

IgG and IgE Antibody Patterns after Immunotherapy with Monomethoxy Polyethyleneglycol Modified Honey Bee Venom

S. L. NORDVALL,^{1,3} T. UHLIN,³ S. ÖHMAN,² J. BJÖRKANDER⁴ H.-J. MALLING,⁵ B. WEEKE,⁵ S. DREBORG,³ Å. LANNER³ and R. EINARSSON³

^{1,2}Depts. of Pediatrics and Dermatology, University Hospital, Uppsala, ³Pharmacia Diagnostics AB, Uppsala,

⁴Asthma and Allergy Research Centre, Dept. of Medicine I, Sahlgrenska Hospital, University of Göteborg, Sweden, and ⁵Allergy Unit, Rigshospitalet, Copenhagen, Denmark

Antibody responses to honey bee venom (HBV) were studied in 13 patients during a 4-month course of immunotherapy with monomethoxy polyethyleneglycol (mPEG) modified venom. There was a rise of HBV-specific IgG antibodies as measured by IgG-RAST in all patients and a slight decrease of IgE antibody in most of them. The IgG-antibody responses during mPEG-HBV treatment as examined by crossed radio-immunoelectrophoresis were directed to phospholipase A, hyaluronidase, acid phosphatase and to another allergen, antigen 1. Thus, despite a high degree of mPEG-modification of HBV, the immunogenicity of the most important HBV allergens was retained.

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

Accepted for publication 22 August 1985

Immunotherapy with potent allergen extracts involves the risk of provoking serious side effects in the hypersensitive patient. This risk can be reduced by the use of modified allergen extracts with reduced allergenicity. Various methods for the modification of allergen preparations have been described. Experience from immunotherapy has been obtained, e.g. with formaldehyde-modified (18, 20) glutaraldehyde-modified (6, 11) and polyethyleneglycol-modified allergen extracts (7, 16, 17). The clinical efficacy of therapy with the modified allergens and the IgG-responses are similar to those with the unmodified preparation. Suppression of IgE antibody responses, such as reported in animal experiments (10), has not been regularly achieved.

Since an IgG antibody response appears to be important for clinical efficacy (15), retained immunogenicity of the components of modified

honey bee venom (HBV) is of interest. We therefore studied the immune response of monomethoxy polyethyleneglycol (mPEG)-modified honey bee venom in patients on therapy with this substance. The main purpose of this study was to learn against which allergenic components IgG antibody responses were obtained.

MATERIAL AND METHODS

Patients and treatment

The study comprised 14 patients aged 24-55 with a history of bee sting allergy, positive skin prick test with bee venom, and positive RAST tests (24). The patients were given therapy as subcutaneous injections with mPEG-HBV. The doses were increased from 15 µg to 400 µg over 4 months. None of the patients was stung by

bees during the treatment period which was in the winter of 1982. Serum was drawn before and after 4 months of therapy and was kept frozen until analysed. One of the treated patients was not analysed due to insufficient serum amounts (24).

Allergen preparations

Venom of honey bee (*Apis mellifera*), lot 00500, obtained by electrical stimulation, was bought from Mr. C. Mraz, Champlain Valley, Middlebury, Vermont. This venom lot was also used for raising a pooled rabbit antiserum, essentially according to the procedure of Harboe & Ingild (5) and also as the antigen in crossed immunoelectrophoresis. The mPEG-HBV preparation used for immunotherapy was also produced from this venom lot.

The mPEG-HBV product was prepared by coupling mPEG succinate of molecular weight 5700 daltons to HBV, employing the mixed anhydride method (22). Activated mPEG is mainly coupled to the epsilon amino groups of the proteins by this method. The degree of modification was determined by analysing the product for its content of mPEG, protein and lysine, and calculating the mPEG/lysine ratio in the modified product.

The mPEG-HBV preparation contained 80 % mPEG, 15 % protein and 0.148 mmol lysine/g dry weight. Of the total number of lysine residues, 94 % were modified with mPEG. The content of mPEG was determined by nuclear magnetic resonance (NMR) technique, and the protein and lysine contents were determined by amino acid analysis. The molecular weight of mPEG-HBV was determined by gel filtration on a calibrated Sepharose 6B column. The average molecular weight of mPEG-HBV was approx. 440,000 d. In RAST inhibition the allergenicity of mPEG-HBV was reduced to 0.5 % of that of the unmodified HBV, and with histamine release assay the allergenicity was reduced to below 1 %. The enzyme activity was also significantly reduced; hyaluronidase enzyme activity approx. 1 % and phospholipase A₂ activity approx. 1 % of the unmodified HBV (23).

Radioimmunoassays

Total IgE was measured by Phadebas PRIST®, and HBV specific IgE and IgG antibody concentrations were determined by Phadebas RAST® and Phadebas IgG RAST® kits (Pharmacia Diagnostics AB, Uppsala, Sweden), respectively. The results were expressed in Phadebas RAST units/ml (PRU/ml) for specific IgE and arbitrary units (U/ml) for specific IgG.

Crossed immunoelectrophoresis (CIE)

CIE was performed on gelbond film (8.2 cm × 8.2 cm) as previously described by Einarsson et al. (3) using 0.025 mol/l TRIS/Tricine buffer (Bio-Rad), pH 8.6 in 1 % agarose (Agarose A, Pharmacia Fine Chemicals, Uppsala, Sweden). In the first dimension electrophoresis, 75 µg bee venom was run at 10 V/cm for 55 min at 15°C. In a second dimension electrophoresis, the separated bee venom proteins were electrophoresed at 2 V/cm for 18 h into an agarose gel containing 14 µl rabbit antiveenom serum per cm² gel.

Crossed radioimmuno-electrophoresis (CRIE)

Allergen-specific IgG detection. IgG-CRIE was performed as previously described (14). The CIE plates were incubated overnight in patient sera diluted 1/200 in 0.05 mol/l phosphate buffer, pH 7.4 containing 0.9 % sodium chloride, 0.3 % Tween and 0.3 % Dextran 10 (PDT). Pretreatment and posttreatment sera were incubated simultaneously. After washing 4 × 30 min using 10 % PDT buffer solution in 0.9 % sodium chloride the plates were re-incubated in a dilution of ¹²⁵I-anti-IgG (72,000 cpm/ml). The plates were washed again 4 × 30 min, the last time in distilled water. Autoradiography was performed with the rapid procedure (21) using Du Pont Cronex-4 X-ray film and Du Pont Lighting Plus intensifying screen in Kodak X-Omatic cassettes at low temperature (-70°C).

Allergen-specific IgE detection. IgE-CRIE was performed as previously described (2), incubating the CIE plates overnight in 10 ml serum diluted

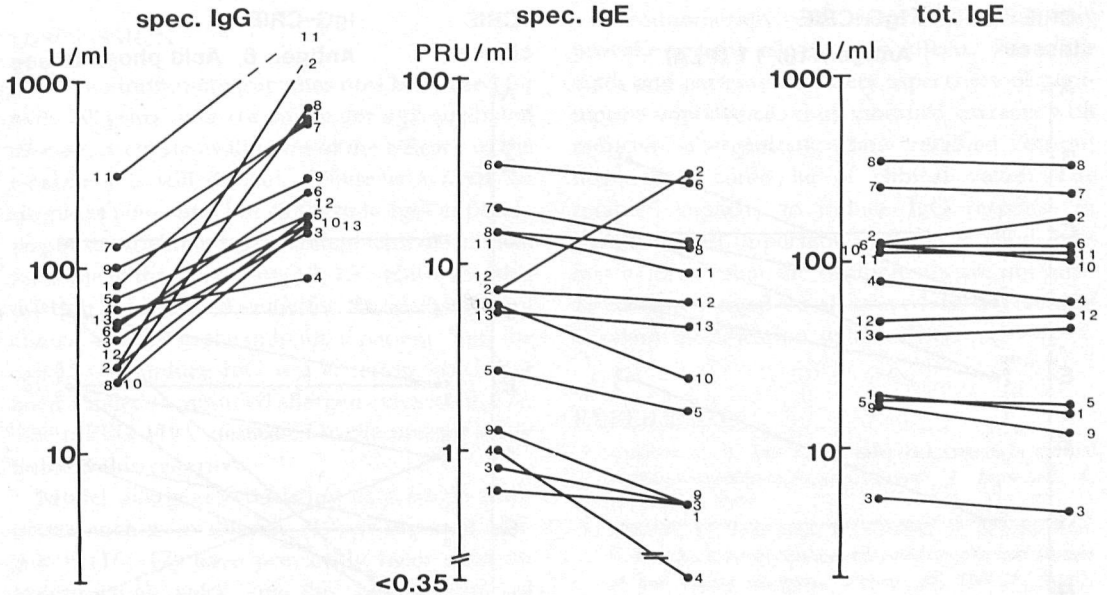


Fig. 1. Antibody responses in 13 patients during 4 months' treatment with mPEG-modified honey bee venom measured with IgG-RAST, RAST, and PRIST.

(1/17) in PDT-buffer solution. After washing, the plates were reincubated overnight in 72,000 cpm/ml ^{125}I -anti-IgE (Pharmacia Diagnostics AB), washed again and after drying autoradiography was performed as described for IgG-CRIE. The amount of serum available was only enough to perform simultaneous experiments in seven of the 13 patients.

Reference systems for CRIE were used as previously described in detail for IgE detection (1) and IgG detection (14). Thus, the degree of radiostaining was semiquantified and expressed in CRIE-classes, A-G, A denoting a high antibody content.

RESULTS

Immunotherapy for 4 months with mPEG-modified HBV caused raised specific IgG antibody levels as measured by IgG-RAST for all patients in the trial (Fig. 1). The degree of radiostaining in IgG-CRIE of the most important allergenic components before and after 4 months of treatment is shown in Fig. 2. The degree of radiostaining in CRIE classes of PLA (precipitate 10/11), hyaluronidase (precipitate

8/9) and of antigen 1 are given. Before immunotherapy, IgG antibody binding above background level to the precipitates containing PLA, was registered in most patients. Radiostaining to hyaluronidase, one isomer of acid phosphatase (precipitate 6) and to antigen 1 was of a lower degree and occurred less frequently. Weak binding to melittin was registered in only a few patients (data not shown). After 4 months of immunotherapy, IgG antibody increases were registered for all of these antigens except melittin. The PLA-specific IgG responses were consistently of 1-2 CRIE classes higher except in one patient, where no increase was registered. The IgG responses to the other important HBV allergens: hyaluronidase, acid phosphatase and antigen 1 were more varied, but apparently strong in some of the patients. Melittin-specific antibodies induced by therapy were not detectable as visible radiostaining.

A small decrease in HBV-specific IgE antibody levels was found, but this was barely reflected in the total IgE level (Fig. 1). The IgE pattern in CRIE was virtually unchanged in the studied patients during immunotherapy and, further, in some patients no decrease in specific IgE could be found (data not shown).

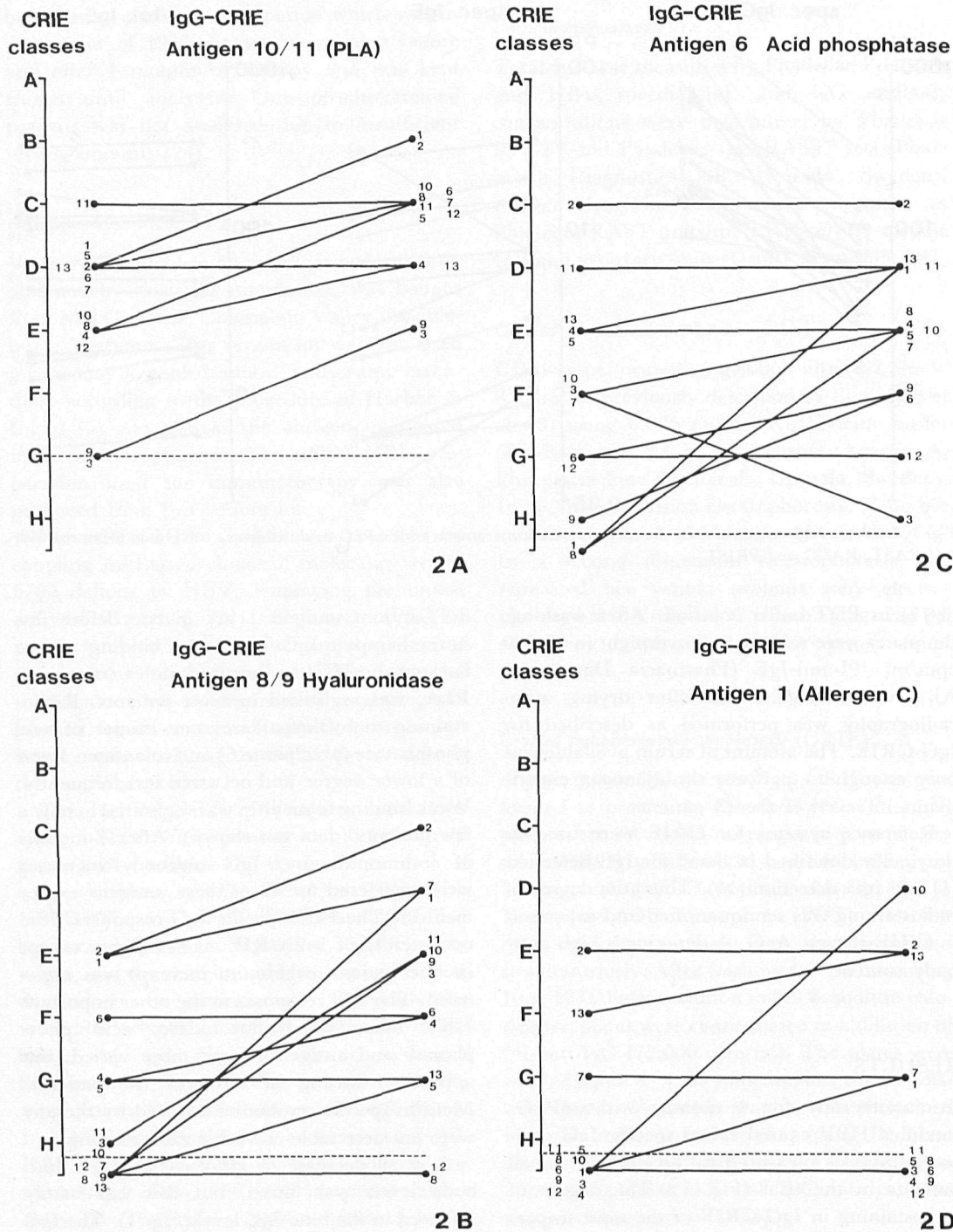


Fig. 2. Antibody responses to individual components of honey bee venom in 13 patients during 4 months treatment with mPEG modified honey bee venom. The antibody levels are given in CRIE classes (A-H) (see text), class A denoting dense antibody radiostaining, H weak radiostaining (low levels). ---- denotes the level of background radiostaining.

DISCUSSION

Although immunotherapy has now been used for over 70 years as a treatment for IgE-mediated disease, accurate evaluation of the efficacy of the treatment is still difficult. There is a need for simple *in vitro* tests, but so far only IgG antibody responses are known to correlate with the clinical outcome of the treatment (13, 15). However, this relation is of limited value for the assessment of clinical efficacy in the individual patient. Yet, the capacity to induce IgG is a criterion which has been applied to modified allergen extracts (9, 17). The mPEG-HBV described in the present study fulfilled this criterion.

Model allergens, consisting of a single compound such as ovalbumin (19) or ragweed antigen E (16, 17) have previously been used for experimental work on the modification of allergens. The degree of modification is easily controlled when simple substances are modified. Previous works have shown that the degree of PEG-modifications appeared to be important for the immunogenicity of the substance, which under extreme conditions could even be abolished by too high a degree of modification (19). Thus, it was important to study the immunogenicity, i.e. the IgG response in man to the various components of an mPEG-HBV preparation.

In treatment with mPEG-HBV distinct serum IgG responses occurred most frequently to the major allergen, phospholipase A. Even though antibody responses to all allergens were not detected in all patients, the data clearly show retained immunogenicity of HBV in man after PEG-modification. The results were similar to those obtained with sera from hyperimmunized bee keepers (12). The data reflect the genetic capacity in man for immune response within the HBV antigen complex. With the described methods, no IgG response at all was detectable to melittin in most hyperimmunized bee keepers. Apparently, the high concentration of melittin in bee venom (4) does not compensate for its low immunogenicity. Melittin is known from other studies to be a weak immunogen and allergen (2, 8), but, occasionally, high IgE response can be detected (12).

Immunotherapy with native allergens may provoke serious allergic side effects. For clinicians and patients the safety aspects are of paramount importance, thus modified extracts with reduced allergenicity, but retained immunogenicity could be of clinical value. The retained capacity to induce IgG response in humans to all important allergens is good indirect evidence that the components are not antigenically changed or destroyed as a result of covalent modification with mPEG.

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. *Allergy* 37, 265-271, 1982.
3. Einarsson, R., Annerhed, A., Karlsson, R., Olsson, B. & Renck, B.: Crossed immunoelectrophoresis analysis of bee venom. *Allergy* 37, 273-278, 1982.
4. Habermann, E.: Bee and wasp venoms. *Science* 177, 314-322, 1972.
5. Harboe, N. & Ingild, A.: Immunization and isolation of immunoglobulins, estimation of antibody titre. *Scand. J. Immunol.* 2, Suppl. 1, 161-164, 1973.
6. Johansson, S. G. O., Miller, A. C. M. L., Mullen, N., Overell, B. G., Tees, E. C. & Wheeler, A. W.: Glutaraldehyde-pollen tyrosine. Clinical and immunological studies. *Clin. Allergy* 4, 255-263, 1974.
7. Juniper, E. F., O'Connor, J., Roberts, R. S. & Hargreave, F. E.: A two year study of Pegalgen (a polyethylene glycol modified ragweed extract) in ragweed rhinoconjunctivitis (Abstract). *J. Allergy Clin. Immunol.* 71, 119, 1983.
8. King, T. P., Sobotka, A. K., Kochoumian, U. & Lichtenstein, L. M.: Allergens of honey bee venom. *Arch. Biochem. Biophys.* 172, 661-671, 1976.
9. King, T. P. & Giallongo, A.: Some immunochemical studies of native and modified allergens. In Boström, H. & Ljungstedt, N. (eds.): Theoretical and clinical aspects of allergic diseases, pp. 215-232. Skandia International Symposium, Almqvist & Wiksell, Stockholm, 1983.
10. Lee, W. Y. & Schon, A. H.: Abrogation of reaginic antibodies with modified allergens. *Nature* 263, 618-619, 1977.
11. Moran, D. M., Wheeler, A. W., Overell, B. G. & Woroniecki, S. R.: Chemical modification of crude timothy grass pollen extract. III. The effect of glutaraldehyde-induced aggregation of antigenic and immunogenic properties. *Int. Arch. Allergy Appl. Immunol.* 68, 460-470, 1977.
12. Nordvall, S. L., Uhlén, 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.
13. Nordvall, S. L., Berg, T., Johansson, S. G. O. & Lanner, Å.: Clinical studies of a purified timothy pollen extract: Desensitization therapy with a purified timothy pollen preparation compared to a crude timothy pollen extract. II. Results of the tests in vitro and their relation to symptoms and tests in vivo. *Int. Arch. Allergy Appl. Immunol.* 67, 132-138, 1982.
14. Nordvall, S. L., Uhlin, T. & Einarsson, R.: Determination of specific IgG antibody by crossed radioimmuno-electrophoresis. *Allergy* 38, 183-189, 1983.
15. Norman, P. S.: A review of immunotherapy. *Allergy* 33, 62-70, 1978.
16. Norman, P. S., Alexander, J. F., King, T. P., Creticos, P. S., Sobotka, A. K. & Lichtenstein, L. M.: Responses to conjugated AgE in ragweed hay fever. (Abstract). *J. Allergy Clin. Immunol.* 69, 99, 1982.
17. Norman, P. S., King, T. P., Alexander, J. F., Kagey-Sobotka, A. & Lichtenstein, L. M.: Immunologic responses to conjugates of antigen E in patients with ragweed hay fever. *J. Allergy Clin. Immunol.* 73, 782-789, 1984.
18. Norman, P. S., Lichtenstein, L. M. & Marsh, D. G.: Studies on allergoids from naturally occurring allergens. IV. Efficacy and safety of long-term allergoid treatment of ragweed hay fever. *J. Allergy Clin. Immunol.* 68, 460-470, 1981.
19. Richter, A. W. & Åkerholm, E.: Antibodies against polyethyleneglycol produced in animals by immunization with monomethoxy polyethyleneglycol modified proteins. *Int. Arch. Allergy Appl. Immunol.* 70, 124-131, 1983.
20. Stull, A., Cooke, R. A., Sherman, W. B., Hebard, S. & Hampton, S. F.: Experimental and clinical study of fresh and modified pollen extracts. *J. Allergy* 11, 439-465, 1940.
21. Uhlin, T. & Einarsson, R.: A rapid procedure for crossed radioimmuno-electrophoresis. *Int. Arch. Allergy Appl. Immunol.* 70, 213-219, 1983.
22. Wie, S. I., Wie, C. V., Lee, W.Y., Filion, L. G. & Schon, A. H.: Suppression of reaginic antibodies with modified allergens. III. Preparation of tolerogenic conjugates of common allergens with monomethoxypolyethylene glycols of different molecular weight by the mixed anhydride method. *Int. Arch. Allergy Appl. Immunol.* 64, 84-99, 1981.
23. Åkerblom, E., Ahlstedt, S. & Björkstén, B.: Allergenic and immunogenic properties of monomethoxy polyethylene glycol (mPEG) modified honey bee venom (HBV). A communication given at XI Int. Congr. Allergy and Clin. Immunol., London, 1982.
24. Öhman, S., Björkander, J., Malling, H.-J., Weeke, B., Dreborg, S. & Lanner, Å.: A preliminary study of immunotherapy with a monomethoxy polyethylene glycol modified honey-bee venom preparation. *Allergy* 41, 81-88, 1986.

Address:

Dr. Roland Einarsson
Pharmacia AB
S-751 82 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.