

# The Immune Response to Bee Venom

## Comparison of the Antibody Response to Phospholipase A<sub>2</sub> with the Response to Inhalant Antigens

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**Abstract.** The antibody response to phospholipase A<sub>2</sub> (PLA<sub>2</sub>) was studied by using radiolabelled antigen-binding techniques for both IgG and IgE antibodies. The results are compared with results for antigen P<sub>1</sub> from *Dermatophagoides pteronyssinus* and for rye I from rye grass pollen. The response to PLA<sub>2</sub> differs from the response to the inhalant allergens in several ways: almost all exposed bee keepers produce IgG antibodies to PLA<sub>2</sub>; among the allergic bee keepers there was no quantitative correlation between IgG and IgE antibodies; and the antibodies to PLA<sub>2</sub> are not produced locally in the nose.

Bee keepers with hay fever did not appear to have an increased incidence of allergic reactions to bee venom. In addition, the bee keepers with hay fever had the same levels of IgG and IgE antibodies to rye I as other individuals with hay fever. However, the bee keepers who had no history of hay fever were found to have high levels of IgG antibodies to rye I. These antibodies did not cross-react with PLA<sub>2</sub> or bee venom, and the results suggest that bee stings may act as an adjuvant for IgG antibodies to pollen antigens.

## Introduction

Bee keepers and their families may be stung frequently during the summer months [1]. Some become allergic to bee venom, whilst others are able to tolerate large numbers of stings without ill effect. It has been shown that the majority of bee keepers make IgG antibodies to bee venom [2-4], while only the allergic bee keepers have high titres of IgE antibodies [2-6]. These antibodies may be directed against a number of bee venom antigens: acid phosphatase [7-9], hyaluronidase [10, 11], phospholipase A<sub>2</sub> (PLA<sub>2</sub>) [12, 13] and melittin [14, 15]. Of these venom components, PLA<sub>2</sub> is the most important allergen, giving rise to the highest frequency and highest titres of both IgG and IgE antibodies to bee venom [11]. PLA<sub>2</sub> is also quantitatively the major protein of bee venom [16, 17]. Previous investigations in our department suggested that the allergic response of bee keepers to bee venom was related to their personal and family history of allergy [18]. In order to study the relationship between bee venom and inhalant allergy in more detail, we have measured IgE and IgG antibodies to two inhalant antigens, antigen P<sub>1</sub>, from *Dermatophagoides pteronyssinus*, and the group I protein from rye grass pollen, rye I, as well as to PLA<sub>2</sub> from bee venom. Antibodies to these antigens were measured using a class specific radiolabelled antigen-binding assay [19, 20]. Antibodies to these allergens in bee keepers were compared with those of hay fever patients, volunteers who were not allergic to grass pollen, and farmers.

## Materials and Methods

### Subjects Studied

137 bee keepers and their families were questioned about their reaction to bee stings and their personal history of allergic disease. 44 had had a general reaction within the past 3 years, 39 a severe local reaction, and 54 no reaction. A general reaction was taken as one or more of the followings: (i) generalized skin rash or swelling; (ii) wheezing or difficulty in breathing; (iii) vomiting, diarrhoea, or abdominal pain; (iv) swelling of the tongue or throat, and (v) loss of consciousness; occurring after not more than 5 simultaneous stings. A severe local reaction was defined as a swelling of 2 or more inches, at the site of a single sting.

The sera from bee keepers were compared with sera from 25 non-allergic laboratory staff, with 45 patients with hay fever, and 19 farmers who did not have hay fever. Histories and skin test results were available for all these individuals. In addition, serum was

available from 44 pregnant mothers who had no history of hay fever but who had not been skin tested.

### Measurement of Antibodies to Rye I, PLA<sub>2</sub>, and P<sub>1</sub>

IgG and IgE antibodies to the purified antigens: rye I from rye grass pollen (kind gift from Dr. D. Marsh, Johns Hopkins University, Baltimore); antigen P<sub>1</sub> (kind gift from Dr. M.D. Chapman, London School of Hygiene and Tropical Medicine, London) and PLA<sub>2</sub> from bee venom (kind gift from Prof. C.A. Vernon, University College, London), were measured by an antigen-binding radioimmunoassay previously described in full [19, 20]. Briefly, 0.1 ml of a dilution (1/12.5, 1/25) of patient's serum was incubated with 7 ng of <sup>125</sup>I-labelled antigen for 4 h at room temperature. Specific antibody was precipitated overnight at 4°C using monospecific goat anti-IgE or anti-IgG. For the IgE antigen-binding assay, 0.1 ml of a 1/100 dilution of PS IgE myeloma serum was added to form an IgE/anti-IgE precipitate. The precipitates were washed 4 times with borate-buffered saline pH 8.0 and the radioactivity counted in a γ-counter (NE 1600, Nuclear Enterprises, Edinburgh, Scotland). The assay was quantitated by reference to a standard curve which was obtained by assaying IgG binding activity in serial two-fold dilutions of a hyperimmune serum pooled from 3 patients sensitive to rye I, and antigen P<sub>1</sub> respectively, and from 1 hyperimmune bee keeper.

### Total IgE and Total IgG

A double-antibody inhibition radioimmunoassay was used to measure total IgE in serum and IgG in nasal secretions [21-23]. Total IgG in serum was measured by the assay of Mancini et al. [24].

### Nasal Secretions

Nasal secretions from 20 bee keepers who had had general reactions to bee venom were collected using filter paper strips (approx. 6×0.8 cm) [25]. The secretions in the filter paper were eluted with 1.0 ml of borate-buffered saline, and both total Ig and specific antibodies were measured in these samples. The methods for assaying nasal secretions and for calculating results have been described in detail elsewhere [25]. Briefly, the specific activity per milligram of immunoglobulin for each antibody in nasal secretions was related to the specific activity in the serum:

$$\frac{\text{nasal IgG antibody}}{\text{nasal IgG}} = \frac{\text{serum IgG antibody}}{\text{serum IgG}}$$

values greater than 1 suggest that the antibody in nasal secretions is locally produced.

## Results

### Antibodies to the Grass Pollen Allergen Rye I in Bee Keepers

The bee keepers were divided into those who had experienced allergic reactions to bee venom, both general and severe local, and those who had not. Further, each of these groups was subdivided into those who had a personal history of hay fever, and those who did not. Antibodies to the grass pollen allergen,

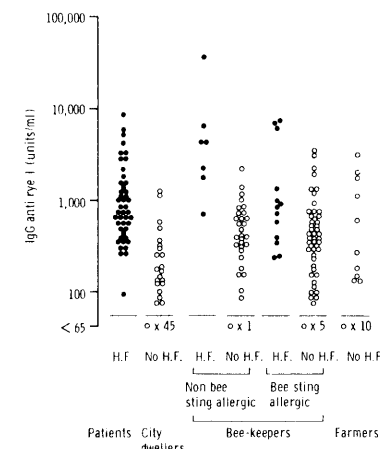


**Fig. 1.** Immunoglobulin G antigen binding activity for rye I in sera from bee keepers compared with results in patients with hay fever and non-allergic controls. The bee keepers were divided into those who had had allergic reactions to bee venom and those who had not. Individuals who had a history of seasonal hay fever and positive skin tests to grass pollen are indicated by solid circles (●). Numbers below the dashed line indicate the number of sera with no detectable IgG-binding activity.

rye I, were measured in these sera and compared with results for several groups of non-bee keepers, hay fever patients, non-allergic pregnant mothers and non-allergic laboratory staff. The results (fig. 1, table I) show that the bee keepers had a similar incidence of hay fever, irrespective of their allergic response to bee venom. This suggests that a personal history of hay fever does not make it more likely that an individual will become allergic to bee venom. The results also revealed the very surprising finding that almost all bee keepers have IgG antibodies to rye I in their serum in quantities similar to those found in patients with hay fever. Not all sera were assayed for IgE antibodies to rye I, but in sera from 45 patients with hay fever 38 (84.4%) had detectable IgE antibodies to rye I (i.e. > 65 units binding activity) while no IgE antibody to rye I was found in sera from 34 bee keepers who had high titres of IgG anti-rye I and no history of hay fever (table I). The increased IgG anti-rye I levels seen in the bee keepers were not paralleled by IgG antibodies to antigen P<sub>1</sub>, since only 16% of the bee keepers

**Table 1.** Binding activity for rye I in sera from bee keepers

|                                    | Binding activity for rye I <sup>1</sup> |                      |       |                     |
|------------------------------------|-----------------------------------------|----------------------|-------|---------------------|
|                                    | % IgG                                   |                      | % IgE |                     |
| Hay fever patients                 | 100                                     | (45/45)              | 84.4  | (38/45)             |
| Non-allergic controls <sup>2</sup> | 31.9                                    | (22/69)              | 0     | (0/22) <sup>4</sup> |
| <b>Bee keepers</b>                 |                                         |                      |       |                     |
| <i>Non-allergic to venom</i>       |                                         |                      |       |                     |
| With hay fever                     | 100                                     | (13/13)              | ND    |                     |
| Without hay fever                  | 90.4                                    | (47/52) <sup>3</sup> | 0     | (0/21) <sup>5</sup> |
| <i>Allergic to venom</i>           |                                         |                      |       |                     |
| With hay fever                     | 100                                     | (7/7)                | ND    |                     |
| Without hay fever                  | 96.7                                    | (29/30) <sup>3</sup> | 0     | (0/13) <sup>5</sup> |

<sup>1</sup> Binding activity was measured by antigen binding radioimmunoassay, figures are the % of sera showing > 65 units binding activity, per milliliter, values in parentheses show the incidence of positive sera.

<sup>2</sup> Non-allergic controls were city dwellers and include sera from 45 pregnant mothers who were not skin tested.

<sup>3</sup> Incidence significantly higher than in non-allergic controls ( $\chi^2 p < 0.001$ ).

<sup>4</sup> Results for skin test negative laboratory workers.

<sup>5</sup> Results for selected sera with high levels of IgG-binding activity.

showed detectable IgG anti-antigen P<sub>1</sub> (data not shown).

In an attempt to determine whether the IgG antibody to rye I was produced locally, in the nose of the bee keepers, we compared the specific activity of IgG antibody to rye I in the nasal secretions of 20 bee venom allergic bee keepers with the specific activity of IgG antibody to rye I in their sera. Antibody levels were high enough to calculate ratios in 11 patients and in these the specific activity of IgG antibody to rye I was higher in the nasal secretions than in the serum (fig. 2), suggesting that the IgG antibodies to rye I in the bee keepers were produced locally, in the nose. Conversely, we found no evidence to suggest that IgG antibodies to PLA<sub>2</sub> or diphtheria toxin were produced locally [25].

#### Specificity of the Anti-Rye I Antibodies in Bee Keepers

The possibility that the IgG antibodies to rye I in bee keepers were due to a direct cross-reaction between bee venom and rye I does not seem to be correct for a variety of reasons. Firstly, the antibodies to rye I were only of the IgG class. Secondly, we did not find

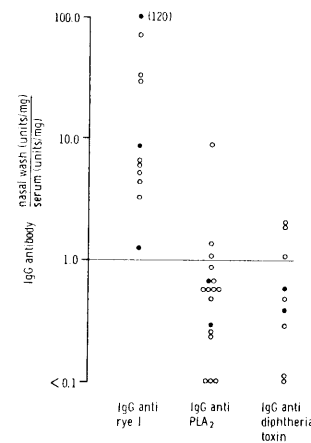


**Fig. 2.** Ratios of the specific activity of antibodies in nasal secretions and serum. For each antigen the IgG-binding activity is related to the total IgG both in secretions and serum. Values greater than 1.0 indicate that a proportion of the antibodies in the secretions must have been produced locally. Results for patients with hay fever are indicated (●).

IgG-binding activity for PLA<sub>2</sub> in non-bee keepers with hay fever. Thirdly, there was no significant correlation between the quantities of IgG anti-rye I and IgG anti-PLA<sub>2</sub> in sera from bee keepers ( $r = 0.017$ ). In addition, we assessed cross-reactivity by using bee venom to inhibit the binding of PLA<sub>2</sub>, rye I and antigen P<sub>1</sub>. Bee venom showed no inhibition of antigen P<sub>1</sub> binding, i.e. < 5% inhibition, in any of six sera tested. By contrast, bee venom inhibited the binding of PLA<sub>2</sub> at a very high dilution; 58–78% inhibition at 1/390,000 dilution of 1 mg/ml bee venom (fig. 3). Bee venom did show some inhibition of rye I binding but this required at least 1,000 times more venom than for PLA<sub>2</sub> and with only one serum did inhibition exceed 50% (fig. 3). These results show that the IgG antibody to rye I in bee keepers cannot be explained simply by a cross-reaction between rye I and bee venom proteins.

#### Exposure to Grass Pollen

An alternative explanation for the high levels of IgG antibody to rye I found in the sera of bee keepers might be that, by working outdoors, their exposure to grass pollen was increased and that such an exposure

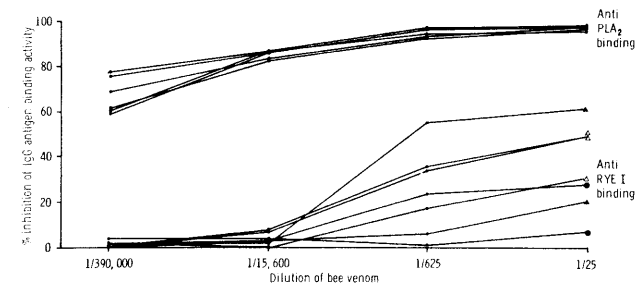


**Fig. 3.** Percentage inhibition of antigen binding for both PLA<sub>2</sub> and rye I by serial dilutions of bee venom. Serum dilutions chosen to give 5–15% binding of the relevant antigen were incubated with dilutions of bee venom undiluted (1 mg/ml) for 3 h, before the addition of radiolabelled PLA<sub>2</sub> or rye I. Sera for the binding of rye I were derived from bee keepers with hay fever (▲), bee keepers without hay fever (●), and non-bee keepers with hay fever (●).

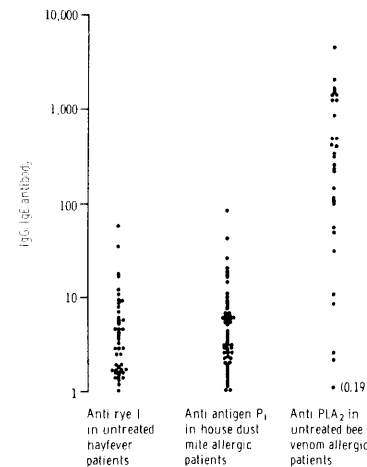


**Fig. 4.** Ratios of IgG-binding activity: IgE-binding activity for rye I, antigen P<sub>1</sub>, and PLA<sub>2</sub> in patients with hay fever, dust mite rhinitis and bee venom allergy. The values for anti-PLA<sub>2</sub> include values extrapolated from anti-PLA<sub>2</sub> RAST results.

#### Comparison of the Relationship between IgG and IgE Antibodies to Injected and Inhaled Allergens

The results for antibodies to rye I, antigen P<sub>1</sub> and PLA<sub>2</sub> were expressed as a ratio of IgG/IgE antibody (fig. 4). The mean ratio for antibodies to rye I in 47 non-bee keeping hay fever patients was 6.34:1, for 60 mite-sensitive patients from Hong Kong this ratio for antibodies to antigen P<sub>1</sub> was 7.63:1, and for 31 allergic bee keepers the ratio of IgG to IgE for PLA<sub>2</sub> was 722:1. Clearly, in the presence of a large excess of IgG antibody it becomes difficult to be sure of the measurements of IgE antibody. However, several techniques have been used to check the results, firstly, increasing the quantity of venom allergen on radioallergosorbent test (RAST), beads and secondly, simple fractionation of sera into IgE-rich fractions using ammonium sulphate [19]. Even if the values for IgE antibody to PLA<sub>2</sub> were slightly underestimated, it is clear that the ratios of IgG:IgE antibody are much higher for this antigen. It seems likely that the 'large' doses of PLA<sub>2</sub>, 10–12 µg/sting favour the production of IgG antibody.

#### Discussion

The present study was designed to compare the antibody response to PLA<sub>2</sub> with the response to two inhalant allergens. The object was to gain more insight into the factors which control whether different individuals will develop IgE antibodies following these different types of antigen exposure. In all sera studied IgE antibodies were never present without IgG antibodies and the quantities of IgG antibody were always greater than the IgE antibodies. However, while the levels of IgG antibody to rye I and antigen P<sub>1</sub> were on-

might lead to increased antibodies to rye I. Since most bee keepers in England keep bees as a part-time hobby their exposure to grass pollen may not necessarily be high. However, we also looked at antibody levels to rye I in the sera of dairy farmers who were not allergic to grass pollen but worked outdoors for at least 6 h/day during the pollen season. Even these sera showed a lower incidence of IgG antibodies to rye I than the bee keepers, although they did show higher levels than the non-allergic controls (fig. 1, table I).

ly about 2- to 10-fold greater than IgE antibody, the IgG antibody to PLA<sub>2</sub> was often 500-fold greater than the IgE antibody. Similarly, for both rye I and antigen P<sub>1</sub> there is an excellent quantitative correlation between IgG and IgE antibodies [19, 26], and this was not apparent for antibodies to PLA<sub>2</sub>. Thus, the antibody response to PLA<sub>2</sub> is distinct from that to inhalant allergens in three respects: (i) there was IgG antibody to PLA<sub>2</sub> in most of the non-allergic bee keepers; (ii) IgG antibody to PLA<sub>2</sub> was often much higher than the IgE antibody and there was no correlation between the two classes, and (iii) the IgG antibodies to PLA<sub>2</sub> did not appear to be produced locally in the nose. The IgG/IgE antibody ratios were often low in subjects who had rhinitis and did not appear to be related to the severity of symptoms [26]. By contrast, IgG/IgE ratios of anti-PLA<sub>2</sub> were often very high in patients who had severe generalized reactions to bee stings. This emphasizes that at present simple measurements of IgE antibodies and blocking antibodies cannot predict the nature of allergic symptoms.

We were surprised to find that most bee keepers made IgG antibodies to rye I, even though they were not allergic to grass pollen, and whether or not they were allergic to bee venom. The only apparent difference between the bee keepers and other non-atopic individuals that could explain these antibodies seemed to be that bee keepers were exposed to, and stung by, bees. This observation could not easily be explained by an increased exposure to grass pollen since we did not find the same incidence of IgG antibodies to rye I in farmers who must have been exposed to much higher levels of grass pollen through their work. Cross-reactivity between PLA<sub>2</sub> and rye I does not explain our findings because (i) the serological evidence does not suggest a cross-reaction; (ii) there is no evidence that non-bee keepers with hay fever are liable to have allergic reactions to bee strings, and (iii) the inhibition of rye I binding by bee venom was modest and required high concentrations of venom. Bee keepers showed a normal antibody response to diphteria toxin, had normal total IgG levels, and were taken as being no different from the rest of the population in their general immune responsiveness. At present, it seems probable that bee venom has a stimulatory or adjuvant-like effect on the IgG antibody response to rye I. The fact that there was no parallel increase in IgG antibodies to antigen P<sub>1</sub> might be explained because the maximum exposure to mite antigens occurs at a different time of year from the bee stings. The al-

ternative explanation is that bee venom can specifically prime for an antibody response to grass pollen. This could occur either because trace amounts of grass pollen are present in certain samples of bee venom or because there was some subtle form of cross-reactivity possibly at a T-cell level. It is possible that cross-reactions might have been found with venom collected at a different time of the year or from an English (rather than Bulgarian) source. *Aalberse et al.* [27] have recently described a cross-reactivity between bee venom and certain foods. However, they report direct cross-reactivity for IgE antibodies, whereas we have found an isolated IgG antibody response in the absence of IgE antibodies.

Our studies did not find any evidence that bee keepers with hay fever were more likely to develop allergic reactions to bee stings, and this was true both for generalized reactions and for severe local reactions. In our earlier study on the relationship between atopic disease and allergy to bee stings [18], we showed that there was a higher incidence of atopy in those bee sting allergic patients with high titres of IgE antibodies to bee venom. Furthermore, when these patients were followed for 6 months it appeared that the atopic bee sting allergic patients were more likely to react to subsequent bee stings. It appears possible that the atopic bee keepers are likely to have higher titres of IgE antibodies and more persistent symptoms if they become allergic to bee venom. In keeping with this view *Settipane et al.* [28] have shown that Hymenoptera sting-sensitive patients with asthma tend to have more serious reactions to stings.

Although it is generally accepted that immediate reactions to bee venom are mediated by IgE antibodies, some studies have suggested that the presence of IgE antibodies to bee venom does not discriminate very accurately between bee keepers who are allergic to bee venom from those who are not. In a recent study 58% of non-allergic bee keepers had detectable IgE antibodies to bee venom although they had had no recent reactions to venom [4]. In two other studies the majority [3] and 40% [6] of non-allergic bee keepers were found to have IgE antibodies to venom without symptoms. However, these results may reflect the ability of bee venom to bind non-specifically to IgG and IgE [4]. Certainly, more recent studies in our laboratory using purified venom antigens to measure IgE antibodies have found a very good correlation between symptoms and IgE antibodies [11].

We would argue that human inhalant allergy re-

presents repeated low-dose immunization, and that atopic individuals are analogous to those strains of mice that respond to low doses by producing persistent IgE and IgG antibody responses [19, 20, 29, 30]. By contrast bee venom 'immunization' is equivalent to high dosage in mice. A large proportion of bee keepers produce high levels of IgG antibody to venom proteins, and there is no apparent correlation between IgG and IgE antibody production. This is in keeping with results in mice immunized with high doses, where the production of IgE antibodies is not related to IgG antibody production, and all strains produce IgG antibody. Those mice that produce IgE following high-dose immunization appear to be those strains of animals who fail to produce IgE class-specific suppressor T cells [31]. By direct analogy with mouse models it seems that suppressor T cells are unlikely to be relevant in inhalant allergy but may well be relevant in controlling IgE antibody production to bee venom allergens. Certainly it appears that the underlying factors that lead some individuals to produce IgG and IgE antibodies to pollen or dust antigens are different from the factors that lead some bee keepers to produce IgE antibody to venom proteins.

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