

IgE and IgG antibody response to purified bee-venom antigens and peptides in four patients who had adverse reactions to immunotherapy

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Summary

The immunological response to individual bee-venom allergens was studied in blood samples collected at frequent intervals from four bee-venom allergic patients who had suffered systemic allergic reactions to injections of bee venom during immunotherapy. All had high IgE antibody levels, at the upper end of the range found in bee-sting allergic patients, and all had antibodies to the minor allergens at the time of the reactions. These did not, however, provide a simple explanation for the reactions that occurred. We were able to observe two interesting phenomena — in one patient IgE antibodies to the individual venom antigens appeared to be 'switched off' sequentially. In another, IgE antibodies to hyaluronidase rose substantially after 4 years of therapy. We believe that these results provide evidence to support the view that the regulation of IgE antibodies is controlled by mechanisms that are both isotype- and antigen-specific.

Introduction

Treatment of immediate hypersensitivity by injection of increasing doses of allergen (immunotherapy) has continued in use since it was first reported by Noon [1] and Freeman in 1911 [2]. In carefully controlled double-blind studies during the 1950s, Frankland and Augustin [3] showed that injections with aqueous grass pollen solutions provided relief of symptoms compared with placebo. Clinical protection was by no means absolute, with complete remission occurring in only a minority of patients who received immunotherapy with pollen vaccines. Conversely, treatment of allergy to bee

stings is much more effective and provides complete protection in the majority of cases [5]. While successful treatment has been shown to correlate with an IgG 'blocking' antibody response, it is more difficult to understand the adverse reactions that still occur during therapy. For this reason, we decided to carry out an intensive study of four patients who had systemic reactions to treatment by measuring their IgE and IgG antibody response to the individual bee-venom allergens.

Subjects and methods

Subjects studied

All four patients studied had a clinical history of systemic allergic reactions to bee stings, the details of which are described in the Results section. All gave a positive intradermal skin test to bee venom at a concentration of 10^{-2} $\mu\text{g/ml}$ or less, and each then received a course of bee venom immunotherapy [6], modified so that the same or a reduced dose was given when a reaction had occurred following the previous injection. Venous blood samples were drawn frequently during treatment, and the separated serum was stored at -20°C .

Antibody assays

IgE antibodies to the purified bee-venom allergens phospholipase A_2 (PLA₂) [7], hyaluronidase (HYAL) [8], acid phosphatase (ACID.P) [9, 10] and melittin (MEL) were measured by the radio-allergosorbent test (RAST) as described previously [11, 12]. The antigen specificity of the RAST was confirmed by inhibition with the other venom antigens. IgG antibodies were measured using an I^{125} radiolabelled antigen-binding immunoassay [13]. The results of both the IgE and the IgG antibody assays were quantitated by reference to a standard curve constructed from the results obtained by assaying dilutions of a serum pool, which have a known IgE and IgG antibody content, as determined by saturation analysis [14]. One unit of IgE antibody is equal to approximately 0.2 ng of IgE, and one unit of IgG antibody to 2.4 ng of IgG. The units of IgG antibody to ACID.P may be artificially low due to difficulties in obtaining precise data from saturation analysis, and were probably at a similar level to hyaluronidase-specific IgG antibodies. No IgG antibody measurements were made for MEL as we have been unable to develop a suitable assay.

Results

Case 1

A 32-year-old amateur beekeeper started keeping bees in 1974 and suffered systemic reactions to a bee sting on at least two occasions in 1975, characterized by generalized urticaria, shortness of breath and laryngeal oedema. In 1976, on intradermal skin testing, he gave a positive reaction to bee venom (10^{-2} $\mu\text{g/ml}$), and to pure phospholipase A_2 (10^{-3} $\mu\text{g/ml}$). Immunotherapy with bee venom was started in May 1977 and, despite some mild reactions, consisting primarily of an erythematous rash, he reached a 100 μg maintenance dose in June 1977. He also had experienced more severe delayed symptoms including nausea, dyspnoea, fever and oedema, and when sting challenged in September 1977, he again had a systemic reaction. Maintenance injections were continued and in March 1978 he was stung without any reaction.

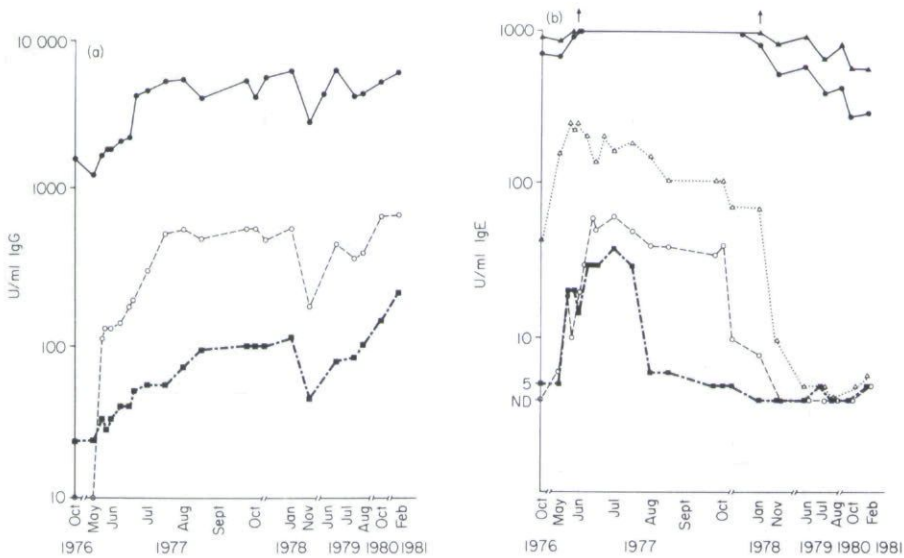


Fig. 1. The IgG (a) and IgE (b) antibody response to individual bee-venom allergens in a patient who had adverse reactions to immunotherapy. While IgG antibodies showed a modest rise, IgE antibodies to individual allergens fell. (○)Hyal, (●) PLA₂, (■) Acid P, (△) Mel, (▲) HBV.

The concentration of IgG antibody to PLA₂ was high initially and rose further during treatment (results not shown), while IgG antibodies to HYAL and ACID.P showed little change. IgE antibodies to each allergen rose, however, during treatment and were elevated at the time he suffered his adverse reactions (results not shown). IgE antibody levels then fell, with antibodies to ACID.P declining most rapidly and those to PLA₂ most slowly.

Case 2

A 25-year-old female beekeeper had received immunotherapy for hay fever but had suffered no adverse reactions to stings. A bee sting in 1970 had been followed by rhinitis and in 1974, after a further sting, she collapsed in what appeared to be an anaphylactic reaction. An intradermal skin test in 1975 gave a positive to bee venom at 10^{-2} µg/ml, and to PLA₂ at 10^{-3} µg/ml. A course of immunotherapy with bee venom was begun in May 1977, but after a number of delayed reactions she suffered a more immediate reaction after an injection of 40 µg bee venom in July. The symptoms of rhinitis and constriction of the throat responded to an injection of 0.3 ml of epinephrine (1% v/v). In August 1977, she had a mild urticarial reaction to a sting challenge and in September had dyspnoea following the second of two 100-µg injections of bee venom. Apart from a local reaction to a maintenance injection in August 1979, she had no further difficulties.

The immunological response of this patient to the different bee venom injections is striking. Her IgG antibodies showed a steady rise for all three allergens (Fig. 1a), which was greatest for hyaluronidase. IgE antibodies were initially high (RAST class 4) and rose further before falling (Fig. 1b). There was a rapid rate of fall in IgE antibodies that occurred independently at different times for each antigen and was also independent of the IgG response. As in case 1, IgE antibodies to PLA₂ were the last to fall.

There was a modest IgG antibody response to PLA₂ and HYAL but not ACID.P (results not shown). Most of her identifiable IgE antibody was directed against PLA₂, which changed little during the course of treatment (results not shown).

Discussion

It is surprising that adverse reactions do not occur more commonly during immunotherapy with bee venom and other agents [15], since a transient rise in IgE antibody levels appears to be the rule rather than the exception. Even those patients who lose their clinical sensitivity to bee venom seldom lose their IgE antibodies or skin reactivity, which tend to persist at a low level. Nevertheless, bee venom immunotherapy is remarkably successful in reversing anaphylactic reactivity. In studying four patients who had treatment difficulties, we hoped to find in what way they differed from individuals who tolerate venom injections without side-effects. We are, however, unable to find a single explanation that could apply to all cases, and it is clear that more than one mechanism must operate.

Sensitivity to the minor venom allergens in cases 1 and 2 may well have played a part in maintaining clinical sensitivity. Case 3 also showed unexplained, transient rises of IgE antibody to more than one component of venom at the time of his adverse reactions to therapy. In case 4, the IgE antibody rise during treatment was particularly brisk, and the fact that IgE to whole venom remained high suggests that she may have been allergic to other unidentified venom antigens. Nevertheless, once her IgG antibody level rose, her reactions to venom injections largely ceased. The persistence or actual increase in the IgE response during treatment may, thus, be a contributory factor. It is notable that three of the four patients with treatment difficulties had high pre-treatment IgE antibody levels to bee venom (RAST class 3 or greater). A slow IgG antibody response or an inadequate ratio of IgG antibody to IgE antibody may also have played a part in cases 1, 3 and 4. This, too, seems an inadequate explanation for the discrepancies that are seen between those who suggest anaphylactic sensitivity and those who do not. Differences in mast cell and basophil releaseability or target organ sensitivity to inflammatory mediators may have also been involved but have yet to be adequately documented. It is clear that the factors responsible for recurrent bee-sting reactions are still imperfectly understood.

While the data on IgE and IgG antibodies to the individual allergens cannot completely explain the clinical response of these patients, they can provide an additional insight into the regulation of these antibody isotypes after repeated immunization. The demonstration in case 1, and more clearly in case 2, that IgE antibodies to bee venom antigens can be 'turned off', on an individual time-scale, regardless of the IgG antibody level, shows that these responses can be regulated independently of each other. A similar observation has been made in an individual with cows' milk allergy (R. Barnetson, pers. comm.) who also lost her IgE antibodies to individual proteins independently of one another. In keeping with the concept of an independent regulation of the IgE response to different antigens, there was also evidence from independent rise and fall of different IgE antibodies in cases 3 and 4. The IgE response to PLA₂ appeared to rise most strongly and be suppressed the least readily in every case. While the immunological response to venom is still poorly understood, a further study of the immune response to individual antigens may help our understanding of the way in which IgE and IgG isotypes are regulated.

References

- 1 Noon L. Prophylactic inoculation for hay fever. *Lancet* 1911; i:1572-4.
- 2 Freeman J. Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine. *Lancet* 1911; ii:814-7.
- 3 Frankland AW, Augustin R. Prophylaxis of summer hay fever and asthma. Controlled trial comparing crude grass pollen extracts with isolated main pollen component. *Lancet* 1954; i:1055-7.
- 4 Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect hypersensitivity. *N Engl J Med* 1978; 299:157-61.
- 5 Lichtenstein LM, Sobotka AK, Valentine MD. A case for venom treatment in anaphylactic sensitivity to hymenoptera sting. *N Engl J Med* 1974; 290:1223-7.
- 6 Golden BDK, Valentine MD, Kagey-Sobotka A, Lichtenstein LM. Regimens of venom immunotherapy. *Ann Intern Med* 1980; 92:620-64.
- 7 Shipolini RA, Callewaert GI, Cotterell RC, Doonan S, Vernon CA. Phospholipase A₂ from bee venom. *Eur J Biochem* 1971; 20:459-68.
- 8 Kemeny DM, Dalton N, Lawrence AJ, Pearce FL, Vernon CA. The purification and characterisation of hyaluronidase from the venom of the honey bee, *Apis mellifera*. *Eur J Biochem* 1984; 139:217-23.
- 9 Kemeny DM. The purification of hyaluronidase from bee venom and the assessment of its importance in bee venom allergy. PhD Thesis, University of London, 1982.
- 10 Barboni M, Kemeny DM, Campos S, Vernon CA. The purification of acid phosphatase from honey bee venom. *Toxicon* 1987; 25:1097-103.
- 11 Kemeny DM, Harries MG, Youlten LJF, 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.
- 12 Kemeny DM, Mackenzie-Mills M, Lessof MH. Allergen solid phase interactions, specificity and cross reactivity. In: Kemeny DM, Lessof MH, eds. *Recent Developments in RAST and Other Solid-Phase Immunoassay Systems*. Amsterdam: Excerpta Medica, 1983:3-16.
- 13 Kemeny DM, Mackenzie-Mills M, Harries MG, Youlten LJF, Lessof MH. Antibodies to purified bee venom proteins and peptides. II. A detailed study of the changes in IgE and IgG antibodies to individual bee venom antigens. *J Allergy Clin Immunol* 1983; 72:376-85.
- 14 Kemeny DM, Lessof MH. The immune response to bee venom: II Quantitation of the absolute amounts of IgE and IgG antibodies by saturation analysis. *Int Arch Allergy Appl Immunol* 1987; 83:113-20.
- 15 Golden BK, Valentine MD. Adverse reactions to venom immunotherapy. In: Levine M, Lockey RE, eds. *Monograph on Insect Allergy*. Library of Congress, U.S.A., 1981:53-6.

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