

Low specific IgE, IgG and lymphocyte reactivity in a group of patients developing anaphylaxis following a honey-bee sting

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Summary

Ten patients who developed severe generalized reactions following a honey-bee sting were investigated for the presence of specific IgE and IgG antibodies, and for lymphocyte reactivity following *in-vitro* honey-bee venom (HBV) stimulation. Five of the patients (high responders) showed high HBV-specific IgE and IgG levels, whereas the other five patients (low responders) showed low HBV-specific IgE and IgG levels. Mononuclear cells from the high responder group incorporated significant amounts of ³H-thymidine when activated with pure bee venom, whereas insignificant lymphocyte proliferation was observed in the low-responder group. It is concluded that, amongst HBV-sensitive patients, a group of low responders exists in whom the mechanism of anaphylaxis cannot be explained.

Introduction

There is now considerable evidence that the development of IgE antibodies (IgE ab) to venom proteins is the underlying cause of anaphylactic reactions to insect stings [1–3]. Since the development of the RAST assay for measurement of specific IgE ab, however, it has become evident that not all patients with clear evidence of systemic reactions following a sting have measurable levels of specific IgE ab in their serum [3–5]. This group of patients with low specific IgE levels has not been extensively investigated, and since high titres of specific IgG ab can interfere with specific IgE measurements, part of this study was undertaken to examine both specific IgG and IgE levels in ten unselected patients who had previously experienced a systemic reaction following a honey-bee sting.

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As there is also considerable evidence that lymphocytes from atopic patients incorporate increased amounts of ^3H -thymidine and produce mediators in response to specific antigens [6, 7], this study also examined lymphocyte responsiveness in honey-bee venom (HBV)-allergic patients. The aim of the study, therefore, was to assess the relationship between HBV-specific IgE levels, and both HBV-specific IgG levels and proliferative activity of lymphocytes activated by pure HBV. The results indicate that a proportion of patients with classical symptoms of acute anaphylactic reactions following a honey-bee sting, have low HBV-specific IgE and IgG levels, and show lack of reactivity in lymphocyte proliferation studies to HBV antigen.

Subjects and methods

Patients

Ten unselected adult patients presenting at a bee-sting allergy clinic were included in this study. None of the patients were receiving medications for concurrent illnesses, and all were assessed within 7–14 days following the last sting. The group consisted of nine males and one female, and their ages ranged from 17 to 43 years. All patients had been stung on a number of occasions and had experienced systemic reactions following a definite honey-bee sting, as evidenced by generalized pruritis, urticaria, wheezing, shortness of breath or shock requiring emergency treatment. All patients were skin-test positive when tested by intradermal injection of 0.1 μg or 1.0 μg of pure bee venom (Pharmacia, Uppsala, Sweden).

Stimulation of mononuclear cells (MN) with pure HBV

Mononuclear cells were obtained from heparinized blood by Ficoll-Hypaque density gradient centrifugation. The MN cells were washed three times in RPMI-1640 containing 10% pooled AB serum (complete medium). Samples of 0.2 ml of $1 \times 10^6/\text{ml}$ cells were cultured in quadruplicate in round-bottom microtitre wells in the presence and absence of various concentrations (0.1–10.0 $\mu\text{g}/\text{ml}$) of pure HBV (Pharmacia, Uppsala, Sweden). The plates were incubated for 5 days at 37°C in 5% CO_2 and 95% air, and ^3H -thymidine (0.5 $\mu\text{Ci}/\text{well}$) was added for the last 16-hr of culture. The cells were then harvested with a MASH II harvester and ^3H -thymidine incorporation was assessed by liquid scintillation counting. Stimulation index (SI) was calculated as follows:

$$\text{SI} = \frac{\text{c.p.m. of MN cultured with HBV}}{\text{c.p.m. of MN cultured in medium}}$$

Antibody measurement

Honey-bee venom specific IgE and IgG ab levels were assayed by the Phadebas RAST® (Pharmacia Diagnostics AB, Uppsala, Sweden) according to the manufacturer's directions, employing pure HBV covalently coupled to paper discs. All serum samples from this group of patients were examined at the same time.

Results

^3H -thymidine incorporation

In preliminary studies it was established that 5–10 μg of HBV was the optimal concentration of bee venom required for maximum lymphocyte activation. The

Table 1. Tritiated thymidine incorporation of MN cells from high- and low-responder patients in the presence of 10 $\mu\text{g/ml}$ of HBV

Patient	Control	10 $\mu\text{g/ml}$ bee venom	Stimulation index
C.R.	1421 $\pm$ 355*	56 875 $\pm$ 772	40.0
T.F.	1450 $\pm$ 435	49 300 $\pm$ 992	34.0
M.E.	1424 $\pm$ 222	38 447 $\pm$ 555	27.0
E.A.	2709 $\pm$ 203	69 616 $\pm$ 617	25.0
P.R.	1478 $\pm$ 235	21 625 $\pm$ 448	14.0
J.F.	1806 $\pm$ 382	4 604 $\pm$ 480	2.5
G.S.	1857 $\pm$ 222	3 818 $\pm$ 290	2.0
P.M.	2598 $\pm$ 275	13 945 $\pm$ 857	5.3
F.T.	1034 $\pm$ 333	4 655 $\pm$ 342	4.5
A.D.	1180 $\pm$ 251	3 540 $\pm$ 448	3.0

* Values are counts per minute $\pm$ s.e.m.

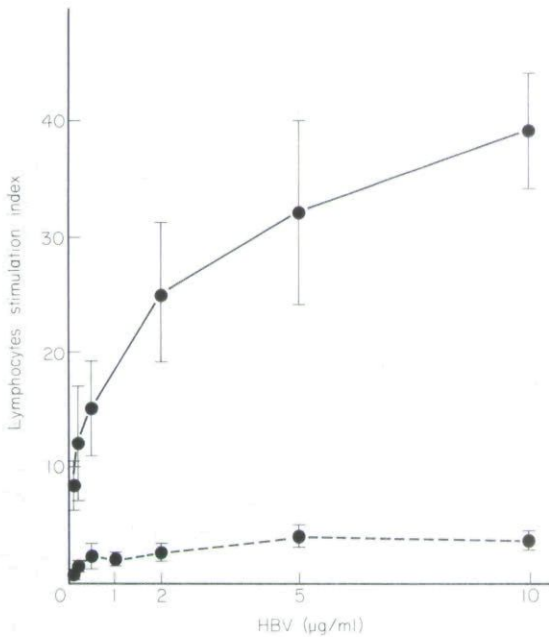


Fig. 1. Incorporation of ^3H -thymidine by MN cells of high- (—●—●) and low- (---●---●) responder HBV-allergic patients stimulated by increasing concentration of HBV. Each curve represents the mean $\pm$ s.e.m. of five patients.

response of MN cells from the ten allergic patients to *in-vitro* stimulation by HBV was assessed, and the results differentiated two distinct groups of patients (Table 1). Mononuclear cells from five patients reacted to HBV in a dose-dependent manner and incorporated ^3H -thymidine even when cultured in the presence of as little as 0.1 μg of the allergen (high responders). The other group consisted of five low-responding patients in whom MN cells reacted minimally to HBV (Fig. 1). Mononuclear cells from normal control individuals had low-stimulation indices when cultured *in vitro* with

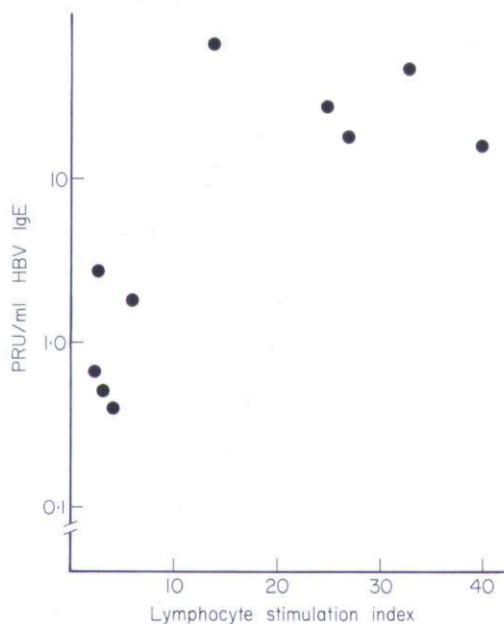


Fig. 2. The relationship of HBV-specific IgE levels with the lymphocyte stimulation index.

HBV (results not shown). These studies were repeated 4 weeks later and a similar pattern of responses emerged, in that the high responders demonstrated a dose-dependent stimulation while the low responders showed minimal response to HBV (results not shown).

HBV-specific IgE and IgG levels in the serum of HBV-sensitive patients

Serum levels of HBV-specific IgE antibodies in allergic patients varied from 0.3 to 87.0 PRU/ml with a mean of 19.87 ± 26.3 (geometric mean $\pm$ s.e.m.). In this regard the patients' IgE levels fell into two groups corresponding to the results of lymphocyte proliferation studies. Whereas the high-responder group had HBV-specific IgE levels between 10.8 PRU/ml and 87.0 PRU/ml (mean 39.0 ± 13.2), the group whose lymphocytes responded poorly to HBV in proliferation studies, showed HBV-specific IgE levels that ranged from 0.3 to 7.1 PRU/ml (mean 2.48 ± 1.24). When HBV-specific IgE levels are plotted against the lymphocyte stimulation index, the two distinct groups are clearly correlated (Fig. 2). A significant difference in HBV-specific IgG levels could also be observed in the low-responder group (20.0–144.7 U/ml, mean 73.2 ± 26.49) as compared with the high-responder group (138.2–421.3 U/ml, mean 254 ± 53.3) (Table 2).

Discussion

This study defines a low-responder group of HBV-sensitive patients, on their low levels of HBV-specific IgE and IgG, and the inability of their MN cells to proliferate after activation with HBV. Although a proportion of HBV-allergic patients have previously been shown to have HBV-specific IgE levels that approximate the values found in normal sera [3, 4], we were surprised to find that five of ten unselected patients with bee-venom allergy fell into this group. We do not suggest, however, that 50% of such

Table 2. Honey-bee venom-specific IgE and IgG values in the low- and high-responder patients

Patient	Response	IgE (PRU/ml)	IgG (U/ml)
P.M.	Low	1.8	20.0
J.F.	Low	2.7	54.3
G.S.	Low	0.5	144.7
W.W.	Low	7.1	20.0
J.T.	Low	0.3	128.0
M.E.	High	10.8	138.6
C.R.	High	46.3	283.2
M.A.	High	27.0	321.3
T.F.	High	25.2	138.2
P.R.	High	87.0	289.2

patients will be low responders, and in other studies low specific IgE levels were found only infrequently [4]. The reason for the ability of these patients to develop positive skin tests or generalized allergic reactions is not known. Reisman *et al.* [7] have pointed out the unexplained inconsistency of the allergic reaction as related to the presence of specific IgE antibody. In the study of Clayton *et al.* [3], fourteen of the twenty-five patients with low IgE levels had positive skin tests employing a dose of venom as low as 0.01 µg/ml. Even in those patients with negative skin tests the anaphylactic symptoms were similar to venom-allergic individuals with demonstrable levels of specific IgE. Similarly, in the present study no difference in the clinical picture of high and low responders could be identified, as regards age, severity of symptoms, medication or period of time since the last sting.

Although the mechanism of anaphylaxis in patients with low IgE is unknown, it has been assumed that in these cases the majority of the IgE is bound to the basophils or mast cells. Alternatively, it is possible that the appearance of IgE antibodies is transient, although in the present study, all patients were assessed 1–3 weeks after developing a systemic reaction, and then retested 4 weeks later. These results are similar to those of Busse and Yunginger [8] who also observed that the severity of systemic reactions and the interval between the reaction and the time of antibody determination was similar in patients with normal, as compared to those with elevated, venom-specific IgE levels. It has previously been suggested that patients with severe anaphylaxis and shock may have low IgE titres, while those with large local reactions may have extremely high titres [9].

Although Parker *et al.* [5] have reported that some patients developing systemic reactions following a bee sting have low initial IgG levels, the present study indicates that of the patients with the lowest specific IgG levels, four fell into the low-responder group. As all of these patients had been stung on a number of occasions it would suggest that this low-responder group cannot mount an adequate immune response against HBV, an observation confirmed in lymphocyte proliferation studies.

It is now well accepted that lymphocytes from atopic patients incorporate increased amounts of ³H-thymidine *in vitro* and produce mediators when activated by specific allergens [6, 10, 11]. Case *et al.* [12] have previously shown that in bee-venom allergic patients, the greatest lymphocyte proliferation occurred in patients with large local reactions, and that proliferation did not occur in all patients with systemic reactions.

The findings in the present study that not all HBV-allergic patients have lymphocytes that respond *in vitro* to specific antigen, is similar to those of Rocklin *et al.* [10] who studied lymphocyte responses of ragweed-sensitive patients. In their study they indicated that patients who are highly allergic do not respond uniformly *in vitro* to antigen E. Of particular interest was their observation that patients with reactive lymphocytes also had higher specific IgE and IgG levels in general, as compared with the group of non-responders, and that symptom scores did not reveal any differences in the severity of symptoms between the sensitive and insensitive patients.

The present study, therefore, indicates that HBV-allergic patients fall into two distinct groups. The high-responder group have raised HBV-specific IgE and IgG levels, and responsive lymphocytes. The low-responder group, however, do not develop specific lymphocyte or antibody responses to HBV, and the mechanism for their development of anaphylaxis is, as yet, unknown. Similarly, the mechanism by which a positive skin-test reaction is elicited is also unknown. The clinical significance of identifying this low-responder group is, at present, unclear.

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