

Clinical and immunological studies of beekeepers

WILMA C. LIGHT, ROBERT E. REISMAN, JOHN I. WYPYCH
and CARL E. ARBESMAN

*Allergy Research Laboratory of the Buffalo General Hospital and the Departments
of Medicine and Microbiology of the State University of New York at Buffalo,
Buffalo, New York*

Summary

Thirty-four beekeepers were interviewed and their blood assayed for the presence of antibodies reacting with bee venom, bee venom phospholipase A (PLA), and whole bee body extract. Following a bee sting, most beekeepers experienced only minimal local tissue reaction. Their serum contained high levels of total antibodies (primarily IgG) reacting to bee venom and phospholipase A. These antibody titres correlated with the frequency of bee stings.

Bee venom and PLA specific IgE antibodies were present in serum of some beekeepers. Beekeepers who had experienced allergic reactions were characterized by low total antibody and high venom specific IgE antibody titres. Bee body IgE antibodies were found in varying degree and did not correlate with levels of venom IgE antibodies. There was no difference in the titres of bee body IgE in the sera of beekeepers with and without systemic reactions.

The data suggest that allergic reactions are mediated by venom specific IgE and immunity is at least in part a function of other antibodies, probably primarily IgG.

Introduction

As one facet of our studies of the problems of allergic reactions due to stinging insects, a clinical and immunological investigation of beekeepers was conducted. In a sense, beekeepers represent the antithesis of insect allergic patients. Although beekeepers frequently receive multiple stings, they typically develop only minimal local swelling, to a degree less than would 'normally' be expected. Studies of this 'immune' population were undertaken in the hope that practical information might be obtained which could be applied to the therapy of the stinging insect allergic individual.

Materials and methods

Clinical data

Thirty-four beekeepers were interviewed. The clinical data obtained included the

Correspondence: Dr Carl E. Arbesman, Allergy Research Laboratory, Buffalo General Hospital, 100 High Street, Buffalo, New York 14203, U.S.A.

following: number of years of beekeeping, numbers of stings per week, incidence of any untoward reactions following stings, the presence of personal atopic disease, and the occurrence of stinging insect allergy in family members.

Immunological studies

Serum was obtained from the thirty-four beekeepers during the summer months, at which time bees were active. The serum was stored at 4°C until examined.

Antigens. Bee venom was obtained from Sigma Chemical Company.* Bee venom phospholipase A was prepared by Dr Willard Elliott (Shepherd, Elliott & Arbesman, 1974) and was relatively free of other minor bee venom components. A 15% whole bee body extract was prepared as previously described (Shulman, Langlois & Arbesman, 1964).

Specific IgE antibodies. The disc radioallergosorbent test (RAST) as modified in our laboratory (Ceska, Eriksson & Varga, 1972; Arbesman, Wypych & Reisman, 1974) was used to determine the presence of specific IgE antibodies reacting with bee venom, phospholipase A and whole bee body extract. Two hundred activated cellulose discs were coupled with either 20 mg of bee venom, 1 mg of phospholipase A, or 300 mg of whole bee body extract.

Total serum antibodies. Total antibodies to bee venom and bee phospholipase A were determined by a competitive radioimmunoassay method developed in this laboratory (Fujita *et al.*, 1975). The majority of antibody detected by this technique is IgG.

Results

Clinical data

Beekeepers varied widely in the number of estimated stings sustained during the season reporting from one sting per year to 225 stings per week. The clinical and immunological data were analysed in relationship to the sting frequency (Tables 1 and 2). Twelve beekeepers received ten or more stings per week during the season, thirteen beekeepers received less than ten stings per week and nine beekeepers were inactive.

Active beekeepers had been engaged in this activity for many years. Inactive beekeepers generally had been involved for shorter time periods and their major limiting factor had been the occurrence of reactions to stings. Three individuals had marked local reactions and six individuals had systemic reactions following bee stings.

Ten out of twenty-seven beekeepers reported that family members had had either systemic or marked local reactions after bee stings. Three beekeepers had a personal history of either hay fever or asthma.

Immunological studies

Total antibodies to bee venom or bee venom phospholipase A (PLA) in the serum of beekeepers ranged from 0.01 µg/ml to 500 µg/ml. The group of beekeepers who received ten or more stings per week had significantly greater levels of these total antibodies when compared to either beekeepers with fewer stings per week, inactive beekeepers, insect sensitive patients or control, non-allergic individuals (Table 2).

* Sigma Chemical Company, St Louis, Missouri.

Table 1. Beekeepers' clinical data. Beekeepers were divided into three groups depending upon sting frequency

	Beekeepers		
	Greater than ten stings/week	Less than ten stings/week	Inactive no. stings
No.	12	13	9
Years beekeeping			
Mean	35	15	12
Range	4-55	1-55	2-43
Reactions			
Systemic	0	0	6
Local	0	0	3
Atopy	0	1	2
Family member with bee sting reaction	3	4	3

Table 2. Beekeepers' immunological data. Beekeepers were divided into three groups, depending upon sting frequency. A fourth group of insect allergic individuals who had systemic reactions following a single bee sting, is used for comparison

Group	Beekeepers			Untreated insect allergic patients
	Greater than ten stings/week	Less than ten stings/week	Inactive no. stings	
No.	12	13	9	18
Total anti bee venom antibody ($\mu\text{g/ml}$)	160 (8-500)	58 (0.18-350)	38 (0.6-300)	34 (0.01-210)
Total anti PLA antibody ($\mu\text{g/ml}$)	129 (2.5-500)	46 (0.03-400)	16 (0.5-80)	15 (0-87.5)
Specific IgE antibody*				
Bee venom	6 (0-52)	3 (0-9)	48 (0-121)	199 (12-876)
PLA	18 (0-124)	9 (0-28)	46 (0-115)	257 (0-1505)
Whole bee body extract	22 (0-102)	10 (0-48)	25 (3-63)	168 (3-930)

* Expressed as RAST %; levels greater than 25% are considered significant.

Among beekeepers stung more than ten times per week, a direct correlation was found between the number of stings and the level of total antibodies. (For bee venom antibodies, $r = 0.722$, $P < 0.01$ and for PLA antibodies $r = 0.628$, $P < 0.05$) (Figs 1 and 2).

Bee venom specific IgE and PLA specific IgE antibodies were found in the sera of a number of beekeepers. Highest levels were found in beekeepers who had had allergic reactions. Much lower levels were present in other beekeepers regardless of sting frequency. Similarly whole bee body IgE antibodies were found in beekeepers but in lower levels than in insect allergic individuals. Interestingly, beekeepers with

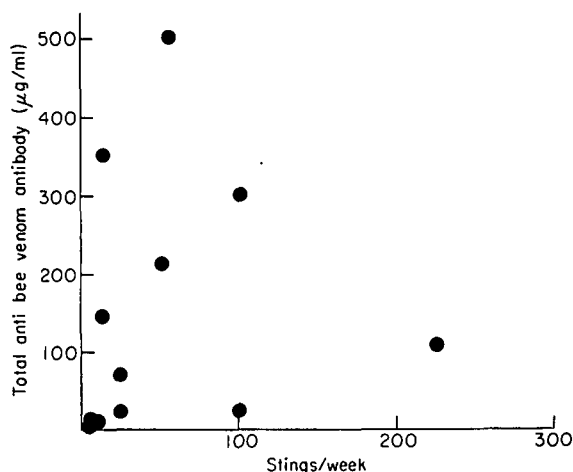


Fig. 1. Relationship of total antibody to bee venom in sera of beekeepers and estimated number of bee stings per week. Total antibody is expressed in $\mu\text{g/ml}$.

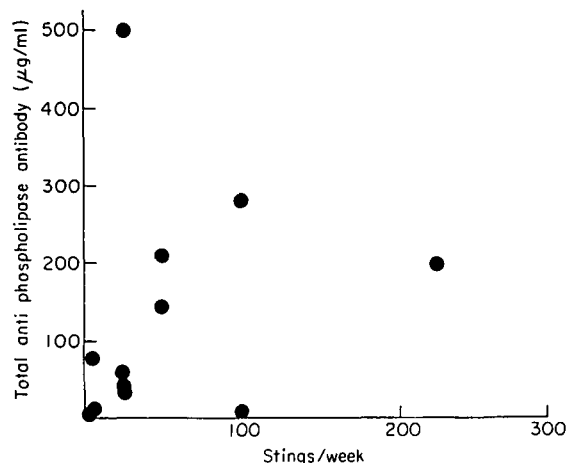


Fig. 2. Relationship of total antibody to phospholipase A in sera of beekeepers and estimated number of bee stings per week. Total antibody is expressed in $\mu\text{g/ml}$.

reactions did not differ from other beekeepers with respect to the whole bee body IgE specific antibodies.

There was a significant correlation ($z = 3.96$) between bee venom specific IgE antibodies and phospholipase specific IgE antibodies (Fig. 3). There was no significant correlation between levels of bee venom and whole bee body IgE antibodies (Fig. 4).

One of the frequently stung beekeepers had high levels of IgE antibodies. Total antibodies were also elevated and presumably responsible for his immune status (Table 3).

In beekeepers with reactions, or insect sensitive patients, the proportion of total antibody relative to the specific IgE antibody is much lower.

Discussion

From a clinical viewpoint beekeepers represent an immune group of individuals who

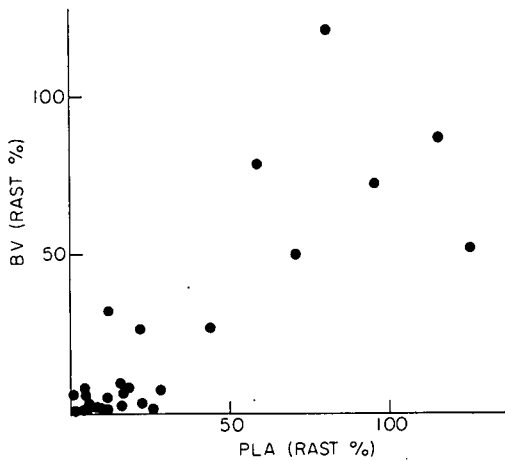


Fig. 3. Relationship of bee venom (BV) and phospholipase A (PLA) IgE specific antibodies in the sera of beekeepers. Titres are expressed as RAST % and titres over 25% are considered significant.

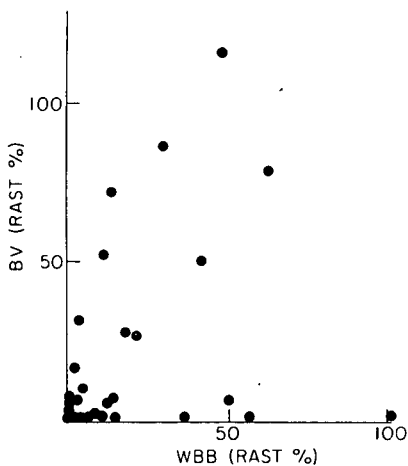


Fig. 4. Relationship of bee venom (BV) and whole bee body (WBB) IgE specific antibodies in the sera of beekeepers. Titres are expressed as RAST % and titres over 25% are considered significant.

Table 3. Total antibodies and IgE antibodies in serum of one nonallergic beekeeper

	Total antibody	RAST %*
Bee venom	210 µg/ml	52
Phospholipase A	210 µg/ml	124

* Greater than 25% is significant.

frequently fail to develop even the erythema and swelling associated with the 'normal' stinging reaction. Immunization occurs as a result of multiple bee stings. Total antibodies to bee venom, primarily a measurement of gamma G, are found in higher titer in their sera. The amount of total antibody correlates with antigen exposure, the number of stings per week.

The presence of elevated IgE antibodies to bee venom or phospholipase A appears to reflect susceptibility to systemic reactions. These antibodies have been detected in the sera of almost all patients who have had systemic reactions following bee stings. It is of interest that some beekeepers also develop IgE antibodies. However, when elevated levels of bee venom specific IgE are also accompanied by concurrent high total antibody levels, the individual appears to be protected. Immunological studies of one patient who received honey bee venom immunotherapy because of extreme sensitivity showed a similar immunological pattern (Busse *et al.*, 1975). Protection against bee venom developed as high titres of gamma G antibody developed, despite the continued presence of elevated bee venom specific IgE. A similar analogy has been found in a study of an individual who had concurrent insulin allergy and insulin resistance (Dolovich *et al.*, 1970).

The occasional development of allergic reactions in beekeepers is somewhat of a paradox. From a mechanistic viewpoint, preferential stimulation of IgE production must occur. Review of the clinical data fails to suggest criteria which might identify such susceptible beekeepers.

Total antibodies to either bee venom or phospholipase A are induced by exposure to bee venom but do not appear to be affected by treatment of individuals with whole bee body extract. As it appears that allergic reactions are primarily induced by bee venom antigens (Reisman & Arbesman, 1975), the use of whole body extracts as therapeutic agents is open to serious question.

Phospholipase A has been identified as a major antigen in bee venom (Sobotka *et al.*, 1974). While there are exceptions, patients reacting to bee venom usually also react to phospholipase A. In this study there was excellent correlation between both IgE antibodies and total antibodies reacting with these two antigens.

IgE antibodies to both bee venom and whole bee body protein were found in some beekeepers. There was a poor correlation between the levels of these antibodies, suggesting that in general they are reacting with different antigenic determinants. In contrast to the findings with bee venom IgE antibodies, the level of whole bee body IgE was similar in beekeepers with and without reactions. This finding supports the importance of bee venom antigens in the etiology of systemic allergic reactions.

As beekeepers are exposed to inhalation of bee 'dust' as well as frequent stings, this finding of antibody production to bee body proteins is not unexpected.

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References

- ARBESMAN, C.E., WYPYCH, J.I. & REISMAN, R.E. (1974) Standardization of stinging insect extracts. *World Health Organization Conference Proceedings, International WHO-IABS Symposium on*

- Standardization and Control of Allergen Administration to Man*, Geneva 1974, vol. 29, p. 249. Karger, Basel.
- BUSSE, W.W., REED, C.E., LICHENSTEIN, L.M. & REISMAN, R.E. (1975) Immunotherapy in bee sting anaphylaxis: Use of honeybee venom. *Journal of the American Medical Association*, **231**, 1154.
- CESKA, M., ERIKSSON, R. VARGA, J.M. (1972) Radioimmunosorbent assay of allergens. *Journal of Allergy and Clinical Immunology*, **49**, 1.
- DOLOVICH, J., SCHNATZ, J.D., REISMAN, R.E., YAGI, Y. & ARBESMAN, C.E. (1970) Insulin allergy and insulin resistance. *Journal of Allergy*, **46**, 127.
- FUJITA, Y., WYPYCH, J.I., WICHER, K., REISMAN, R.E. & ARBESMAN, C.E. (1975) In vitro reaction of antibodies to ragweed. I. A radioimmunoassay for measuring antibody levels. *International Archives of Allergy and Applied Immunology* (in press).
- REISMAN, R.E. & ARBESMAN, C.E. (1975) Stinging insect allergy: Current concepts and problems. *Pediatric Clinics of North America*, **22**, 185.
- SHEPHERD, G.W., ELLIOTT, W.B. & ARBESMAN, C.E. (1974) Fractionation of bee venom. I. Preparation and characterization of four antigenic components. *Preparative Biochemistry*, **4**, 71.
- SHULMAN, S., LANGLOIS, C. & ARBESMAN, C.E. (1964) The allergic response to stinging insects I. Preparation of extracts and their biochemical characterization. *Journal of Allergy and Clinical Immunology*, **35**, 446.
- SOBTKA, A., FRANKLIN, R., VALENTIN, M., ADKINSON, N.F. & LICHTENSTEIN, L.M. (1974) Honeybee venom: Phospholipase A as the major allergen. *Journal of Allergy and Clinical Immunology*, **53**, 103.

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