

Venom-specific IgE and IgG antibodies as a measure of the degree of protection in insect-sting-sensitive patients

R. URBANEK,* U. KRAUSS,* J. ZIUPA† and G. SMEDEGÅRD*

* *Universitäts-Kinderklinik, Freiburg*, and † *Medizinische Universitätsklinik, Freiburg, West Germany*

(Received 23 April 1982; accepted for publication 24 April 1982)

Summary

Venom-specific IgE and IgG antibodies were measured in the sera of bee-venom-sensitive patients during a 3-year hyposensitization period. The level of specific IgG antibodies initially increased, and 2 months after the start of therapy, this increase was on average five-fold. A concomitant but non-significant increase in specific IgE antibodies was also observed initially. Later during the treatment period specific IgE antibodies showed a continuous decline and after 3 years the level was one third of the pre-treatment level. Venom-specific IgG antibodies, however, remained above the pre-treatment level. The disappearance of the severe allergic reactions was related to the level of both IgE and IgG antibodies as demonstrated in seventy-six bee sting challenges. In patients with specific IgG antibody levels above 400 u/ml, no severe reactions were observed even if the patient had levels of specific IgE antibodies of RAST class 3 or 4. These data suggest that the relationship between venom-specific IgE and IgG antibodies permits an evaluation of the state of immunity to insect stings.

Introduction

Clinical trials have demonstrated that allergen-immunotherapy with appropriate antigens results in a reduction or elimination of allergic symptoms. Production of specific IgG antibodies, or 'blocking antibodies' appears to correlate to the clinical improvement (Lichtenstein, Valentine & Sobotka, 1979; Norman, 1980; Norman, 1981; Patterson, 1981). Since insect venom allergy offers well-defined conditions, we investigated hypersensitivity to bee venom and its treatment. The aim of the study was to find out whether or not in the course of a prospective hyposensitization treatment conducted over a number of years, an increase in the specific IgG antibodies and/or a fall in the specific IgE antibodies could be related to a reduction or cessation of the allergic symptoms. In addition the specific IgG level was sought which might allow clinical protection to be assessed prospectively.

Correspondence: PD Dr R. Urbanek, Universitäts-Kinderklinik, Mathildenstr. 1, 78/Freiburg, West Germany.

Material and methods

Patients

Hyposensitization of bee venom allergic patients (forty-eight children aged 4 to 16 years and one adult) was performed over a 3-year period. A rush treatment was initially used followed by administration of 100 µg bee venom (Reless® Bienengift, [Pharmalgen®] Pharmacia Diagnostics AB, Sweden) at monthly intervals (Urbanek, Karitzky & Forster, 1978). Blood samples for antibody analyses were withdrawn before and 60, 180, 270, 360, 540, 720 and 1080 days after the start of treatment. The sera were stored at -20°C until analysed.

Challenge with a live bee placed on the forearm was employed on seventy-six occasions (seventeen non-treated patients and forty-nine treated patients of which ten were challenged twice). The resulting reactions were classified clinically in four categories (Table 1).

Antibody analyses

Venom-specific IgE and IgG antibodies were measured with Phadebas IgG RAST®, respectively (Pharmacia Diagnostics AB). Undiluted sera were used for the IgE determinations whereas for the IgG measurements the sera were diluted 200 times or 1000 times if values above 400 u/ml were expected.

As a part of this investigation Phadebas IgG RAST® was used to determine venom-specific IgG in bee keepers ($n=19$) and in non-allergic subjects ($n=9$). In addition the inter-assay coefficient of variation for this method was assessed using four patient's sera. The tests were performed in duplicate on twelve occasions. Finally the IgG values obtained by the RAST were compared with values obtained with ELISA. The details of this method have been reported earlier (Forster & Urbanek, 1979). In summary, 1 µg bee venom was fixed in the holes of microtitre plates. Serial dilutions of test sera in foetal calf serum were placed into the holes and incubated. The plates were washed and incubated with peroxidase-conjugated anti-human IgG. Following another washing O-phenylenediamine and H₂O₂ was added to phosphate buffer and the reactions were stopped by addition of HCl. Antibody titres were estimated by photometry (Titertec, Flow).

Statistics

Confidence intervals (95%) for the probability of having severe reactions were calculated assuming bi-nomial distribution.

Table 1. Classification of sting reactions

Grade	Reaction
0	Normal reaction
I	excessive local reaction (swelling extending over two neighbouring joints)
II	Mild general reaction (e.g. urticaria, Quincke oedema, generalized pruritus, nausea)
III	severe general reaction (e.g. dyspnoea, unconsciousness, collapse, shock)

Results

Hyposensitization with bee venom resulted in an initial increase in the level of specific IgG antibodies (Fig. 1). There was also in most patients a concomitant increase in specific IgE antibodies. After 6 months of therapy IgE antibody levels fell below, while the levels of IgG antibodies remained above the pre-treatment level. During the following years, IgE antibody level continued to decrease and 3 years after the start of treatment the average level was one third of the level obtained before treatment. Specific IgG antibodies however still remained above pre-treatment level at this time.

In seventy-six challenges to bee sting, both IgG and IgE antibodies were compared with the clinical reaction developed. The patients were divided into four groups according to the level of specific IgE and IgG antibodies at the time of challenge (Fig. 2). Among the twenty patients who had high amounts of specific IgE antibodies (RAST class ≥ 3) and low levels of specific IgG antibodies (≤ 400 u/ml) 40% responded with severe (Grade III) reactions. In contrast, none of the eighteen patients who had both high IgE and IgG antibody levels, responded with severe reactions. The distribution of the severity of the reactions in the patients having low IgE/low IgG ($n=32$) and low IgE/high IgG ($n=6$) is also demonstrated in Fig. 2. Statistical evaluation of the probability (95% confidence intervals) of developing a 'grade-III' reaction was between 19–64% for a patient who had high IgE/low IgG. High IgE together with high IgG provided a risk for severe reactions of less than 19%. Corresponding values for the groups of patients having low IgE/low IgG was less than 11% and low IgE/high IgG less than 46%. (This high value is due to the small number of patients in the latter group.)

The inter-assay coefficient of variation for the four sera analysed by IgG (mean 26, 175, 538 and 1076 u/ml, respectively) varied between 13.2 and 15.3%. Seven of the nine non-allergic subjects had venom-specific IgG levels below 20 u/ml. Two subjects

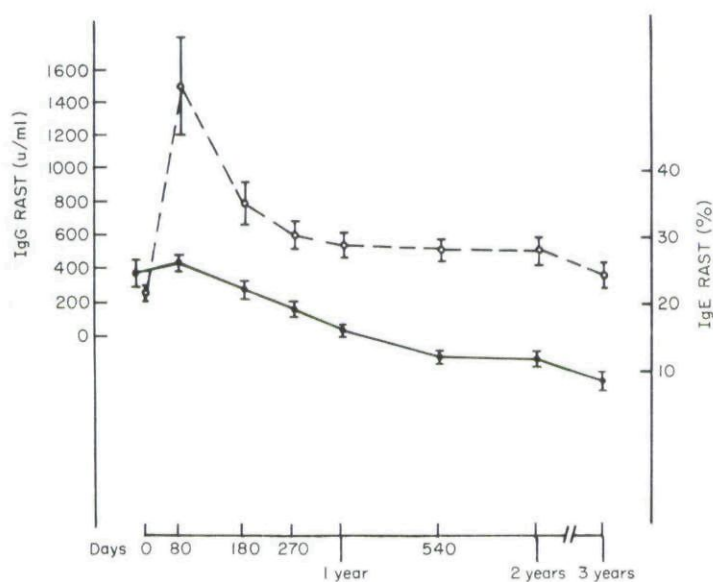


Fig. 1. Specific IgG and IgE antibodies against bee venom during hyposensitization. ---, IgG RAST (u/ml); —, IgE RAST (% of total activity).

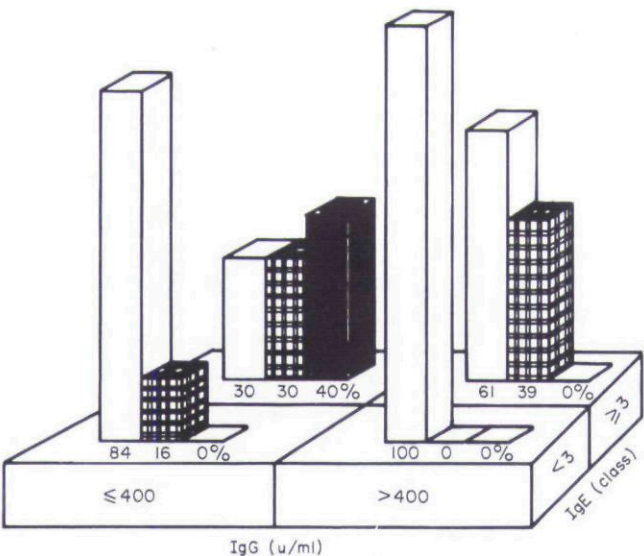


Fig. 2. Prognostic significance of specific IgG and IgE antibodies in bee venom allergy, severity of reaction: □, ○-I; ▨, II; ■, III.

possessed IgG values of 27 and 31 u/ml, respectively. The same subjects had elevated IgG levels using ELISA.

Venom-specific IgG antibodies were found in all bee keepers (Fig. 3). The bee keepers who had the lowest amounts reported unusually marked local reactions following bee stings. One of these bee keepers had only been a bee keeper for 1 year.

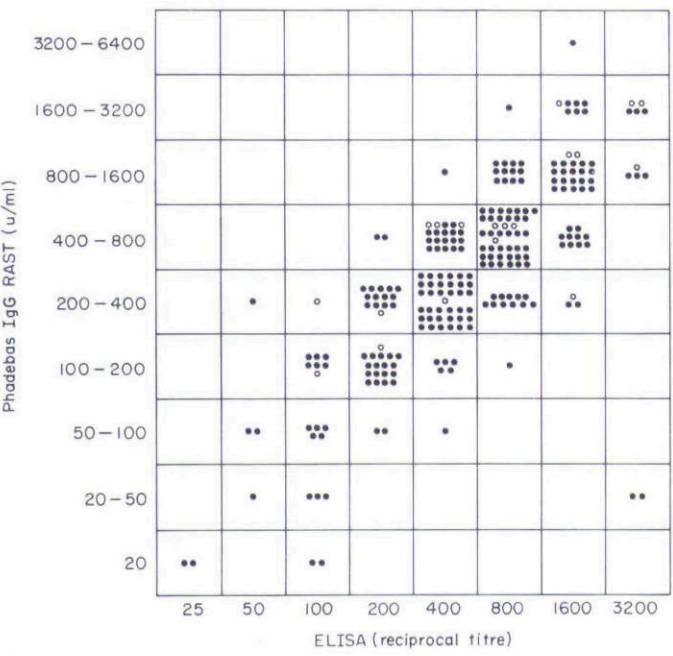


Fig. 3. Bee venom-specific IgG antibodies, correlation between Phadebas IgG RAST and ELISA. ●, IgG levels in bee venom allergic patients; ○, IgG levels in bee keepers.

Figure 3 also shows the correlation between IgG RAST and ELISA. The IgG values for the RAST are given in intervals, corresponding to the two-fold dilution steps of the ELISA. As seen in the Fig. 3 there is a good correlation between the methods.

Discussion

Hyposensitization with bee venom resulted in increased serum concentrations of venom-specific IgG antibodies. The most marked rise was noted during the first 3 months of treatment, the phase of highest allergen administration. A dose-dependent influence of allergen-immunotherapy on the IgG levels is detected mainly after rush hyposensitization (Golden *et al.*, 1981; Lichtenstein *et al.*, 1979). It can be assumed that measurement of the specific IgG antibody level at regular intervals allows an assessment of the effect of the allergen-immunotherapy. In order to reach a significant rise in the IgG antibody level, preferably above 400 u/ml, it is advisable to administer as high a dose of allergen as possible in the initial phase of hyposensitization. The similarity in the IgG levels observed in successfully hyposensitized patients and bee keepers is not surprising. Bee keepers receive many bee stings and therefore develop a natural immunity (Light *et al.*, 1975; Urbanek *et al.*, 1980).

The results of comparison of the venom-specific IgG and IgE antibody levels have a predictive value. In the initial phase of treatment the levels of both specific IgE and IgG antibodies rose, followed by a fall after approximately 6 months. Specific IgG, however, remained above the pre-treatment level whereas specific IgE was suppressed. Following a consequent hyposensitization treatment IgE levels were practically undetectable in five patients, though the protective specific IgG antibody persisted. In such cases the measurement of antibody levels provides a clear indication for the cessation of the hyposensitization treatment.

In the search for a protective IgG level, it is generally accepted that the probability that protection exists is greater the higher the IgG level. In addition to the level of specific IgG it is necessary to know the level of specific IgE antibodies and the previous reactions to natural insect stings. The higher the IgE levels and the degree of clinical sensitization, the more important it is to reach a high IgG antibody level.

The majority of patients will be protected by a specific IgG antibody level of > 400 u/ml but at lower degrees of clinical sensitivity lower levels are adequate. In the course of seventy-six bee sting challenges, severe (grade III) systemic reactions were not observed in patients with specific bee venom IgG antibodies > 400 u/ml. Mild allergic reactions were however observed in the presence of high IgE antibody levels (RAST class 3 and 4) but the reactions were always weaker after hyposensitization treatment than before.

It is possible to control the individual antibody response to the hyposensitization treatment. Hyposensitization treatment with pure insect venom results in a disappearance or a distinct reduction of the allergic reaction following bee sting. An evident specific IgG antibody response after an adequately high allergen dosage is important for the therapeutic success. Regular measurements of the specific IgE and IgG antibody levels permits an evaluation of the degree of protection from insect stings.

Acknowledgment

This study was supported by Deutsche Forschungsgemeinschaft, Grant Nr U + 12/3.

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