

A comparative study of IgG subclass antibodies in patients allergic to wasp or bee venom

Wilson AB, Deighton J, Lachmann PJ, Ewan PW. A comparative study of IgG subclass antibodies in patients allergic to wasp or bee venom. Allergy 1994; 49: 272–280. © Munksgaard 1994.

IgG1 and IgG4 antivenom antibody responses were compared in groups of patients who had experienced systemic reactions to wasp (*Vespula* spp.) or bee stings. Pretreatment serum IgG4 antibody levels were low in both groups, but IgG1 antibodies were significantly raised in bee-allergic patients ($P < 0.002$), probably reflecting their greater exposure to stings than wasp-reactive patients. No direct or indirect relationships were found, in untreated bee or wasp patients, between IgG1, IgG4, or IgE antibody levels and the severity of a patient's last systemic reaction to a sting. After a 12-week course of venom immunotherapy (VIT), IgG1 antibodies increased significantly only in wasp-sensitive patients ($P < 0.001$), although both groups responded with marked increases in venom-specific IgG4 ($P < 0.01$). Wasp-allergic subjects who responded to VIT with high production of specific IgG4 showed the greatest increases (pre- to post-VIT) in IgE antibodies ($P < 0.05$). This group also demonstrated a direct correlation ($P < 0.05$) between post-VIT levels of IgE and IgG1 antibodies, a finding contrary to an IgE-immunoregulatory role for IgG1. High levels of venom-specific IgG1 alone, or in combination with IgG4, were not protective in three patients who suffered repeated adverse reactions to bee VIT, showing that absolute levels of IgG subclass antivenom antibodies are not reliably indicative of clinical responsiveness in individual patients.

A. B. Wilson, J. Deighton,
P. J. Lachmann, P. W. Ewan

Molecular Immunopathology Unit, Medical
Research Council Centre, Cambridge, UK

Key words: bee venom; Hymenoptera; IgG subclasses; immunotherapy; wasp (*Vespula* spp.) venom.

Dr A. B. Wilson
MIP Unit, MRC Centre
Hills Road
Cambridge CB2 2QH
UK

Accepted for publication 6 July 1993

Treatment of severe allergic reactions by administering gradually increasing doses of the offending allergen was introduced more than 80 years ago by Noon (26), who used pollen extracts for the relief of hay fever. The highest success rates for this form of treatment are currently found with insect venom immunotherapy (VIT) (10). However, the immunologic processes by which symptoms of hypersensitivity are alleviated are not yet clearly defined. A reduction in IgE antibody production is obviously desirable, but this, alone, is not the decisive factor in successful therapy, since patients become hyporesponsive, or totally unresponsive, at a time when IgE antibody levels are still high. Other factors, therefore, must contribute to the successful outcome of treatment.

The most consistent response during immunotherapy (IT) with inhalant or parenteral allergens is an increased production of IgG antibodies which are primarily restricted to the IgG1 and IgG4 subclasses (1, 5, 6, 29). Allergen-specific IgG1 increases for a

relatively short time early in the course of treatment, and is followed by a greater and more persistent rise in IgG4 antibodies. In some reports, a correlation was noted between levels of IgG4 antibodies and the effectiveness of treatment with either insect venoms (29) or inhalant allergens such as those of house-dust mite (20, 24). Earlier studies had shown that IgG antibodies are capable of blocking IgE-mediated histamine release *in vitro* (18). This property was subsequently attributed to IgG4, which was found to inhibit IgE-dependent reactions, both *in vitro* and *in vivo* (25, 30). Further evidence supporting a protective role for IgG (?IgG4) came from two studies in which patients receiving bee VIT were protected from treatment-induced systemic reactions by the passive administration of gamma globulin fractions of nonallergic beekeepers' serum (2, 23).

The attribution of a protective role to IgG4 antibodies, however, remains controversial. Studies by Djurup et al. involving treatment of allergy to grass

pollen (8) and to insect venom (7) indicated that an early, rapid rise in specific IgG4, or a high posttreatment level, was associated with failure of IT. These authors also reported a correlation between high IgG1 antibody levels and reduction in specific IgE, suggesting that IgG1 may function as a negative feedback for IgE antibody synthesis. Another investigation (21) revealed an association between increased IgG1 antibodies and a decrease in clinical response to ryegrass pollen. More recently, Müller et al. (22) found that patients who reacted to a sting challenge in the course of bee VIT had higher prechallenge levels of IgG and IgG4 antibodies than nonreactors, and concluded that no single antibody estimation (G, G1, G4, or E) was predictive of responsiveness in any subject. Likewise, several groups have found no clear relationship between IgG4 (or IgG1) antibody levels and the ability of allergic patients to tolerate a bee or wasp sting (13, 19).

The work presented here forms part of a larger investigation of IgG antibody responses in patients allergic to bee or wasp venom. In a parallel study (9) we found that serum levels of total IgG antivenom antibodies do not show any clear correlation with protection from systemic reactions to stings. The present report details our findings on IgG1 and IgG4 antibody responses in patients undergoing VIT. Patients treated with bee or wasp venom are compared, and IgG subclass antibody levels are examined in relationship to venom-specific IgE antibody production and to the severity of clinical hypersensitivity reactions.

Material and methods

Patients

The study included 33 patients attending the insect sting allergy clinic (Addenbrooke's Hospital, Cambridge, UK) after experiencing systemic reactions to either bee or wasp stings. None of these patients had previously received IT. Diagnosis was made on the basis of clinical history combined with results of intradermal skin tests using amounts of venom equivalent to 1/25 of a sting ($\sim 2 \mu\text{g}$ bee venom protein or $\sim 0.2 \mu\text{g}$ wasp venom protein), and of assays measuring venom-specific serum IgE. Levels of IgE antibee and antiwasp venom were determined by a radioallergosorbent test (RAST; Pharmacia, Uppsala, Sweden) which gave results expressed in Phadebas RAST units/ml (PRU/ml). After diagnosis, each patient received subcutaneous provocation with venom equivalent to 1/12 of a sting, except for patients in whom unusually high sensitivity was expected and a lower dose was used.

Of the patients studied, 16 (11 female and five male, age range 32–58 years) were allergic to wasp venom, and 17 (10 female and seven male, age range

10–58 years) were hypersensitive to bee venom. Details were taken of the last generalized (systemic) reaction (GR) of each patient to a bee or wasp sting, and these were graded 1–8 as follows: 1, very severe: prolonged loss of consciousness; 2, severe: brief loss of consciousness, fainting, significant respiratory difficulty; 3, fainting, collapse, moderate to severe dyspnoea; 4, dyspnoea, light-headedness; 5, urticaria, angioedema, mild dyspnoea, abdominal symptoms; 6, urticaria, mild dyspnoea, or vomiting; 7, pruritus, urticaria, angioedema; 8, pruritus and/or mild urticaria, or minimal angioedema.

Immunotherapy regimen

All 16 of the wasp-reactive patients and 9/17 bee-sensitive patients proceeded to VIT. The latter group included nine beekeepers, six relatives or neighbours of beekeepers, and one fruit grower. Treatment consisted of paired injections (at 30-min intervals) of incremental doses of venom, administered weekly for the first 8 weeks, followed by single weekly injections up to the 12th week. Patients were then maintained on 3- or 6-monthly booster injections. During the initial 12-week course, doses of bee or wasp venom were increased from amounts equivalent to 1/50 of a sting up to two stings per injection. In cases of adverse systemic reactions, the dose schedule was modified. One bee-sensitive subject (RB) and two subjects (FR and PB) allergic to wasp venom underwent rush IT in which the 12-week course of VIT was condensed into a period of 2.5–3.5 h. This treatment was followed by weekly boosters for 4 weeks (RB, FR) or 12 weeks (PB), and then monthly injections over a period of 3 months (patient RB) or 7 months (FR), and subsequent maintenance on injections every 3 months.

Blood samples were taken from each patient, for serum separation, before and at the end of the 12-week course. Postinitial course sera from rush IT patients were obtained approximately 12 weeks after treatment began. All sera were stored at -20°C .

Venom preparations

Our own aqueous preparations were used as immunogens for IT. Bees or wasps were induced, by mild electric stimulation, to sting into a closed vessel containing phosphate-buffered saline (PBS), pH 7.4, with preservatives, 0.5% phenol and 20% propylene glycol. These venoms were analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and each was compared with two commercially available venom preparations (Fig. 1). All three honeybee venoms (tracks 5–7) appeared to be similar, except for the albumin added to Pharmedgen (Pharmacia) preparations. Our wasp venom

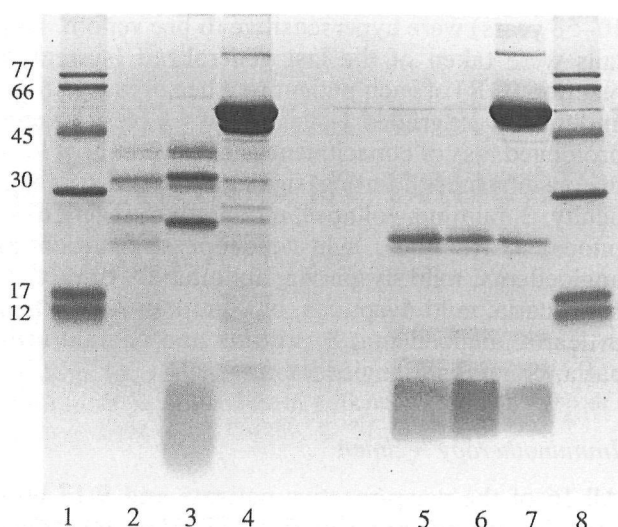


Fig. 1. Comparison of our own wasp and bee venoms with other, commercial, venom preparations by SDS-PAGE. Proteins separated on 10–20% polyacrylamide gradient under nonreducing conditions. Gel stained with Coomassie brilliant blue. Wasp venoms (tracks 2–4): 2 = *Vespula* spp., European mix (Vespa Lab.); 3 = our own wasp venom; 4 = *Vespula* spp. venom in albumin diluent (Pharmalgen). Bee venoms (tracks 5–7): 5 = honeybee, *A. mellifera* (Sigma); 6 = our own honeybee venom; 7 = Pharmalgen bee venom in albumin diluent. Tracks 1 and 8: mol.-mass markers (mol. mass $\times 10^{-3}$).

(track 3) contained the three principal allergens (antigen 5, phospholipase, and hyaluronidase) plus a 60-kDa band and low-mol.-mass peptides. In contrast, commercial *Vespula* venoms (tracks 2 and 4) which had been collected by venom sac extraction rather than by electrostimulation showed additional bands, presumably corresponding to sac proteins. The wasp venom (Vespa Laboratories, Spring Mills, CA, USA) in track 2 had been dialysed and lacked low-mol.-mass components.

Venom preparations used for patient skin-testing and therapy were standardized by measurement of their phospholipase activity with a haemolytic assay. For this, 5- μ l aliquots of titrated venom were placed in wells (3 mm diameter) in plates containing 1% washed guinea pig erythrocytes in borate-buffered saline with 1% agarose and 25 μ g/ml poly-L-lysine. After 2 h at room temperature (RT), areas of lysis were measured with an image analyser. This allowed estimation of new venom concentrations as fractions of undiluted whole bee or wasp venom. Absolute protein levels could not be measured because of assay interference by venom preservatives.

For the IgG subclass antibody assays, we used commercially available venoms. These were freeze-dried wasp venom (*Vespula germanica*, *V. vulgaris*) from Vespa Laboratories, Spring Mills, CA, USA (Lot no. 04268910YT), and bee (*Apis mellifera*) venom obtained from Sigma Chemical, Poole, UK.

The bee venom was depleted of melittin by passage through Sephadex G-100 (Pharmacia). Reasons for melittin removal are twofold; IgG antibodies against melittin are difficult to assay (14) and, since melittin represents 50% of the dry weight of bee venom, its presence on the ELISA plate would reduce the assay sensitivity for antibodies to the principal allergens in bee venom (phospholipase A₂, hyaluronidase, and acid phosphatase).

Reagents

WHO/IUIS standard reference monoclonal antibodies (Oxoid) specific for human IgG1 (clone NL16, HP6012) and IgG4 (RJ4, HP6011) were purchased from Unipath (Bedford, UK). Purified (>95%) human myeloma proteins IgG1 κ and IgG4 κ were obtained from the Binding Site (Birmingham, UK) and Calbiochem (Nottingham, UK), respectively. Foetal calf serum (FCS) was supplied by Globepharm (Esher, UK) and was heat inactivated at 56°C for 30 min before use. Goat antimouse IgG-alkaline phosphatase conjugate (adsorbed with human serum proteins), Tween 20, and *p*-nitrophenyl phosphate (*p*-NPP) were obtained from Sigma.

ELISA for IgG1 and IgG4 antibodies in patients' sera

Standard reference antisera against bee and wasp venom were prepared by pooling sera from 17 subjects with high-level IgG antibodies against bee venom, including allergic and nonallergic beekeepers, and from 15 patients producing IgG antiwasp venom as a result of IT. Levels of IgG1 and IgG4 antibodies in the standard pooled sera were quantified by a competitive ELISA modified from that developed by Rieben & Blaser (27) for measuring serum antibodies against bee venom phospholipase A₂. Briefly, antibodies bound from the standard human serum pools against bee- or wasp-venom-coated microtitre plates were reacted with monoclonal anti-IgG1 (NL16) or anti-IgG4 (RJ4) in the presence of titrated amounts of the corresponding purified human IgG subclass myeloma protein. The concentrations of bound IgG1 or IgG4 antivenom antibodies were then calculated from the derived inhibition curves. In triplicate assays, amounts of antibodies in the antiwasp venom pool were determined for IgG1 as (mean $\pm$ SD) 12.0 $\pm$ 0.7 μ g/ml and for IgG4 as 54.0 $\pm$ 2.9 μ g/ml. The standard pool antbee venom serum contained IgG1 antibodies at 15.6 $\pm$ 0.1 μ g/ml and an IgG4 antibody level of 107.2 $\pm$ 5.3 μ g/ml.

For quantification of antibodies in patients' sera, microtitre plates (96-well M129B; Dynatech, Billingshurst, UK) were coated with wasp venom (5 μ g/ml) or with melittin-depleted bee venom (10 μ g/ml)

in 0.05 M carbonate-bicarbonate buffer, pH 9.6, (100- μ l aliquots/well) overnight at 4°C. The plates were then washed three times with PBS containing 0.05% Tween 20 (PBST), and were blocked for 1 h at RT with 5% heat-inactivated FCS in PBST. For each plate, the appropriate reference human antiserum was double-diluted in PBST-FCS, with initial dilutions from 1/200 to 1/800 according to the type of venom (bee or wasp) and IgG subclass antibody under assay. Test sera expected to have low levels of antibodies (e.g., pretreatment bleeds) were titrated from 1/25 dilution, and others from 1/100 or higher. All dilutions were performed in duplicate, and negative control serum and "blanks" without human serum were included on every plate. After 5 h at RT, plates were washed three times with PBST. Monoclonal anti-IgG1 (NL16, 1/1000) or anti-IgG4 (RJ4, 1/4000) diluted in PBST-FCS was added, and left overnight at 4°C. The plates were washed three times before reacting with alkaline phosphatase-conjugated goat antimouse IgG (1/600 in PBST-FCS) for 4 h at RT. After three washes with PBST and a final wash with Tris (0.05 M)-buffered sodium chloride (0.15 M), pH 7.4, the *p*-NPP enzyme substrate (1 mg/ml in diethanolamine buffer, pH 9.8) was added. Plates were incubated for 1 h at either RT (bee venom assays) or at 37°C (wasp venom assays). Reactions were stopped by the addition of 50 μ l/well 3 M sodium hydroxide, and their absorbance at 405 nm was measured with a Bio-Rad microplate reader. Concentrations of IgG1 or IgG4 antibodies were determined (μ g/ml) from the standard reference antisera by linear regression analysis. The assays were sensitive to 1–2 ng/ml antibody, and gave intra-assay coefficients of variation (% CV) ranging from 2.1 to 7.9 and interassay % CV of 6.4–16.6.

Results

Pretreatment levels of IgG1 and IgG4 antivenom antibodies

Levels of venom-specific IgG1 and IgG4 were determined, before treatment, in sera of 16 wasp-allergic and 17 bee-allergic patients. As seen in Fig. 2, the amounts of IgG subclass antibodies in these patients varied widely, particularly within the bee-sensitive group. In the two patient groups, those reacting to bee stings presented initially with significantly higher mean levels of venom-specific IgG1. In bee patients, the geometric mean value (95% confidence intervals (CI) for IgG1 antibodies was 9.4 μ g/ml (5.2–17.2), as compared with 2.1 μ g/ml (1.1–4.1) in wasp-sensitive subjects (t-test for small samples; $P < 0.002$). No significant difference was found between the two groups in levels of IgG4 antibodies, which averaged 2.6 μ g/ml (1.0–6.9) in bee-allergic

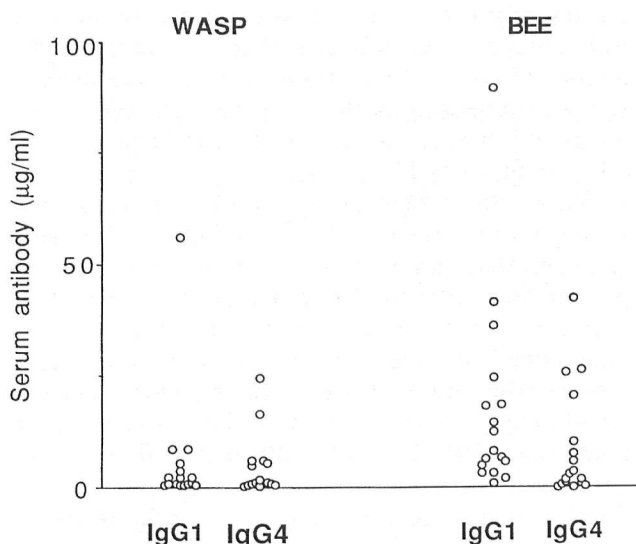


Fig. 2. Pretreatment serum levels of IgG1 and IgG4 antivenom antibodies in 16 wasp-allergic and 17 bee-allergic patients.

patients and 1.8 μ g/ml (0.8–3.8) in those reacting to wasp stings.

The relative concentrations of IgG1 and IgG4 anti-bee or antiwasp venom antibodies in the serum of each untreated subject are shown in Fig. 3. Rather surprisingly, IgG4 antibodies predominated in 6/16 wasp patients and in 6/17 bee patients. Dominance of the response by IgG4 was not necessarily linked to high overall levels of IgG antibody. In fact, the patient with the highest total of antivenom IgG in each group (patients CO, wasp; JS, bee) had 95% antibody of the IgG1 isotype.

IgG subclass antibody levels and previous exposure to stings

A history was taken from each patient presenting at the clinic, to obtain details of the numbers of stings received and the time over which they had occurred. One bee-sensitive person, a beekeeper (HK), claimed to have had more than 200 stings, a number which far exceeded those reported by other patients in either the bee group (range 2–42 stings) or the wasp group (range 1–10 stings). The data from this subject were therefore excluded from statistical analyses.

Wasp-allergic patients tended to have been stung over a longer period (five patients, <2 years; four, 2–19 years; and seven, 20–30 years) than bee-reactive patients (eight, <2 years; six, 2–19 years; and two, 20–25 years). Moreover, comparison of total numbers of stings received showed that, on average, wasp patients had been stung on fewer occasions (mean $\pm$ SD, 4.5 $\pm$ 3.8 stings) than had the bee-allergic patients (15.6 $\pm$ 13.2 stings). By use of

log transformed data, necessitated by the unequal SD values for total sting numbers, a significant difference ($P < 0.001$) was found between numbers of stings experienced by the wasp-sensitive (geometric mean, 95% CI; 3.2 stings, 2.0–5.1) and bee-sensitive (10.7 stings, 8.5–13.5) groups.

Within the patient groups, only one statistically significant relationship was found between IgG subclass antibody levels and the variables of sting exposure (including total number of stings, length of time exposed, mean number of stings per year, and time since last sting). This was a direct correlation between the total number of stings and the levels of circulating IgG4 antibodies found in bee-allergic patients ($r = 0.506$; $P < 0.05$). When this group of patients was subdivided on the basis of having a predominance of either IgG1 ($n = 10$) or IgG4 ($n = 4$) antivenom antibodies, the latter group had experienced a significantly higher number of stings ($P < 0.05$).

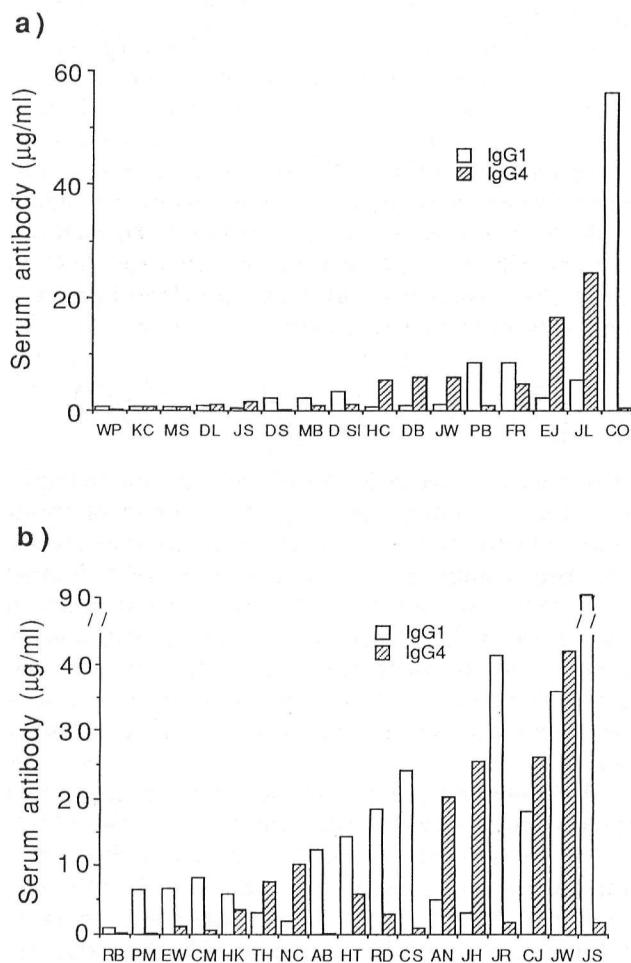


Fig. 3. Comparison of IgG1 and IgG4 venom-specific antibody levels in individual untreated subjects who had experienced systemic reactions to a) wasp, or b) bee stings.

Effect of immunotherapy on IgG1 and IgG4 antibody production

Changes in IgG1 and IgG4 antibody levels induced by the 12-week course of VIT in wasp- and bee-allergic patients are shown in Fig. 4. After therapy, IgG1 antibodies were significantly raised ($P < 0.001$; one-tail t-test based on null hypothesis) in patients treated with wasp venom, increasing to a geometric mean level (95% CI) of 8.9 $\mu\text{g/ml}$ (5.5–14.4). In patients receiving bee venom, however, the mean level of specific IgG1 was 13.8 $\mu\text{g/ml}$ (7.2–26.5), which was not significantly different from concentrations in pretreatment sera. As expected, the levels of antivenom IgG4 rose significantly ($P < 0.01$) in both wasp- and bee-allergic groups, and were measured as 22.9 $\mu\text{g/ml}$ (11.6–45.1) and 33.1 $\mu\text{g/ml}$ (13.9–79.1), respectively. The difference between the changes in pre- to posttreatment levels of IgG1 (but not of IgG4) antibodies in wasp and bee patients was statistically significant (ANOVA; $P < 0.02$).

IgG subclasses and IgE antivenom antibodies

Serum levels of IgE antibodies against bee or wasp venom were measured by RAST, both before treatment and on completion of the initial 12-week course of VIT. Serum concentrations of venom-specific IgE corresponding to the full range of RAST classes (0, 1, 2, 3, and 4) were found in both the bee- and wasp-allergic patient groups. Absolute levels of IgE antibodies for each subject, pre- and posttreatment, were calculated in PRU/ml. One bee-sensitive patient (HT) had a much higher initial level of venom-specific IgE (> 100 PRU/ml) than others in the group, and was omitted from statistical analyses.

Before IT, IgE antibody levels ranged from 0.23 to 52.5 PRU/ml in wasp-sensitive patients, and from 0.24 to 16.8 PRU/ml in bee patients. Pretreatment

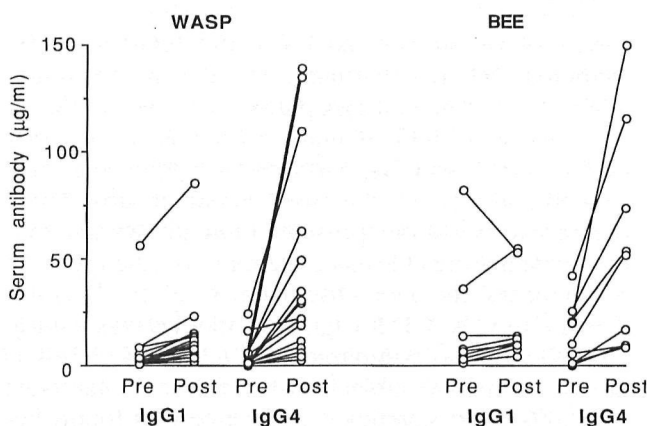


Fig. 4. Changes in IgG subclass antibody levels induced by 12-week course of IT with wasp or bee venom.

geometric mean values (95% CI) for specific IgE were 1.68 PRU/ml (0.62–4.57) for wasp-allergic subjects, and 2.04 PRU/ml (0.51–8.11) for those reactive to bee stings. Post-VIT, IgE antivenom antibody levels rose in some patients (11/16 wasp; 4/9 bee) but fell in others, giving geometric mean values (95% CI) of 2.45 PRU/ml (0.88–6.82) in wasp patients' sera, and 1.77 PRU/ml (0.51–6.14) in those of bee patients. Overall, there was no significant change in IgE antibody levels in either of the patient groups during the 12-week course of therapy.

Nonparametric statistical analysis (Spearman's rank test) was used to examine interrelationships between antibody isotypes. No correlation was found between pretreatment levels of IgE antibodies and those of either IgG1 or IgG4 subclasses. After IT, there was a positive correlation between serum concentrations of IgE and IgG1 antibodies in the wasp patient group ($r_s = 0.582$; $P < 0.05$), but no other relationships were found. The changes occurring in IgE antibodies during treatment were also examined in relation to levels of IgG1 and IgG4 antibodies (pre- and post-VIT). The only significant correlation which emerged was that between the change in IgE and the posttreatment levels of IgG4 antibodies in wasp-reactive patients ($r_s = 0.613$; $P < 0.05$). This indicated that subjects responding to VIT with high levels of IgG4 also showed the greatest increases in venom-specific IgE.

Antibody levels and clinical hypersensitivity

For examination of any influence of IgG1 or IgG4 (?protective) antibodies on allergic reactions to insect venoms, responsiveness of patients was assessed in two ways; either by the extent of their last GR to a bee or wasp sting, or by their record of adverse reactions during VIT.

Sting-induced GR were graded 1–8 in order of diminishing severity, as detailed in Material and methods. The pattern of patients' reactivity, together with pretreatment levels of IgG1/IgG4 and IgE antivenom antibodies, is shown in Fig. 5. There was no apparent interrelationship between IgG1 or IgG4 with IgE antibodies and the severity of reactions to stings. In addition, the analysis of data by contingency tables showed no correlation (Fisher's exact test) between low ($< 5 \mu\text{g/ml}$) or higher serum concentrations of either IgG1 or IgG4 antibodies and the development of mild (grades 5–8) or severe (grades 1–4) systemic reactions.

During the initial course of VIT, a number of patients developed GRs to venom inoculations. In the bee-sensitive group, one patient (JS) developed repeated reactions (urticaria, asthma) early in the course of conventional treatment, while another patient (JW) had reactions (fall in blood pressure, light-

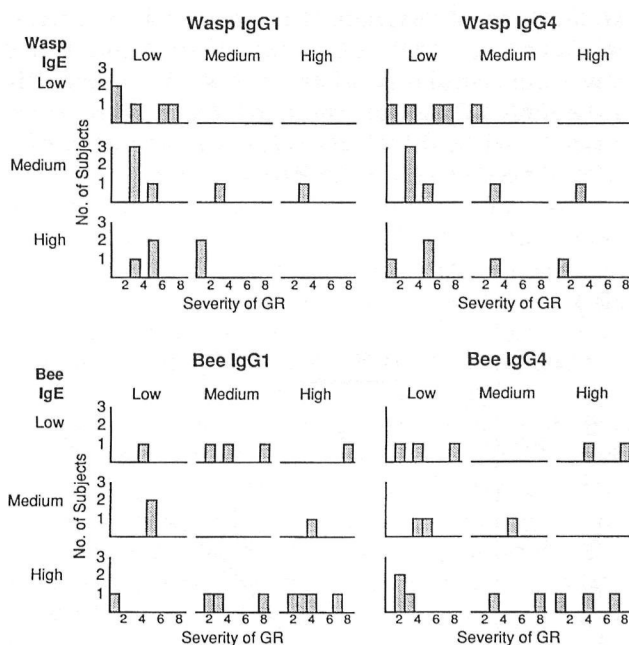


Fig. 5. Interrelationship of IgG1 and IgG4 pretreatment serum concentrations (low, $< 5 \mu\text{g/ml}$; medium, $5\text{--}15 \mu\text{g/ml}$; high, $> 15 \mu\text{g/ml}$) with IgE antivenom antibodies (RAST classes: low, 0–1; medium, 2; high, 3–4) and severity of patients' last generalized reaction (GR; graded severe–mild, 1–8; see Material and methods). One bee-allergic patient was omitted because of incomplete data.

headedness, rhinitis, urticaria) to higher doses of venom, which continued during maintenance therapy (Fig. 6). A third patient (AN) developed severe GRs during bee VIT. Two of these bee-allergic patients who experienced severe GRs had moderate to high pretreatment levels of IgG4 antibee venom antibodies (AN, $20.4 \mu\text{g/ml}$; JW, $42.1 \mu\text{g/ml}$), while the third was lower (JS, $1.7 \mu\text{g/ml}$). All three patients also had elevated levels of IgG1 antibodies (AN, $5.0 \mu\text{g/ml}$; JW, $36.1 \mu\text{g/ml}$; JS, $89.7 \mu\text{g/ml}$). Patient JW continued to experience adverse reactions during maintenance therapy despite having even higher antibody levels (IgG1, $54.8 \mu\text{g/ml}$; IgG4, $115.7 \mu\text{g/ml}$) after the 12-week course. This patient, like the other two with severe adverse reactions to bee VIT, had high levels of venom-specific IgE which was RAST class 4, with 16.8 PRU/ml pre-VIT and 18.9 PRU/ml posttreatment. The IgG4:IgE antibody ratio in this patient increased (pre- to post-VIT) from 2.23 to 6.13. In contrast, four wasp-sensitive patients (MB, DB, KC, and JL) with high IgE venom-specific antibodies (RAST classes 3 or 4; $4.9\text{--}52.5 \text{ PRU/ml}$) and low to moderate IgG4 antibody levels of $0.7, 0.9, 6.1$, and $24.4 \mu\text{g/ml}$ (giving IgG4:IgE ratios of $0.017, 0.135, 0.052$, and 5.02 , respectively) experienced only mild reactions during conventional VIT. Both of the wasp-allergic patients (FR and PB) who developed severe GRs were receiving rush VIT

at the time of reaction. They each had moderate levels of IgG1 (FR, 8.6 $\mu\text{g/ml}$; PB, 8.7 $\mu\text{g/ml}$) and lower concentrations of IgG4 (FR, 4.8 $\mu\text{g/ml}$; PB, 1.0 $\mu\text{g/ml}$). Their IgE antibody levels were either moderate (PB, RAST class 2; 0.7 PRU/ml) or high (FR, RAST class 4; 23.5 PRU/ml).

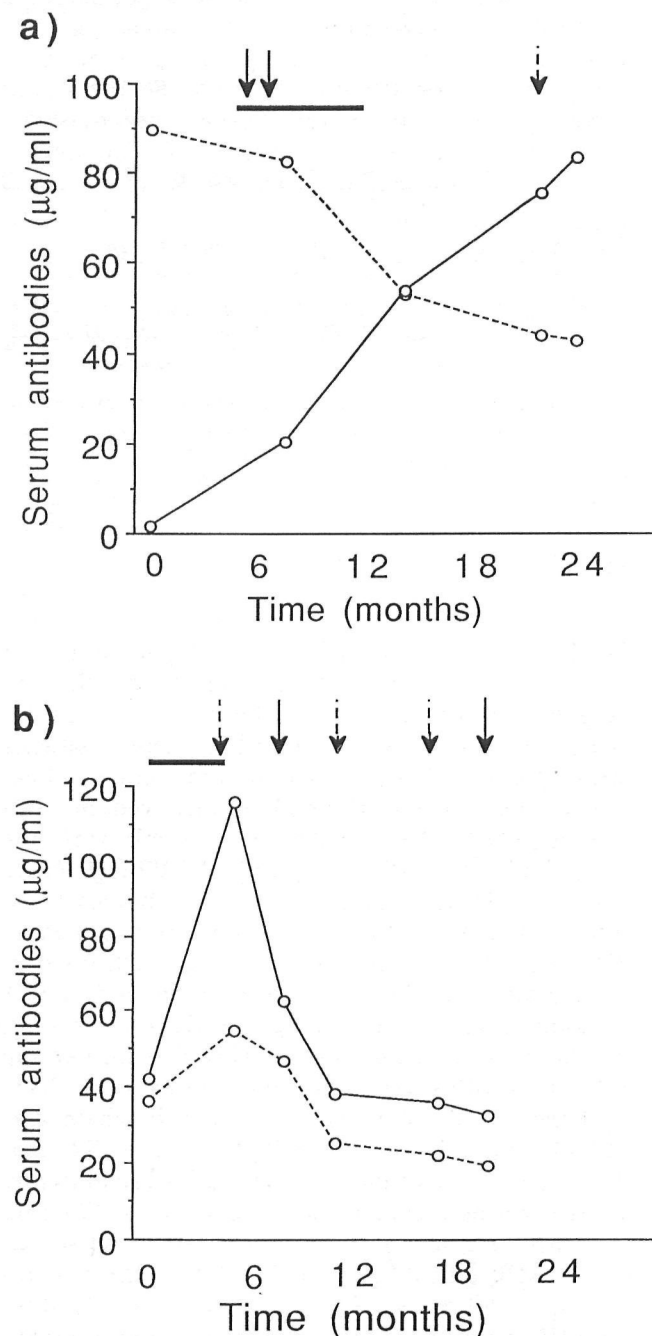


Fig. 6. Two-year profiles of IgG subclass antibodies (IgG1 $\circ$ ----- $\circ$, IgG4 $\circ$ ----- $\circ$) in sera of two subjects, a) JS and b) JW, who experienced repeated systemic reactions during conventional regimen bee VIT. Period of initial course VIT indicated by bold line; occurrence of adverse reactions shown as severe ($\downarrow$) or mild ($\downarrow$).

Discussion

Our previous observations on IgG antibodies (9) and those of Kemeny et al. (15) showed differences in the pattern of venom-specific IgG responses between patients allergic to bee or wasp stings. Most wasp-sensitive patients presented with low serum levels of IgG antibodies which increased significantly with VIT, but those responding to bee stings had high initial levels of antivenom IgG which changed little with treatment. From the present IgG subclass antibody assays, it is apparent that these differences are primarily reflected in the IgG1, rather than in the IgG4, antibody responses of bee- and wasp-sensitive patients. Levels of antivenom IgG1 were significantly higher ($P < 0.002$) in the untreated bee-allergic group and rose proportionately little with VIT, while wasp-allergic patients had low starting levels of IgG1 antibodies which increased significantly after treatment ($P < 0.001$). Both groups, however, showed significant rises in IgG4 antibody production after VIT ($P < 0.01$).

The differences in pretreatment levels of IgG1 antibodies in bee and wasp patients presumably reflected the extent of their prior exposure to venom antigens, since the bee-allergic group had been stung more frequently ($P < 0.001$) and, in general, over a shorter period of time. No direct correlation could be found for individual patients, however, between IgG1 antibody levels and their history of insect stings, although IgG4 antibody production in bee-sensitive subjects appeared to reflect the number of stings experienced ($P < 0.05$). Any association between antibody levels and number or frequency of stings is probably more difficult to show for wasp-allergic patients since the amount of protein injected per sting is much more variable for wasps than for bees (11).

Examination of antivenom antibodies in pre-VIT sera showed no correlation between levels of either IgG1 or IgG4 and IgE antibodies. Similarly, after VIT, no relationship was found between these antibody isotypes in bee-allergic patients. Those who had undergone wasp VIT, however, showed a direct correlation between posttreatment levels of IgG1 and IgE venom-specific antibodies ($P < 0.05$). This finding is contrary to the model for IgG1 downregulation of IgE allergen-specific antibodies proposed by Djurup et al. (7, 19) in their studies of different regimens for VIT. From their model, it would be expected that an increase in production of IgG1 antibodies would shortly be followed by a fall in specific IgE. Djurup et al.'s report (7) shows IgE antibody levels beginning to fall less than 1 month after the initial rise in IgG1 antibodies. It is almost certain, therefore, that if such a correlation had occurred in our patients, it would have been apparent by the end of the 3-month

course of VIT. It could still be argued that rising levels of IgG4 antibodies in these patients would compete with IgG1 and prevent the formation of IgE-regulatory immune complexes. However, we observed (results not shown) that IgE antibody levels increased or remained unchanged in three patients with moderate to high pretreatment IgG1 antiwasp venom antibodies (8.6–56.1 µg/ml) and lower levels of venom-specific IgG4 (0.5–4.8 µg/ml). This indicates that a predominance of IgG1 antibodies does not necessarily lead to a reduction in allergen-specific IgE. More detailed studies of antibody responses made by each patient to individual venom proteins, e.g., by immunoblotting methods (12), are required to give further information on this aspect of IgE regulation.

The present work supports other findings (13, 22) that absolute levels of IgG1 and IgG4 antibodies are of questionable value in predicting a patient's degree of clinical hypersensitivity. No correlation was found between IgG subclass antibodies, either alone or in combination with IgE antibody levels, and the severity of a patient's last systemic reaction to a bee or wasp sting, nor were they reliable in predicting susceptibility to adverse reactions during VIT. Most patients receiving conventional regimen wasp VIT tolerated the treatment course well, with only mild systemic or local reactions regardless of their IgE antibody levels and whether they had high or low initial levels of antivenom IgG1 or IgG4. In the bee-sensitive group, those responding adversely to VIT had high venom-specific IgE (RAST classes 3–4), as found in another study on reactors to bee VIT (14). They also had high levels of IgG4 and/or IgG1 antibodies. As shown in Fig. 6, two patients who experienced repeated GRs to bee venom had very high serum concentrations of IgG1 antibodies (JS, 89.7 µg/ml; JW, 54.8 µg/ml) at the time of reactions, and one of them (JW) had an extremely high level of IgG4 antibody (115.7 µg/ml). A third bee-sensitive patient (AN) also had severe systemic reactions despite relatively high serum levels of IgG4 (20.4 µg/ml) and IgG1 (5.0 µg/ml) antibodies. Thus, although the increase in level of IgG4 antibody correlated with reduced sensitivity to bee venom in patient JS, high levels of antivenom IgG4 were not protective in patients JW or AN. Patient JW also had an IgG4:IgE antibody ratio much greater than that seen in wasp-sensitive patients who had correspondingly high venom-specific IgE, and who experienced few problems with VIT.

It is apparent from these findings that high levels of circulating IgG1 or IgG4 antibodies are not, *per se*, protective against anaphylactic reactions to venom inoculation. If IgG antibodies have a role in the abatement of allergic responses, as suggested by passive immunization (2, 23), then other, qualitative

properties of these antibodies must be involved. These are likely to include changes in the relative functional affinities of IgG1, IgG4, and IgE antibodies, as described by Devey et al. (4) in their study of patients receiving bee VIT. Alternatively, there is evidence that production of IgG and IgE specific for a particular allergen is independently regulated and may be switched on or off at different times (14), conceivably leading to a situation where there may be missing specificities in IgG antibodies, as compared with the IgE repertoire (23). Additional possibilities are that long-term immunotherapy induces the production of IgG autoantibodies, either as anti-idiotypes reactive with allergen-specific antibodies (3, 17), or as specific anti-IgE (28), although a role for the latter was not supported in a recent investigation on wasp VIT patients (16). Because of differences in the sites of reaction, IgG antibodies may have a more significant role in protection against allergy to parenteral antigens, such as insect venom, as opposed to inhalant allergens. In any event, we believe that a clearer definition of any protective effect of IgG antibodies will come from further analyses of their qualitative properties rather than from straightforward quantification.

Acknowledgment

This work was supported by the Medical Research Council of Great Britain.

References

1. AALBERSE RC, VAN DER GAAG R, LEEUWEN J. Serologic aspects of IgG antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J Immunol* 1983; 130: 722–6.
2. BOUSQUET J, FONTEZ A, AZNAR R, ROBINET-LEVY M, MICHEL F-B. Combination of passive and active immunization in honeybee venom immunotherapy. *J Allergy Clin Immunol* 1987; 79: 947–54.
3. CASTRACANE JM, ROCKLIN RE. Detection of human auto-anti-idiotypic antibodies (Ab2). II. Generation of Ab2 in atopic patients undergoing allergen immunotherapy. *Int Arch Allergy Appl Immunol* 1988; 86: 295–302.
4. DEVEY ME, LEE SR, RICHARDS D, KEMENY DM. Serial studies on the functional affinity and heterogeneity of antibodies of different IgG subclasses to phospholipase A₂ produced in response to bee-venom immunotherapy. *J Allergy Clin Immunol* 1989; 84: 326–30.
5. DEVEY ME, WILSON DV, WHEELER AW. The IgG subclasses of antibodies to grass pollen allergens produced in hay fever patients during hyposensitization. *Clin Allergy* 1976; 6: 227–36.
6. DJURUP R. The subclass nature and clinical significance of the IgG antibody response in patients undergoing allergen-specific immunotherapy. *Allergy* 1985; 40: 469–86.
7. DJURUP R, MALLING H, SØNDERGAARD I, WEEKE B. The IgE and IgG subclass antibody response in patients allergic to yellow jacket venom undergoing different regimes of venom immunotherapy. *J Allergy Clin Immunol* 1985; 76: 46–55.
8. DJURUP R, ØSTERBALLE O. IgG subclass antibody response in grass pollen-allergic patients undergoing specific immunotherapy. *Allergy* 1984; 39: 433–41.

9. EWAN PW, DEIGHTON J, WILSON AB, LACHMANN PJ. Venom-specific IgG antibodies in bee and wasp allergy: lack of correlation with protection from stings. *Clin Exp Allergy* 1993; 23: 647-60.
10. GOLDEN DBK, KAGEY-SOBOTKA A, VALENTINE MD, LICHTENSTEIN LM. Dose dependence of Hymenoptera venom immunotherapy. *J Allergy Clin Immunol* 1981; 67: 370-4.
11. HOFFMAN DR, JACOBSON RS. Allergens in Hymenoptera venom. XII. How much protein is in a sting? *Ann Allergy* 1984; 52: 276-8.
12. JEEP S, MEYSEL U, KUNKEL G. IgE, IgG, IgG1 and IgG4 patterns in yellow jacket allergic patients during immunotherapy with a venom depot extract. *Clin Exp Allergy* 1992; 22: 297-302.
13. KAMPELMACHER MJ, VAN DER ZWAN JC. Provocation test with a living insect as a diagnostic tool in systemic reactions to bee and wasp venom: a prospective study with emphasis on the clinical aspects. *Clin Allergy* 1987; 17: 317-27.
14. KEMENY DM, KAGEY-SOBOTKA A, LICHTENSTEIN LM, LESSOF MH. IgE and IgG antibody response to purified bee-venom antigens and peptides in four patients who had adverse reactions to immunotherapy. *Clin Allergy* 1988; 18: 79-84.
15. KEMENY DM, LESSOF MH, PATEL S, YOULTEN LJF, WILLIAMS A, LAMBOURN E. IgG and IgE antibodies after immunotherapy with bee and wasp venom. *Int Arch Allergy Appl Immunol* 1989; 88: 247-9.
16. KEMENY DM, TOMIOKA H, TSUTSUMI A, KOIKE T, LESSOF MH, LEE TH. The relationship between anti-IgE auto-antibodies and the IgE response to wasp venom during immunotherapy. *Clin Exp Allergy* 1990; 20: 67-9.
17. KHAN RH, SZEWCZUK MR, DAY JH. Bee venom anti-idiotypic antibody is associated with protection in beekeepers and bee sting-sensitive patients receiving immunotherapy against allergic reactions. *J Allergy Clin Immunol* 1991; 88: 199-208.
18. LICHTENSTEIN LM, HOLTZMAN NA, BURNET LS. A quantitative *in vitro* study of the chromatographic distribution and immunoglobulin characteristics of human blocking antibody. *J Immunol* 1986; 101: 317-24.
19. MALLING H-J, DJURUP R, SØNDERGAARD I, WEEKE B. Clustered immunotherapy with yellow jacket venom. Evaluation of the influence of time interval on *in vivo* and *in vitro* parameters. *Allergy* 1985; 40: 373-83.
20. MCHUGH SM, LAVELLE B, KEMENY DM, PATEL S, EWAN PW. A placebo-controlled trial of immunotherapy with two extracts of *Dermatophagoides pteronyssinus* in allergic rhinitis, comparing clinical outcome with changes in antigen-specific IgE, IgG and IgG subclasses. *J Allergy Clin Immunol* 1990; 86: 521-32.
21. MOSS RB, HSU Y-P, KWASNICKI JM, SULLIVAN MM, REID MJ. Isotypic and antigenic restriction of the blocking antibody response to ryegrass pollen: correlation of rye group I antigen-specific IgG1 with clinical response. *J Allergy Clin Immunol* 1987; 79: 387-98.
22. MÜLLER U, HELBLING A, BISCHOF M. Predictive value of venom-specific IgE, IgG and IgG subclass antibodies in patients on immunotherapy with honeybee venom. *Allergy* 1989; 44: 412-18.
23. MÜLLER UR, MORRIS T, BISCHOF M, FRIEDLI H, SKVARIL F. Combined active and passive immunotherapy in honeybee-sting allergy. *J Allergy Clin Immunol* 1986; 78: 115-22.
24. NAKAGAWA T, KOZEKI H, KATAGIRI J, et al. Changes of house dust mite-specific IgE, IgG and IgG subclass antibodies during immunotherapy in patients with perennial rhinitis. *Int Arch Allergy Appl Immunol* 1987; 82: 95-9.
25. NAKAGAWA T, DE WECK AL. Membrane receptors for the IgG4 subclass on human basophils and mast cells. *Clin Rev Allergy* 1983; 1: 197-206.
26. NOON L. Prophylactic inoculation against hay fever. *Lancet* 1911; i: 1572-3.
27. RIEBEN R, BLASER K. Quantification of IgG and IgG4 antibodies to bee venom phospholipase A₂ by competitive inhibition in ELISA. *J Immunol Methods* 1989; 119: 1-8.
28. SHAKIB F, MILLS CS, POWELL-RICHARDS A. The recognition of a recombinant human Fc_γ fragment by the subclasses of IgG autoanti-IgE: disproportional subclass distribution of complexed autoantibody. *Int Arch Allergy Appl Immunol* 1992; 97: 243-8.
29. URBANEK R, KEMENY DM, RICHARDS D. Subclass of IgG anti-bee venom antibody produced during bee venom immune therapy and its relationship to long-term protection from bee stings and following termination of venom immunotherapy. *Clin Allergy* 1986; 16: 17-22.
30. VIJAY HM, PERELMUTTER L. Inhibition of reagin-mediated PCA reactions in monkeys and histamine release from human leukocytes by human IgG4 subclass. *Int Arch Allergy Appl Immunol* 1977; 53: 78-87.

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.