

Sub-class of IgG anti-bee venom antibody produced during bee venom immunotherapy and its relationship to long-term protection from bee stings and following termination of venom immunotherapy

R. URBANEK*, D. M. KEMENY† and D. RICHARDS

**University Children's Clinic, University of Freiburg, Freiburg, West Germany and*

†*Department of Medicine, Guy's Hospital Medical School, London SE1 9RT*

(Received in original form 28 November 1985 and in revised form 6 March 1986; accepted for publication 7 March 1986)

Summary

The IgG sub-class antibody response to bee venom in the four sub-classes was investigated in ten patients during and after venom immunotherapy. All patients tolerated a bee sting challenge 1, 2 and 3 years after the start of treatment as well as 1 and 2 years after treatment was stopped. Anti-phospholipase A₂ (PLA₂) antibodies were of IgG1 and IgG4 sub-class and rose early in treatment, IgG1 anti-PLA₂ fell to pre-treatment levels after 3 years in contrast to IgG4 anti-PLA₂ levels, which remained high during maintenance therapy and declined relatively little in the 2 years after the termination of treatment. This data shows that IgG4 antibodies are maintained in the absence of monthly maintenance injections and suggests that they may provide long lasting clinical protection from insect stings.

Introduction

Treatment of bee sting allergy by incremental injections of bee venom is highly effective in preventing subsequent allergic reactions in sensitive patients [1,2]. Such treatment results in the formation of bee venom-specific IgG antibodies and a rise in their concentration in the serum is associated with clinical improvement [3–6]. Bee venom-specific IgG antibody can both inhibit the release of histamine from sensitized peripheral blood leucocytes *in vitro* (P. Clinton, Department of Biochemistry, Imperial College, unpublished observations) and reduce the reactions to bee venom *in vivo* [7]. The sub-class of IgG antibody to bee venom that is produced is restricted to IgG sub-

classes 1 and 4 (D.M. Kemeny, unpublished observations). Aalberse and coworkers [8] showed that newly stung novice beekeepers first produced a predominantly IgG1 antibody response to bee venom phospholipase A₂ (PLA₂) which was superseded by IgG4 antibody to PLA₂ following repeated stings. High IgG4 antibody levels have also been demonstrated following grass pollen immunotherapy [9] and a similar change from IgG1 to IgG4 during a course of grass pollen immunotherapy was reported [10].

It was the aim of this study to find out if specific IgG sub-class antibodies were suitable indicators of continued protection from bee stings after maintenance injections of bee venom were stopped. We were also interested in seeing if the live bee sting challenge altered the IgE or IgG sub-class antibody response once therapy had been stopped.

Materials and methods

Patients studied

Children and adolescents (aged 6–20 years) who had a history of severe systemic allergic reactions to bee stings were studied over a period of 5 years. All patients gave a positive intradermal skin-prick test (<2 mm weal) to 100 µg or less of bee venom (Pharmalgen, Pharmacia, Sweden) and a positive bee venom radioallergosorbent test (RAST). Treatment with bee venom was by rush regimen and the maintenance dose (100 µg) was reached after 7 days and was continued by monthly injections of this amount of venom for 3–4 years. All patients were sting challenged annually after they had reached the maintenance dose. Venom immunotherapy was discontinued after 3–4 years and all patients re-stung at 1 and 2 years after discontinuation of treatment.

Reagents

Bee venom phospholipase A₂ (PLA₂) was a kind gift from Professor C. A. Vernon (University College, London). Bee venom used for diagnosis and treatment (Pharmalgen) was purchased from Pharmacia PLC, Sweden. Mouse monoclonal anti-human IgG1–4 (McAb) was purchased from Unipath (formerly Seward) Ltd, England anti-IgG1 (Clone NL16) anti-IgG4 (Clone RJ4). Alkaline phosphatase-conjugated rabbit anti-mouse IgG (AP-anti-MIG) and p-nitrophenyl phosphate were purchased from Sigma Ltd, U.S.A. Rabbit anti-PLA₂ antibodies were raised and isolated as described previously [11]. Microtiter plates (M129/A) were purchased from Dynatech Ltd, U.K. All other reagents were purchased from BDH Ltd, U.K.

IgG sub-class antibody assay

IgG sub-class antibodies were measured using a modified sandwich ELISA [11–13] which was adapted to quantitate IgG sub-class antibodies (D.M. Kemeny, unpublished observations). Briefly, microtiter plates were coated with 100 µl of affinity purified rabbit anti-PLA₂ (1 µg/ul) in pH 9.6 sodium carbonate/bicarbonate buffer (0.1 M at 4°C overnight). The following day, unbound anti-PLA₂ was washed off. All washes were performed three times with phosphate-buffered saline (PBS) 0.05 M, 0.15 M NaCl pH 7.4 containing 0.5% Tween 20 (wash solution). PLA₂ (100 µl/100 ng/ml) was then incubated with the antibody-coated plates for 1 hr at 4°C in assay diluent (PBS/0.5% horse serum). All buffers contained 0.5% v/v phenol red (40 mg/100 ml) and 0.5% v/v naphthalene black (60 mg/100 ml) which imparts a lilac colour at alkaline pH and a green colour at neutral pH, and facilitates pipetting samples (use of colours taken

from Dr R.C. Aalberse, Redcross, Amsterdam). Following incubation of PLA₂ with anti-PLA₂-coated microtiter plates, unbound PLA₂ was washed off as above and 100 μ l patients' serum diluted 1/50–1/50 000 in assay diluent added. After 2 hr at 4°C unbound material was washed off and 100 μ l of a 1/1000 dilution of McAb specific for IgG1 or IgG4 added (we have not found appreciable amounts of IgG2 or IgG3 antibody to bee venom proteins). After 1 hr at 4°C the plates were washed again and 100 μ l of 1/500 AP anti-MIG added. After 1 hr at 4°C this too was washed off twice in PBS/0.5% Tween 20 and once with distilled water to remove traces of phosphate. The substrate (100 μ l, 1.0 mg/ml p-nitrophenyl phosphate in pH 9.6 diethanolamine buffer) was then added and colour developed for one and a half hours at 37°C. The reaction was stopped by addition of 50 μ l of NaOH (3 M) and the optical density read in a Multiskan spectrophotometer (Flow Ltd, U.K.) at 405 nm. Results were read from a reference curve which contained a known amount of IgG antibody to PLA₂ (24 μ g/ μ l). The reference curve was taken from dilutions of the reference serum whose antibody was detected with a 'Pan-IgG'-reactive McAb. Results are expressed as units/ml where one unit equals 0.24 ng/ml IgG anti-PLA₂. IgG antibodies to whole bee venom were measured as described previously [14] and also expressed as arbitrary units/ml.

Specific IgE

Specific IgE antibodies were measured by the radioallergosorbent test (RAST) using phadebas RAST (Pharmacia, Sweden) according to the manufacturer's instructions. Results are expressed as percentage counts per minute bound.

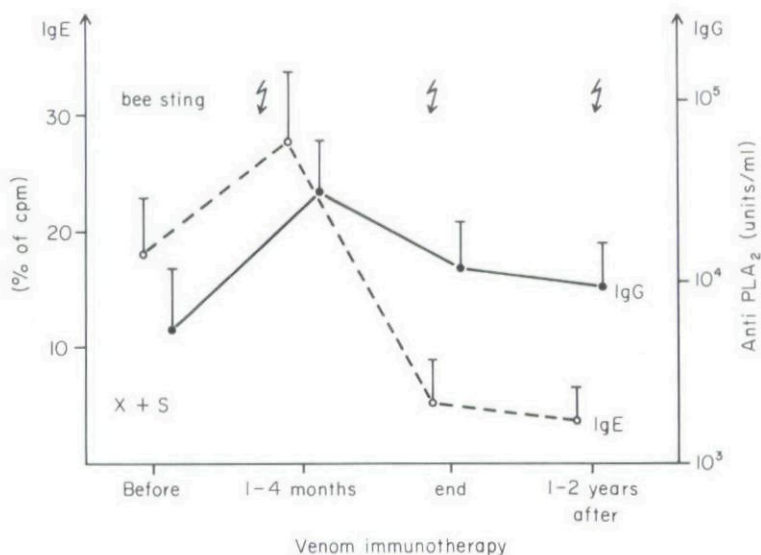


Fig. 1. The pattern of IgE (---) and IgG (—) antibody to bee venom during and after bee venom immunotherapy showing that specific IgE antibody levels fall to below pre-treatment levels following treatment, while specific IgG antibodies are maintained. The results for specific IgE antibody are expressed as % counts per minute bound (cpm) and for IgG antibody are in arbitrary units/ml. The data shown is the mean of ten patients $\pm$ standard deviation.

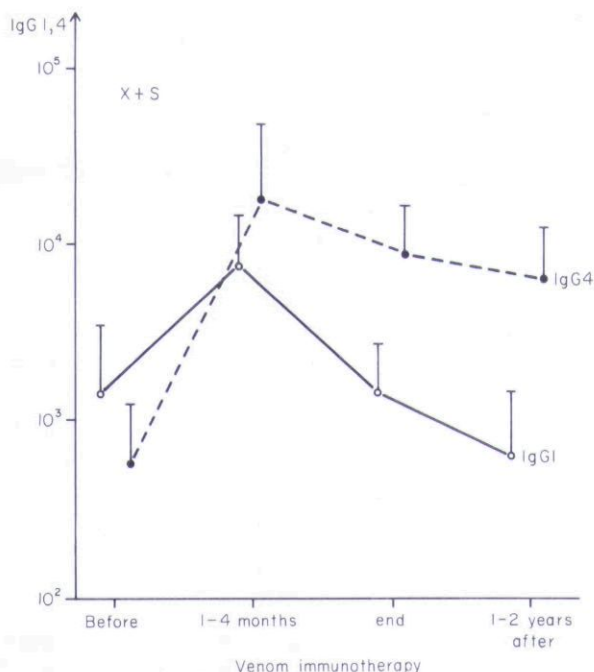


Fig. 2. Mean IgG1 (—) and IgG4 (---) antibodies $\pm$ standard deviation, to bee venom PLA₂ during immunotherapy.

Table 1. The mean IgG, IgG1 and IgG4 anti-PLA₂ antibody levels expressed as arbitrary units/ml, in ten patients during and after bee venom immunotherapy

	Anti-PLA ₂ antibody		
	IgG	IgG1	IgG4
Pre-treatment	5519	1400	560
3 months	31870	6800	18000
3 years	10785	1450	9400
End of treatment			
5 years	9325	1000	5800

Results

Using a rush immunotherapy regimen all patients reached the standardized maintenance dose of 100 μ g 7 days after the start of treatment. None reported any adverse reactions to the injections and all tolerated a live bee sting challenge 1, 2 and 3 years after the start of treatment and at 1 and 2 years after the termination of immunotherapy.

Following an initial rise in venom-specific IgE antibodies these fell to below pre-treatment levels in all patients (Fig. 1) and remained low subsequently. Venom-specific IgG antibodies to whole bee venom rose and subsequently fell. Termination of therapy after 3 years had little effect on specific IgE or IgG antibody levels and the live stings

too did not affect these, although samples were not taken shortly after the stings when a boost in antibody levels might be expected.

The sub-class of IgG antibody to bee venom PLA₂ was restricted to IgG1 and IgG4. We were unable to detect any IgG2 or IgG3 anti-PLA₂ activity (results not shown). The pattern of IgG sub-class antibody response to PLA₂ is shown in Fig. 2. During the early phase of treatment, the concentrations of IgG1 and IgG4 antibodies both rose although the increase in IgG4 antibody was much greater (Table 1). Continued injection of bee venom was paralleled by a marked fall in IgG1 antibody to pre-treatment levels while high IgG4 antibody levels, in contrast, persisted. The proportion of IgG antibody in each sub-class changed from predominantly IgG1 pre-treatment (7/10) to predominantly IgG4 post-treatment (9/10 (Fig. 2).

Discussion

The protective effect of bee venom immunotherapy may depend partly on IgE antibody suppression and partly on the production of venom-specific IgG antibodies [14]. These antibodies can block *in vitro* release of histamine from sensitized leukocytes (P. Clinton, Department of Biochemistry, Imperial College, unpublished observations) and reduce the response to venom challenge in sensitive patients [7]. This study was undertaken to investigate the effect of stopping bee venom immunotherapy on the immune response in ten children whose venom-specific IgE antibody levels had fallen to below pre-treatment levels. All patients tolerated an annual bee sting challenge during the 3 years of therapy and for 2 years post-treatment. The question, therefore, was whether the protective effect of IgG antibody could be characterized in terms of the IgG sub-class response. The sub-class of IgG antibody to bee venom PLA₂ was therefore measured and its relation to the long-term clinical protection provided by the treatment studied.

Studies by Aalberse and coworkers [8] using binding of I¹²⁵ radiolabelled PLA₂ to precipitated IgG sub-class protein, and in our laboratory with a modified ELISA technique (D.M. Kemeny unpublished observations) showed that IgG anti-PLA₂ activity was restricted to IgG1 and IgG4. During immunotherapy, both IgG1 and IgG4 antibody rose although the IgG4 antibody response was generally greater (Table 1).

By the time treatment was stopped, IgG1 antibodies had fallen to pre-treatment levels but the concentration of IgG4 antibodies was still raised. Similar findings have been reported by Aalberse *et al.* [8] in novice beekeepers and in our laboratory in allergic and non-allergic beekeepers (D.M. Kemeny, unpublished observations). All of the patients studied here remained clinically protected as shown by the sting challenge given at 1 and 2 years after termination of treatment. This lasting protection was associated with raised IgG4 antibodies, although we cannot exclude the possibility that the stings themselves helped to maintain this immunity. We have recently shown that IgG4 antibody is highly effective in blocking histamine release from the leucocytes of bee venom allergic patients (P. Clinton, unpublished observations).

It has been suggested that IgG4 antibodies might play a role in the pathogenesis of allergic reactions [15] based upon the presence of high IgG4 levels in asthmatics and in a single case following bovine factor VIII infusion [16]. IgG4 antibodies to bovine factor VIII have, however, been shown to block the ability of bovine factor VIII to promote clotting [17]. Our data and that of others [8–10] supports the view that IgG4

antibodies have a blocking rather than a sensitizing role. Indeed our results indicate that the IgG4 antibody level may be a valuable indicator of the final outcome of venom immunotherapy.

Acknowledgments

We would like to thank Miss Clare Langan for her excellent typing of this manuscript. This work was supported by Grant Deutsche für Schungsgeinschaft Ur 12/5-1.

References

- 1 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.
- 2 Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Lichtenstein LM. A controlled study of immunotherapy in insect hypersensitivity. *N Engl J Med* 1976; 157: 61.
- 3 Sobotka AK, Valentine MD, Ishizaka K, Lichtenstein LM. Measurement of IgG-blocking antibodies: development and application of a radioimmunoassay. *J Immunol* 1976; 117:84-90.
- 4 Kemeny DM, Mackenzie-Mills M, Harries MG, Youlten LJF, Lessof MH. Antibodies to purified bee venom proteins and peptides II. A detailed study of changes in IgE and IgG antibodies to individual bee venom antigens. *J Allergy Clin Immunol* 1983; 72:376-85.
- 5 Urbanek R, Forster J, Karitzky D, Ziupa J. The prognostic significance of specific IgG antibodies in insect sting allergy. *Eur J Pediatr* 1985; 136:31-4.
- 6 Urbanek R, Forster J, Kuhn W, Ziupa J. Discontinuation of bee venom immunotherapy in children and adolescents. *J Pediatr* 1985; 107:367-72.
- 7 Lessof MH, Sobotka AK, Lichtenstein LM. Protection against anaphylaxis in hymenoptera-sensitive patients by passive immunisation. *Monogr Allergy* 1977; 12:253-6.
- 8 Aalberse RC, Gaag Vander R, Leeuwen Van J. Serologic aspects of IgG4 antibodies. 1. Prolonged immunisation results in an IgG4-restricted response. *J Immunol* 1983; 130:722-6.
- 9 Giessen van der M, Herman WL, Kernbeck van G, Aalberse RC, Dieges PH. Subclass typing of IgG antibodies formed by grass pollen allergic patients during immunotherapy. *Int Archs Allergy Appl Immunol* 1976; 50:625-40.
- 10 Djurup R, Osterballe R. IgG subclass antibody response in grass pollen-allergic patients undergoing specific immunotherapy. *Allergy* 1984; 39:433-41.
- 11 Kemeny DM, Urbanek R, Samuel D, Richards D. Increased sensitivity and specificity of a sandwich ELISA for measurement of IgE antibodies. *J Immunol Methods* 1985; 78:217-26.
- 12 Urbanek R, Kemeny DM, Samuel D. Use of enzyme-linked-immunosorbent assay for measurement of allergen-specific antibodies. *J Immunol Methods* 1985; 79:123-31.
- 13 Kemeny DM, Urbanek R, Samuel D, Richards D. Improved sensitivity and specificity of sandwich, competitive and capture enzyme-linked immunosorbent assays for allergen-specific antibodies. *Int Archs Allergy Appl Immunol* 1985; 77:198-200.
- 14 Forster J, Urbanek R. Enzyme-linked immunosorbent assay of allergen-specific IgG antibodies in bee sting allergic patients hyposensitized with pure bee venom. *Klin Wochenschr* 1979; 57:421-2.
- 15 Gwynn CM, Morrison-Smith J, Leon Leon G, Stanworth DR. IgE and IgG4 subclass in atopic families. *Clin Allergy* 1979; 9:119-23.
- 16 Shakib F, Stanworth DR. IgG4: A possible mediator of anaphylaxis in a haemophilic patient. *Clin Allergy* 1979; 9:597-603.
- 17 Anderson BR, Terry WD. Gamma G4-Globulin antibody causing inhibition of clotting factor VIII. *Nature* 1968; 217:174-5.

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.