

Hymenoptera sting hypersensitivity: IgE, IgG and haemagglutinating antibodies to bee venom constituents in relation to exposure and clinical reaction to bee stings

U. MÜLLER, S. G. O. JOHANSSON and C. STREIT

Medical Department, Zieglerspital Bern, Switzerland, and Blodcentralen, Akademiska Sjukhuset, Uppsala, Sweden

(Received 11 November 1977; accepted for publication 29 November 1977)

Summary

High levels of IgE antibodies (Ab) to bee venom constituents were mainly found in patients with bee sting hypersensitivity, whereas the sera of bee keepers usually contained high levels of IgG Ab and haemagglutinins, representing blocking antibody activity.

In bee keepers there was a positive correlation between the degree of severity of an adverse reaction to bee stings and IgE Ab, a negative one between the severity of reaction and IgG Ab and haemagglutinins, a positive one between the number of stings per season and IgG Ab and haemagglutinins, and a negative one between the number of stings and IgE Ab. This suggests a mutual dependence of the production of IgE Ab and blocking Ab in a regularly exposed population. No such correlation was found in bee sting-allergic patients, indicating that other factors influence the severity of reaction in this only sporadically exposed group.

The values of IgG Ab and haemagglutinins correlated well with each other, with IgG Ab giving a better correlation with the severity of reaction and number of stings than the haemagglutinins.

Introduction

High levels of serum IgE antibodies (Ab) to bee venom have been observed in patients with bee sting allergy, whereas the sera of bee keepers usually contain high titres of blocking antibodies (Reisman *et al.*, 1974; Light *et al.*, 1975; Müller, Spiess & Roth, 1977). The object of this study was to investigate a possible correlation between these serological parameters, the degree of exposure to bee venom and the severity of local or general allergic reactions.

Materials and methods

Sera

These were collected from forty patients with generalized type I allergic reactions to bee stings—mainly urticaria, Quincke's oedema, asthma and anaphylactic shock—

Correspondence: Dr U. Müller, Medical Division, Zieglerspital, 3007 Bern, Switzerland.

before hyposensitization therapy was started. Patients with a reaction time of more than 30 min between the sting and the first allergic symptoms were excluded. Sera of forty bee keepers were collected at the end of the bee season, i.e. in August and September. As a control group, the sera of twenty Swedish blood donors without a history of recent bee sting and hymenoptera sting hypersensitivity were collected for IgG Ab studies. Fifty-two similarly selected Swiss blood donors served as a control group for the studies of IgE Ab and haemagglutinins.

According to the intensity of the local or general reaction the patients and bee keepers were divided into seven groups (Table 1).

The number of stings per season was also recorded, amounting from five to 1000 in bee keepers, but only rarely exceeding one in patients.

Table 1. Classification of reactions to bee stings

Grade of reaction	Patients (number)	Bee keepers (number)	Signs and symptoms
(1) No reaction	0	11	No local or general reaction
(2) Local reaction	0	20	Slight to moderate local reaction of less than 24 hr duration
(3) Severe local reaction	1	7	Local reaction exceeding 10 cm and 24 hr duration
(4) Slight general reaction	7	1	Generalized urticaria, itching, malaise and anxiety
(5) Moderate general reaction	7	1	Any of the above plus two of following: angioneurotic oedema, dizziness, constriction of chest, abdominal pain, nausea, vomiting, diarrhoea
(6) Severe general reaction	17	0	Any of the above plus two of following: dyspnoea, dysphagia, hoarseness or thickened speech, confusion, feeling of impending disaster
(7) Shock reaction	8	0	Any of the above plus two of following: cyanosis, fall in blood pressure, collapse, incontinence, unconsciousness

IgE Ab to bee venom

These were estimated by the Phadebas RAST, (Pharmacia Diagnostics AB, Uppsala, Sweden). Results were expressed in parts per thousand (p.p.t.) of reference C of the Phadebas RAST reference system.

IgG Ab to phospholipase A (Phos. A)

IgG Ab to Phos. A, one of the major allergens of bee venom (Sobotka *et al.*, 1976; Hoffman & Shipman, 1976), were measured by a technique based on the adsorption of IgG to a Sepharose-Protein A complex followed by the addition of ^{125}I -labelled allergen (Johansson *et al.*, 1974).

A 0.5 ml aliquot of Sepharose-protein A (Pharmacia Fine Chemicals, Uppsala) diluted 1/10 in test buffer (0.1 M phosphate buffer, pH 7.4, containing 0.2% bovine

serum albumin, 0.1% Tween 20 and 0.05% NaN_3) was incubated with 0.1 ml of the serum sample diluted 1/10 in test buffer under vertical rotation for 2–3 hr at room temperature. At higher dilutions, the serum sample was diluted in a 10% solution in the test buffer of normal serum lacking the allergen-specific IgG Ab. After centrifugation and two washings with saline, containing 0.1% Tween 20, 0.1 ml of the ^{125}I -labelled PLA preparation, corresponding to 80,000 to 100,000 ct/min, was added. Phos. A (Sigma Chemical Company, St Louis, Missouri) was labelled according to the chloramine T method of Hunter & Greenwood (1962), 20 μg with 1 mCi ^{125}I . After incubation overnight, the Sephadex–protein A complex was washed five times and the bound radioactivity was measured in a gamma scintillation counter. The results were expressed in p.p.t. of a reference serum obtained from a bee keeper.

Haemagglutinating antibodies to Phos. A

These were determined using tanned human red cells, O Rh-negative coated with PLA according to the technique described by Arbesman *et al.* (1960). The results are given in titres of the serum dilution at which the end-point of haemagglutination occurred (Spiess, 1978).

Statistical evaluations

The following questions were analysed. (a) Is there a difference between the mean values of the three serological parameters studied, namely IgE Ab, IgG Ab and haemagglutinins in patients and bee keepers? Because the distribution of the single values was not normal (Gaussian), the mean values were compared by the Wilcoxon rank-sum test. (b) Is there a correlation between the three serological parameters, IgE Ab, IgG Ab and haemagglutinins? (c) Is there a correlation between the severity of an allergic reaction and the three parameters? (d) Is there a correlation between the number of stings and the three parameters? This question was studied in bee keepers only, as patients were usually stung very infrequently.

To study questions (b), (c) and (d), the values of IgE Ab, IgG Ab haemagglutinins and the number of stings were transformed logarithmically.

Results

Table 2 shows the values for the IgE Ab, IgG Ab and haemagglutinins in patients and bee keepers. Mean values of all three parameters differ significantly in the two groups, patients showing high levels of IgE Ab and low levels of IgG Ab and bee keepers low levels of IgE Ab and high levels of IgG Ab and haemagglutinins as a rule. In blood donors, IgE Ab lay below 1000 p.p.t. and IgG Ab below 500 p.p.t. of the respective reference serum, and haemagglutinins were not detectable, as a rule.

We found a good correlation between the IgG Ab and haemagglutinins in both bee keepers ($r = 0.686$; $P < 0.00001$) and bee sting-allergic patients ($r = 0.704$; $P < 0.00001$). A negative correlation was found between IgE Ab and IgG Ab ($r = -0.42$; $P < 0.006$) and IgE Ab and haemagglutinins ($r = -0.34$; $P < 0.03$) in bee keepers, but no correlation between these parameters was detected in patients. A positive correlation exists between the degree of severity of a reaction to bee stings ($r = 0.714$; $P < 0.00001$) and IgE Ab, and a negative one between the degree of reaction and IgG Ab ($r = -0.35$; $P < 0.025$) in bee keepers, but not in patients. No correlation was found between degree of reaction and haemagglutinins.

In bee keepers, a positive correlation between the number of stings and IgG Ab

Table 2. Values for specific IgE, IgG and haemagglutinating antibodies in patients and bee keepers

Subjects (for IgE Ab)	RAST (p.p.t. of reference C)					MV (mean values)	P
	0-500	501-1000	1001-2500	2501-5000	> 5000		
Patients	4	7	12	11	6	2713*	≤ 0.00001
Bee keepers	20	6	11	2	1	1034*	
Blood donors	38	12	1	1	0	459	

Subjects (for IgG Ab)	IgG RIA (p.p.t. of reference)					MV	P
	0-500	501-1000	1001-5000	5001-10000	> 10000		
Patients	21	5	11	3	0	1451*	≤ 0.00001
Bee keepers	8	0	16	13	3	4599*	
Blood donors	20	0	0	0	0	—	

Subjects (for haemagglutination)	Haemagglutinin titre (l/n)					MV	P
	0-10	20-40	80-160	320-1280	> 1280		
Patients	26	6	2	5	1	129*	≤ 0.002
Bee keepers	14	8	5	12	1	330*	
Blood donors	48	3	1	0	0	5.5	

* Shows the comparison being made for the significance test.

($r = 0.389$; $P < 0.02$) and the number of stings and haemagglutinins ($r = 0.325$; $P < 0.04$) was found, and a negative one between the number of stings and IgE Ab ($r = -0.482$; $P < 0.002$).

Discussion

The serological findings in patients and bee keepers correspond to previous observations (Light *et al.*, 1975; Müller *et al.*, 1977) and indicate that sporadic exposure to bee stings rather induces IgE Ab responsible for allergic reactions of type I, while regular massive exposure as in bee keepers leads preferably to formation of blocking antibodies.

A good correlation between HA and IgG Ab was observed in both groups. While the radioimmunoassay determines IgG Ab of the subclasses IgG1, IgG2 and IgG4, the haemagglutination technique detects IgM Ab as well. This fact is thought to be responsible for the disagreement between the values obtained with the two methods in some individuals.

The negative correlation between IgE Ab and IgG Ab observed in bee keepers points to some mutual dependence of the IgE Ab and IgG Ab production in this population, which cannot be demonstrated in the only sporadically exposed population of patients.

While a positive correlation between IgE Ab and the degree of severity and a negative one between IgG Ab and the degree of severity was observed in bee keepers, no correlation between serological parameters and it could be detected in patients.

This difference could be due to the fact that bee keepers had almost exclusively grade 1–3 reactions, that is local reactions, while most patients had grade 4–7 reactions, that is general reactions. This would mean that IgE Ab and IgG Ab correlate with DS in local reactions, but not in general reactions to bee stings. Alternatively, the different exposure modalities in the two groups could also be responsible for this discrepancy.

Mean values of IgE Ab were significantly higher in general than in local reactions, but there was a lack of correlation between the IgE Ab and the degree of severity in general allergic reactions, indicating that other factors influence the severity of a general allergic reaction. Forming a quotient of IgE Ab/IgG Ab or IgE Ab/haemagglutinins resulted in no better correlation to the degree of severity, suggesting that blocking antibodies are not this factor, at least in non-treated patients. This does, however, not exclude a protective function of blocking antibodies (Lessof, Sobotka & Lichtenstein, 1976). When only those patients were studied whose blood samples were taken within 6 months after the last allergic reaction (Müller *et al.*, 1977) there was also no correlation between IgE Ab and degree of severity.

The correlation between serological parameters and the degree of exposure observed in bee keepers suggests that a high degree of exposure induces a high level of blocking antibodies, which in turn interferes with IgE Ab formation. In fact, IgE antibodies are found in a large proportion of bee keepers of recent standing (Müller *et al.*, 1977), while long-standing bee keepers rather have high levels of blocking antibodies (Spiess, 1978).

The radioimmunoassay for IgG Ab was better suited for the estimation of blocking antibodies than the haemagglutination technique, giving a better correlation with the degree of exposure to the antigen and also with the degree of protection, as indicated by the negative correlation between IgG Ab and degree of severity in bee keepers. Determination of the IgG Ab thus seems to be of value for the monitoring of immunotherapy with venom in hymenoptera-allergic patients.

Acknowledgments

We thank Mr M. Schafroth and Mr B. Flury, Institute for Mathematical Statistics, University of Berne, for helpful discussions and the survey of statistical analyses, and Mrs L. Wafler-Tschudi for skilled technical assistance. Test material for the IgE determination was kindly supplied by Pharmacia Diagnostics AB, Uppsala, Sweden. The study was supported in part by the Swedish Medical Research Council, grants No. 16X-105 and 16P-4910.

References

- ARBESMAN, C.E., ROSE, N.R., KAUTER, S.Z. & BEEDE, R.B. (1960) Immunologic studies of ragweed-sensitive patients. I. Specificity and sensitivity of hemagglutination reactions. *Journal of Allergy*, **31**, 317.
- HOFFMAN, D.R. & SHIPMAN, W.H. (1976) Allergens in bee venom. I. Separation and identification of the major allergens. *Journal of Allergy and Clinical Immunology*, **58**, 551.
- HUNTER, W.M. & GREENWOOD, F.C. (1962) Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature*, **194**, 495.
- JOHANSSON, S.G.O., FOUCARD, T., HJELM, H. & SJOEKVIST, J. (1974) Determination of specific IgG-antibody to soluble antigens by adsorption to protein A-Sepharose. *Scandinavian Journal of Immunology*, **3**, 881.
- LESSOF, M.H., SOBOTKA, A.K. & LICHTENSTEIN, L.M. (1976) Protection against anaphylaxis in hymenoptera-sensitive patients by passive immunization. *Journal of Allergy and Clinical Immunology*, **57**, 246.

- LIGHT, W.C., REISMAN, R.E., WYPYCH, J.I. & ARBESMAN, C.E. (1975) Clinical and immunological studies in bee-keepers. *Clinical Allergy*, **5**, 389.
- MÜLLER, U., SPIESS, J. & ROTH, A. (1977) Serological investigations in hymenoptera sting allergy: IgE and haemagglutinating antibodies against bee venom in patients with bee sting allergy, bee keepers and non-allergic blood donors. *Clinical Allergy*, **7**, 147.
- REISMAN, R.E., WYPYCH, T.I., TEAGLE, N. & ARBESMAN, S.E. (1974) Stinging insect hypersensitivity. III. Detection and clinical significance of IgE antibodies to insect venom in man. *Journal of Allergy and Clinical Immunology*, **53**, 110.
- SOBOTKA, A.K., FRANKLIN, R.M., ADKINSON, N.F., VALENTINE, M., BAER, H. & LICHTENSTEIN, L.M. (1976) Allergy to insect stings. II. Phospholipase A, the major allergen in honey bee venom. *Journal of Allergy and Clinical Immunology*, **57**, 29.
- SPIESS, J. (1978) *Serologische Untersuchungen bei Bienenstichallergie*. Thesis, Bern. (In press.)

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.