

Detailed IgG and IgE antibody patterns during immunotherapy with honey bee venom

T. UHLIN,¹ S. L. NORDVALL,² S. ÖHMAN,³ and R. EINARSSON,¹

¹Pharmacia AB, Uppsala, ²St Göran's Hospital, Stockholm, and ³Department of Dermatology, University Hospital, Uppsala, Sweden

Fourteen patients with a known honey bee venom (HBV) allergy were followed during 1-2 years of immunotherapy. HBV-specific IgG antibody levels increased in all patients but one. HBV-specific IgE antibodies decreased slightly during the first year of therapy. The ratio HBV-specific IgG-/IgE showed a marked increase during the first year for most of the patients, and a further increase during the second year in the four patients followed that long. As could be expected an increased radiostaining was found after 1 year of treatment to all important allergens in IgG CRIE, but after 2 years a sustained or increased radiostaining was obtained to phospholipase (PLA) alone. A decreased radiostaining might more easily be seen with weaker immunogens.

Key words: IgE; IgG; CIE/CRIE; honey bee venom immunotherapy.

Accepted for publication 1 December 1986

Bee sting allergy constitutes a considerable threat to the allergic subject and causes disabling anxiety to many people. Immunotherapy with pure venom has been shown to be an effective means to reduce the risks of life-threatening reactions (11) and is now used extensively. A major immunological effect of immunotherapy is the rise of allergen-specific IgG (23). Venom-specific IgE levels also often increase in the initial phase of the therapy but later decline in spite of the continued immune stimulus (4, 29). Bee venom is a complex mixture made up of several proteins, peptides and different low molecular weight components with varying biochemical and pharmacological effects (7). It could be anticipated that many of these substances would elicit an immunologic response on synthesis of antibodies of different classes.

Separation of the individual components is required in order to study the antibody response to the components of this complex mixture. By applying crossed immunoelectrophoresis (CIE) and crossed radioimmuno-electrophoresis (CRIE) the antigenic and allergenic composition of the mixture have been established (2, 5). Previously the antibody patterns in bee keepers have been

studied using these immunological methods (22). The present study deals with the detailed antibody response towards clinically important allergens during the course of regular bee venom immunotherapy.

MATERIAL AND METHODS

Patients

Fourteen patients of both sexes aged 25-68 years with a history of an allergic reaction to bee sting were included in the study (Table 1). Eleven of the subjects belonged to bee keeper families. All patients except one had a positive RAST reaction ≥ 0.35 PRU/ml (Phadebas RAST Unit per ml) but all were skin test positive (Table 2).

Blood samples were drawn at regular intervals and serum was kept frozen (-18°C) until analysed. Antibody examinations with IgE RAST, IgG RAST and crossed immunoelectrophoresis were performed using sera obtained before the start of immunotherapy,

after 1 year of active therapy, and in four patients after 2 years of treatment.

Honey bee venom

Honey bee venom (*Apis mellifera*, lot 00500) collected by electrical stimulation was obtained from Mr. C. Mraz, Champlain Valley, Middlebury, Vermont. This bee venom source material was used for the manufacturing of Pharmalgen bee venom (Pharmacia Diagnostics AB, Uppsala, Sweden) used in this study.

Antisera

Antisera to bee venom proteins were raised in rabbits by multiple bi-weekly intracutaneous immunizations with approximately 300 µg bee venom source material in Freund's complete adjuvant, essentially as described by Harboe & Ingild (8).

Treatment

Pharmalgen bee venom was used for immunotherapy. Eight patients followed a rush treat-

ment schedule (3) and six a schedule with weekly injections (16). The maintenance dose was 100 µg given once monthly for both groups.

Radioallergosorbent test (RAST)

HBV-specific IgE and IgG antibody concentrations in the patient sera were determined by Phadebas RAST® kit (Pharmacia Diagnostics Uppsala, Sweden). The results were expressed in Phadebas RAST Units/ml (PRU/ml) for IgE and U/ml for IgG.

Crossed immunoelectrophoresis (CIE)

CIE was performed on gelbond film essentially as previously described (5) using a 0.05 mol/l TRIS/Tricine buffer (Bio-Rad), pH 8.6 in 1 % Agarose (Agarose A, Pharmacia Fine Chemicals). In the first dimension electrophoresis 75 µg bee venom was run for 55 min, 10 V/cm at 15°C. The second dimension electrophoresis was performed for 18 h using 14 µl rabbit antiserum per cm² gel.

Crossed radioimmuno-electrophoresis (CRIE)

Allergen-specific IgG and IgE detection. IgG-CRIE and IgE-CRIE were performed as previously described (2, 21). The CIE plates were incubated over night in patient serum diluted 1/200 (IgG) and 1/17 (IgE), respectively. After washing, using 10 % PDT buffer solution in 0.9 % sodium chloride, the plates were re-incubated in a dilution of ¹²⁵I-anti-IgG or ¹²⁵I-anti-IgE (72 000 cpm/ml). Autoradiography was performed with the rapid procedure (26) using Du Pont Cronex-4 X-ray film, Du Pont Lighting Plus intensifying screen in Kodac X-Omatic Cassette at low temperature (-70°C). CRIE classification was obtained by sequential exposure according to a previously described procedure (26).

RESULTS

Patients

The maintenance dose of 100 µg was reached within 6-7 weeks in all but three patients. Two

Table 1
Pretreatment sting reactions according to Mueller (19)

Grade	No. of patients
1. Slight general reaction	5*
2. General reaction	7
3. Severe general reaction	1
4. Shock reaction	1

* Including two patients with large local reactions

Table 2
Skin prick test classification

End point conc. (µg/ml)	No. of patients
100	-
33	2
10	7
3	1
1	4

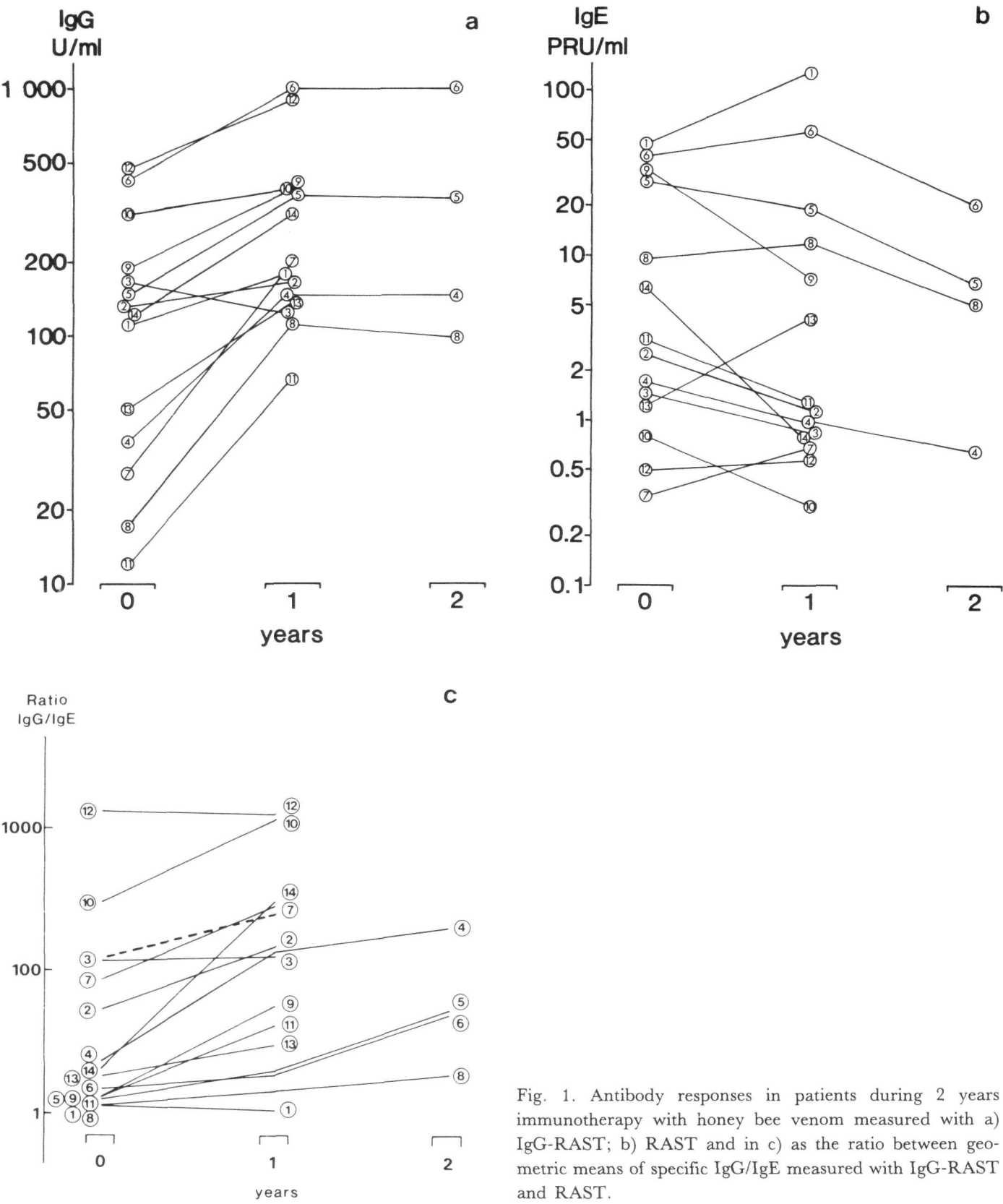


Fig. 1. Antibody responses in patients during 2 years immunotherapy with honey bee venom measured with a) IgG-RAST; b) RAST and in c) as the ratio between geometric means of specific IgG/IgE measured with IgG-RAST and RAST.

patients reached the maintenance dose 1 and 10 months later. In one patient the top dose could never be reached due to asthmatic reaction. Six patients had an accidental sting from honey bee during the first year of treatment with only a normal small local reaction.

HBV-specific antibodies

Individual values of HBV-specific IgG antibodies during treatment are shown in Fig. 1a. After 1 year of immunotherapy with honey bee venom, the HBV-specific IgG antibody levels measured with IgG-RAST showed a

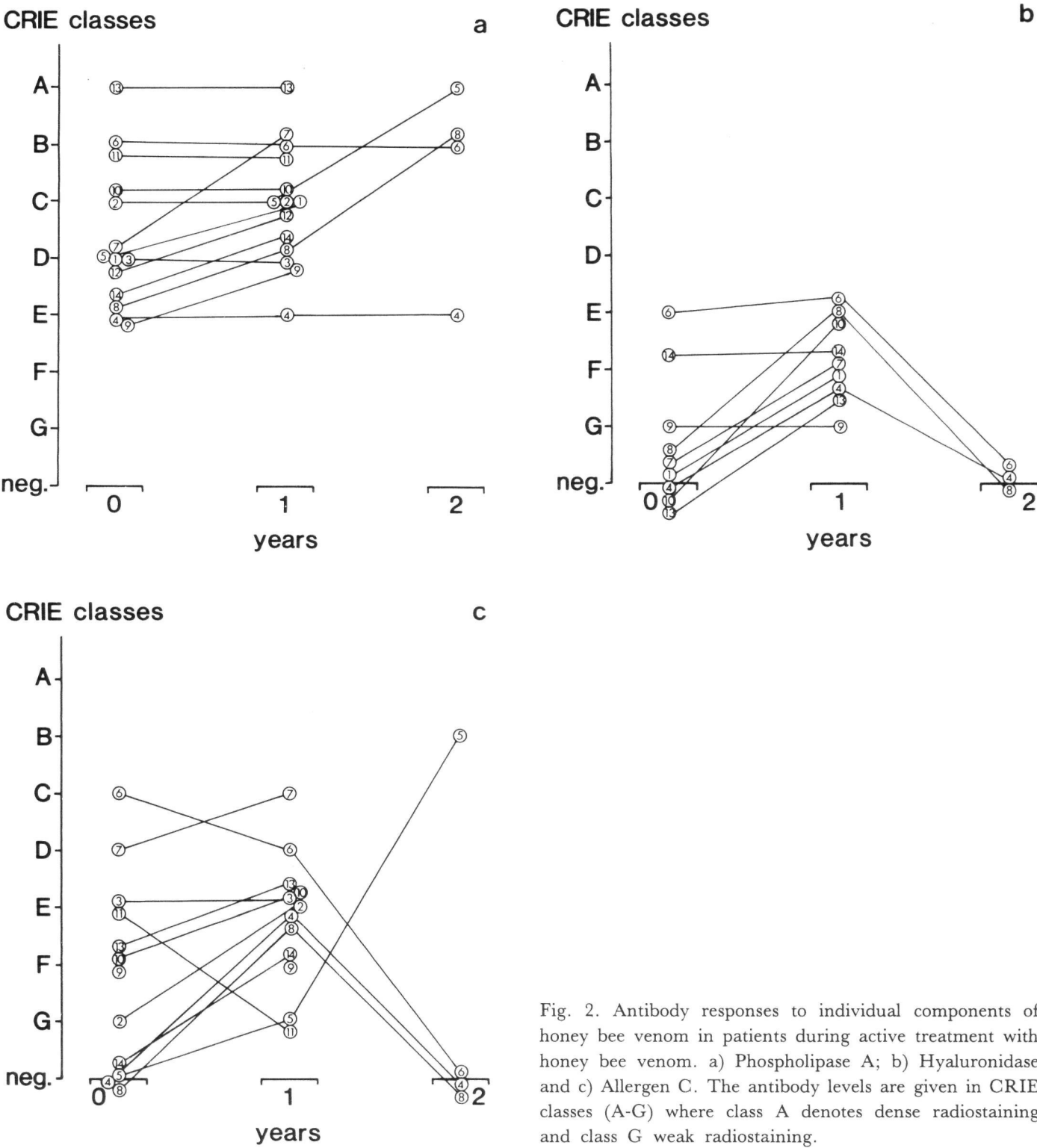


Fig. 2. Antibody responses to individual components of honey bee venom in patients during active treatment with honey bee venom. a) Phospholipase A; b) Hyaluronidase and c) Allergen C. The antibody levels are given in CRIE classes (A-G) where class A denotes dense radiostaining and class G weak radiostaining.

rather steep increase in all patients but one. Only four of the patients were studied after another year of treatment and the HBV-specific IgG serum concentrations were not significantly changed during the observation period. Individual values of HBV-specific IgE antibodies during treatment are demonstrated in Fig. 1b. HBV-specific IgE antibody levels as measured by RAST decreased slightly during the first year of therapy. The four

patients' sera which were analysed after one more year showed a further slight decrease. Fig. 1c demonstrates the individual and mean values of the ratio HBV-specific IgG/IgE during the course of therapy. For most of the patients there was a marked increase in the antibody ratio during the first year of treatment. The four patients studied after 2 years exhibited a further increase in the quotient HBV-specific IgG/IgE.

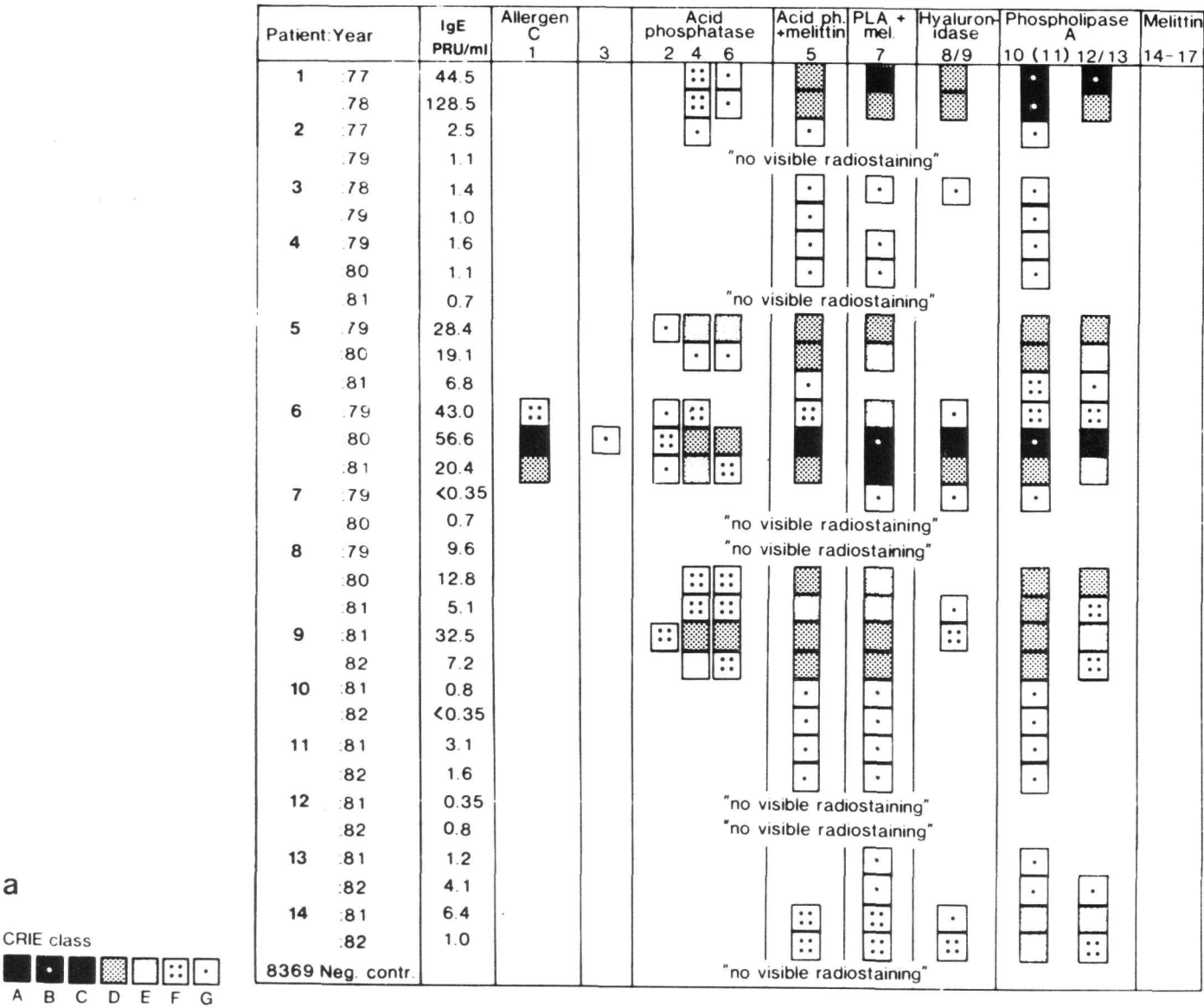


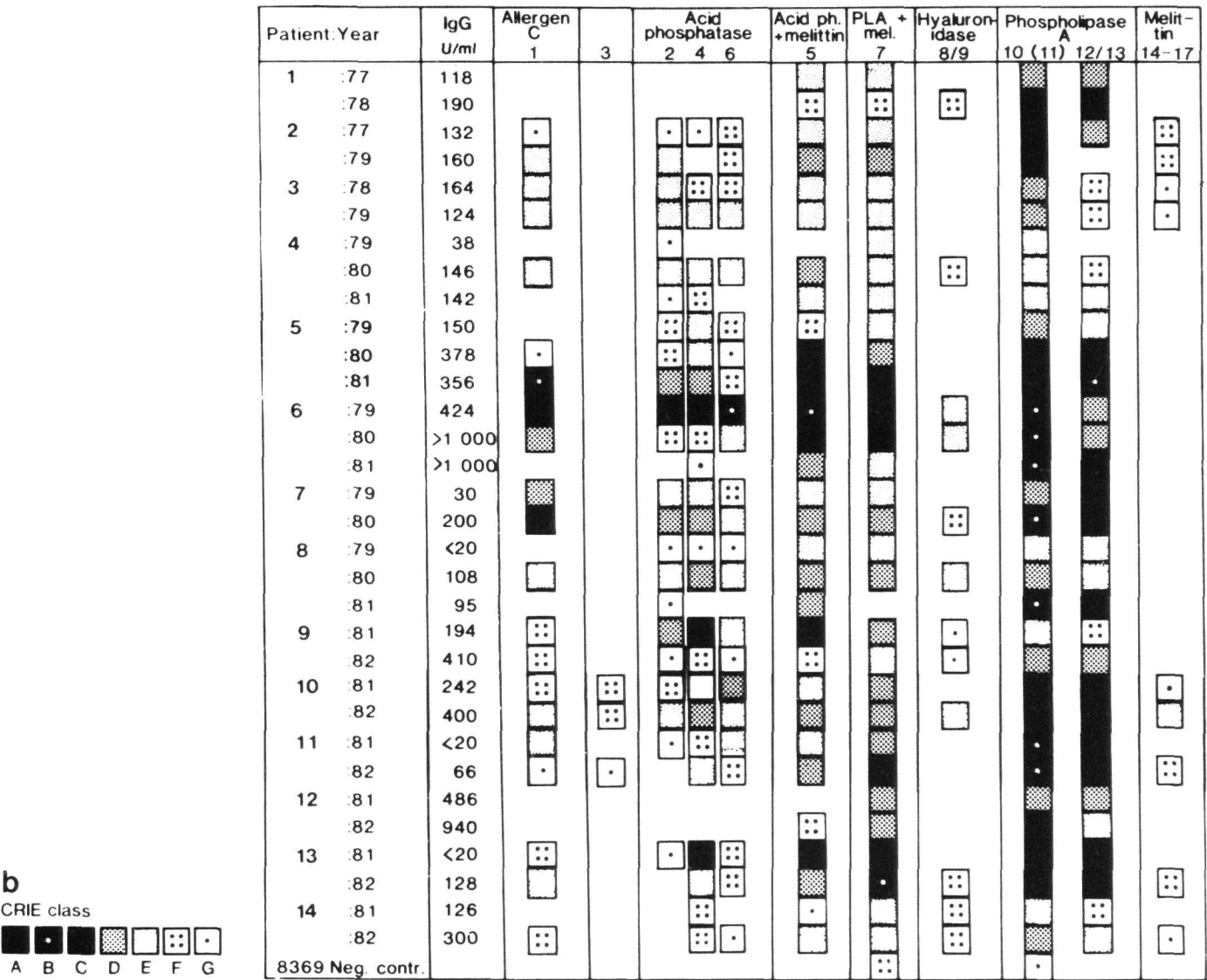
Fig. 3. Allergogram showing CRIE radiostaining of different precipitates in a CIE reference pattern of bee venom before and after treatment with honey bee venom for 1 or 2 years. a) Specific IgE binding to bee venom components; b) Specific IgG binding to bee venom components. The intensity of the radiostaining is shown as hatched squares symbolizing the CRIE classes (A-G).

CRIE analysis

The IgG-CRIE radiostaining data of some individual bee venom immunoprecipitates before and after immunotherapy are given in Fig. 2a-c. Only data from the precipitate considered to be the most representative of the multiple forms are given, i.e. the radiostaining from "antigen 10(11)" represents PLA (Fig. 2a). This precipitate gave, in most cases, a heavy radiostaining prior to starting active treatment. In the majority of cases, stronger radiostaining was found during the therapy. Radiostaining of hyaluronidase of the pretreatment serum was

low or absent (Fig. 2b). Clearly raised antibody levels were found after 1 year of treatment, but after 2 years the levels had decreased to the pretreatment level. The radiostaining of antigen 1 (Fig. 2c) representing allergen C (2, 10) showed a similar pattern, indicating antibody concentrations which initially rose followed by subsidence during the second year of therapy. One of the patients contrasted in that the serum showed continually rising antibody levels as reflected by an increasing degree of radiostaining.

The detailed IgE- and IgG-CRIE patterns of all patients studied are summarized in Fig.



3a-b. The most important allergen was PLA, but hyaluronidase, acid phosphatase, and antigen 1 also gave radiostaining in some patients. None of the patients showed detectable radiostaining to melittin. Small changes in specific IgE antibody concentrations during therapy were not registered as changed radiostaining in CRIE (within the specific CRIE class), whereas more pronounced changes were registered quantitatively by decreased CRIE scores to PLA. From the IgG-CRIE patterns (Fig. 3b) it is obvious that treatment causes a significant increase in the degree of radiostaining for specific IgG antibodies, and this observation is especially pronounced for the multiple forms of PLA. Most allergens in HBV (hyaluronidase, acid phosphatase and allergen C) show an increased radiostaining during the course of therapy, reflecting increases in the level of specific IgG.

DISCUSSION

Hymenoptera sting allergy differs from inhalant allergy in that the allergen dose is considerably higher and in the route of antigen presentation. There is also a simultaneous presence of toxic and vasoactive compounds which may favour non-specific sensitization. Honey bee venom has also been suggested to act as an adjuvant for IgG antibodies to grass pollen antigens in bee keepers (14). Furthermore, an atopic individual is not necessarily developing a bee sting allergy more often than individuals of the general population unless frequently stung (18, 24). In spite of this, in more than half of repeatedly stung bee keepers, IgE antibodies to bee venom can be detected by using sensitive radioimmunoassay techniques (22). Only in a few, however, do IgE antibody levels become elevated enough to cause clinical allergy. We know

from other allergen systems that parenteral injections of allergens may cause IgE antibody synthesis, but this may be regarded as a common part of a humoral immune response.

Immunotherapy involves repeated injections of high doses of potent immunogens. There is normally a rise of IgG "blocking" antibodies whereas the corresponding IgE antibodies are unchanged or decreased. The aim of the therapy is clinical protection but no simple parameter for this is yet established. Allergen-specific IgG antibody levels, however, provide a rough indication of protection against systemic reactions (6, 16, 23, 28). Golden et al. (6) have suggested that an absolute level of more than 5 µg specific IgG per ml (approximately 150 IgG-RAST Units) provides statistical evidence of clinical protection. Patients not reaching that level might be at a higher risk when re-stung. Most of the patients included in this study reached a level of 150 IgG RAST U/ml after 1 year of therapy and consequently should be protected. About 40% of our patients were stung after reaching the maintenance dose without any signs of systemic reactions. The determination of total allergen-specific IgG antibodies, however, does not give the complete answer, since IgG antibody responses to one or several components may be absent or of low degree even with a strong total allergen-specific IgG antibody response. Such data may provide a putative explanation for treatment failures as has been suggested (10, 13) and thus detailed studies of the antibody responses are of value.

The ratio IgG/IgE increased in all treated patients but three, where it remained unchanged. In two of these patients it was difficult to reach the maintenance dose, but one of them was accidentally stung before reaching the top dose with only a normal reaction. The third patient had the highest pretreatment level of specific IgG in the group studied and this patient might thus have reached an adequate immune response already before initiation of therapy. In general, the IgG/IgE ratio might be a better indicator of clinical improvement than specific IgE and IgG measured separately.

Many of the pretreatment sera in this study gave strong radiostaining in IgG-CRIE to the

major bee venom allergen, PLA. This fits with our own previous findings and is not surprising since sensitization causing IgE development is known to include IgG antibody synthesis. There is probably a bias in that many of the patients were chosen for immunotherapy due to an increased risk of getting stung since they belonged to bee keeper families. This fact could also explain the elevated pretreatment PLA-antibody levels. Regarding the other allergens, elevated specific IgG levels were registered primarily to antigen 1, considered to represent allergen C (1, 10), and also to some extent to hyaluronidase.

As could be expected, increased radiostaining was found after 1 year of treatment to all important allergens in IgG-CRIE, but after 2 years sustained or increased radiostaining was obtained to PLA alone. Thus, regarding the less important allergens, hyaluronidase and antigen 1, the patients examined after 2 years of treatment showed decreasing radiostaining except in one case. Decreasing IgG levels in spite of higher dosages during the course of immunotherapy have been registered by others (16, 28). This may not be an unfortunate result, but could instead represent a lessened tendency towards immune response once the desired peak level has been reached during the course of therapy. Specific IgG and IgE diminishing together might then be regarded as a more beneficial sign, and it may stand to reason that this therapeutic effect is more easily obtained to weaker immunogens, such as hyaluronidase, than to substances with strong immunogenic capacity such as PLA. At this point, however, the data are preliminary and need to be confirmed.

REFERENCES

1. Aukrust, L. & Aas, K.: A reference system in crossed radioimmuno-electrophoresis. *Scand. J. Immunol.* 6, 1093-1099, 1977.
2. Aukrust, L., Einarsson, R., Öhman, S. & Johansson, S. G. O.: Crossed radioimmuno-electrophoretic studies of bee venom allergens. *Allergen* 37, 265-271, 1982.
3. Berg, T. & Johansson, S. G. O.: *In vitro* diagnosis of atopic allergy. II. IgE and reaginic antibodies during and after rush desensitization. *Int. Arch. Allergy* 41, 434-442, 1971.
4. Clayton, W. F., Reisman, R. E., Georgitis, J. W.,

- Wypych, J. I. & Arbesman, C. E.: Effect of prolonged venom immunotherapy on serum venom-specific IgE and IgG. *Clin. Allergy* 13, 301-307, 1983.
5. Einarsson, R., Annerhed, A., Karlsson, R., Olsson, B. & Renck, B.: Crossed immunoelectrophoresis analysis of bee venom. *Allergy* 37, 273-278, 1982.
6. Golden, D. B. K., Meyers, D. A., Kagey-Sobotka, A., Valentine, M. D. & Lichtenstein, L. M.: Clinical relevance of the venom specific immunoglobulin G antibody level during immunotherapy. *J. Allergy Clin. Immunol.* 69, 489-493, 1982.
7. Habermann, E.: Bee and wasp venoms. *Science* 177, 314-322, 1972.
8. Harboe, N. & Ingild, A.: Immunization and isolation of immunoglobulins, estimation of antibody titre. *Scand. J. Immunol.* 2, Suppl 1, 161-164, 1973.
9. Hoffman, D. R., Gillman, S. A., Cummins, L. H., Kozak, P. P. & Oswald, A.: Correlation of IgG and IgE antibody levels to honey bee venom allergens with protection to sting challenge. *Ann. Allergy* 46, 17-23, 1981.
10. Hoffman, D. R., Shipman, W. H. & Babin, D.: Allergens in bee venom. II. Two new high molecular weight allergenic specificities. *J. Allergy Clin. Immunol.* 59, 147-153, 1977.
11. Hunt, K. J., Valentine, M. D., Sobotka, A. K., Benton, A. W., Amodio, F. J. & Lichtenstein, L. M.: A controlled trial of immunotherapy in insect hypersensitivity. *N. Engl. J. Med.* 299, 157-161, 1978.
12. Jarisch, R., Yman, L., Boltz, A., Sandor, I. & Janitsch, A.: IgE antibodies to bee venom, phospholipase A₂, melittin and wasp venom. *Clin. Allergy* 9, 535-541, 1979.
13. Kemeny, M. D., MacKenzie-Mills, M., Karries, M. G., Youtlen, L. J. F. & Lessof, M. H.: 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.* 72, 376-385, 1983.
14. Kemeny, D. M., Miyachi, S., Platts-Mills, T. A. E., Wildins, S. & Lessof, M. H.: The immune response to bee venom. Comparison of the antibody response to phospholipase A₂ with the response to inhalant antigens. *Int. Arch. Allergy Appl. Immunol.* 68, 268-274, 1982.
15. King, T. P., Sobotka, A. K., Kochoumian, L. & Lichtenstein, L. M.: Allergens of honey bee venom. *Arch. Biochem. Biophys.* 172, 661-671, 1976.
16. Lichtenstein, L. M., Valentine, M. D. & Sobotka, A.: Insect allergy: The state of the art. *J. Allergy Clin. Immunol.* 64, 5-12, 1979.
17. Light, W. C., Reisman, R. E., Wypych, J. I. & Arbesman, C. E.: Clinical and immunological studies of bee keepers. *Clin. Allergy* 5, 389-395, 1975.
18. Miyachi, S., Lessof, M. H. & Kemeny, D. M.: Evaluation of bee sting allergy by skin tests and serum antibody assays. *Int. Arch. Allergy Appl. Immunol.* 60, 148-153, 1979.
19. Mueller, H. L.: Diagnosis and treatment of insect sensitivity. *J. Asthma Res.* 3, 331-333, 1966.
20. Müller, U., Spies, J. & Roth, A.: Serological investigations in hymenoptera sting allergy: IgE and haemagglutinating antibodies against bee venom in patients with bee sting allergy. *J. Allergy Clin. Immunol.* 7, 147-154, 1977.
21. Nordvall, S. L., Uhlin, T. & Einarsson, R.: Determination of specific IgG antibody by crossed radioimmuno-electrophoresis. *Allergy* 38, 183-189, 1983.
22. Nordvall, S. L., Uhlin, T., Einarsson, R., Johansson, S. G. O. & Öhman, S.: Bee keepers' IgG and IgE antibody responses to bee venom studied by means of crossed radioimmuno-electrophoresis. *Clin. Allergy* 14, 341-350, 1984.
23. Norman, P. S.: A review of immunotherapy. *Allergy* 33, 62-70, 1978.
24. Settupane, G. A., Newstead, G. J. & Boyd, G. K.: Frequency of Hymenoptera allergy in an atopic and normal population. *J. Allergy Clin. Immunol.* 50, 146-150, 1972.
25. Sobotka, A. K., Franklin, R. M., Adkinson, N., Valentine, M., Baer, H. & Lichtenstein, L. M.: Allergy to insect stings. II. Phospholipase A. The major allergen in honey bee venom. *J. Allergy Clin. Immunol.* 57, 29-40, 1976.
26. Uhlin, T. & Einarsson, R.: A rapid procedure for crossed radioimmuno-electrophoresis. *Int. Arch. Allergy* 70, 213-219, 1983.
27. Urbaneck, R., Karitzky, K. & Forster, J.: Hyposensibilisierungsbehandlung mit reinem Bienengift. *Dtsch. Med. Wochenschr.* 103, 1656-1660, 1978.
28. Urbaneck, R., Krauss, U., Ziupa, J. & Smedegård, G.: Venom-specific IgE and IgG antibodies as a measure of the degree of protection in insect-sting-sensitive patients. *Clin. Allergy* 13, 229-234, 1983.
29. Wütrich, B., Arrendal, H. & Lanner, Å.: Monitoring of venom-specific IgE and IgG antibodies during immunotherapy. In: Johansson, S. G. O. (ed): *International Allergy Symposium: Diagnosis and treatment of IgE mediated diseases*. Uppsala, September 24-26, 1980. *Excerpta Medica* 95-106, 1981.

Address

Roland Einarsson
Pharmacia AB
Box 604
S-751 Uppsala
Sweden

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.