they represent 2, 0.4 and 15% of venom proteins and peptides. Like bee venom, the vespid venoms have hyaluronidase as a minor component. But, unlike in bee venom, phospholipase is a minor component of the vespid venom. Antigen 5, which has no counterpart in bee venom, is the major component of vespid venom.

Allergenic activity assays by histamine release from leukocytes of sensitive donors showed that these three antigens are all active as allergens, but that antigen 5 is clearly the major allergen.

Immunodiffusion studies with rabbit anti-venom specific sera have shown that there is no cross reaction between vespid and bee venom antigens. However, there is cross reaction between similar antigens of the three vespids. For example, antigen 5 from white-faced hornet formed precipitins with anti-white-faced hornet venom sera as well as with anti-yellow hornet and anti-yellowjacket venom sera. Antigen 5 from yellow hornet formed precipitin with anti-yellow hornet and anti-yellowjacket sera, but not with anti-white-faced hornet sera. Antigen 5 from yellowjacket formed precipitins only with its own specific sera. In addition to these immunological cross reactions, the three antigen 5s showed very similar amino acid compositions.

Similar immunodiffusion studies have shown that hyaluronidases from the three vespids exhibited even greater cross reaction than antigen 5s, while phospholipase from the three vespids showed less cross reactions. These findings help to explain the observation that a majority of vespid venom sensitive individuals showed multiple sensitivity to different vespid venom antigens. That is to say that a person who is sensitized to only one vespid venom on natural exposure can show varying degrees of reactivity to other vespid venoms. This phenomenon appears to be a consequence of multiple allergens in each venom which are immunologically cross reactive and to which people develop sensitivity of varying degrees.

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Effect of Polymerization and Modification on the Immunochemical Reactivity of Cod Allergen M

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Allergen M belongs to a large group of fish muscle calcium binding proteins (parvalbumins), characterized by their ability to bind 2 mol Ca^{2+} /mol protein. The primary structure of Allergen M as well as several other members of parvalbumins has been established (2). The two Ca^{2+} -coordinations as well as the internal salt bridge between Arg-75 and Glu-81 are essential for the stability of the molecular conformation (3). An extensive cross reactivity was demonstrated between the two polypeptide fragments TM 1 and TM 2 (residues No. 1–75 and 76–113, respectively) (1). The findings could be interpreted in terms of a three-domain molecular model

similar to that postulated for carp pI 4.25 (3). Allergen M was accordingly suggested to have an analogous domains arrangement as predicted by the high sequence homology of the two molecules. The amino acid inter-periodicity of the three domains suggested that the immunochemical reactivity must be effected by repetitions of the active sequence within the Allergen M.

This abstract summarizes the effects of amino-acid modification and polymerization of Allergen M and the fragments on the activity.

The modification of the side chains of the selected amino acids are cited in Table 1. The relative elution

Table 1

Comparison of the main data on immunochemical reactivity, electrophoretic mobility, and gel filtration for the various amino acid modifications of Allergen M. A close agreement exists between the tests of IgE mediated reactivity (RAST and RAST inhibition) and IgG reactivity (Farr test and precipitation tests)

Derivative	Direct RAST (c.p.m.)	(%)	RAST inhibition (%)	Farr test (%)	Max. immune- PPT. (Turbid- ity, 340 nm)	Electro- phoretic mobility (mm/30 min)	Relative elution volume
TFA-Lys-All M	6682	48	30	61	0.42	19	0.41
Acetyl-Tyr-All M	7486	54	30	62	0.47	20	0.29
1, 2-CHD-Arg-All M	8865	64	57	51	0.52	12	0.38
EDTA-All M	9756	71	n.d.	n.d.	0.66	13	0.31
Oxindole-Tyr-All M	10 773	78	74	50	0.73	12	0.28
-Amidine-All M	11 357	83	63	n.d.	0.74	16	0.28
Native All M	13 741	100	80	31	0.73	10	0.28

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