

IgE Antibody Studies in a Case of Generalized Allergic Reaction to Human Insulin

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A patient developed a generalized reaction to purified bovine, purified porcine and human insulin. High levels of IgE antibody to bovine, porcine, human emp and human crb insulin were demonstrated. These fell dramatically following desensitization with human crb insulin. All four types of insulin gave superimposable RAST inhibition curves indicating a common allergen determinant.

Key words: insulin allergy; RAST; human insulin.

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CLINICAL ASPECTS

IgE-mediated allergy can develop to allergens unique to the insulin molecule. This case was rapidly and successfully desensitized using human insulin produced with the recombinant DNA technology.

Generalized reactions to insulin are rare. There is usually a history of previous insulin therapy interrupted by a period of treatment with diet alone or with oral hypoglycaemic agents. The cause of the allergy and the nature of the antigen is obscure. Zinc has been implicated (5) for although it is not included in the formulation of short-acting insulins, it is present entrapped in the insulin molecule. The clinical syndrome resembles that produced by foreign proteins and includes urticaria, swelling of face and eyelids, painful joints and occasionally, dyspnoea and dysphagia.

METHODS

IgE antibodies to insulin were determined by RAST (10) essentially as described (7). One allergen-disc was incubated with 50 μ l serum neat or diluted in a RAST-negative, normal

serum for 3 h at room temperature. After washing 100 μ l ^{125} I-labelled anti-IgE of De2 specificity (3) corresponding to 50,000–70,000 cpm was added to each tube for an overnight incubation. The discs were then washed again and the count rates of the discs were determined. The results were expressed in PRU/ml as derived from the Phadebas® RAST Reference System (Pharmacia Diagnostics AB, Uppsala, Sweden).

Commercially available discs with bovine and porcine insulin (Allergo-Discs® MIAB, Sweden) were used. Discs with human insulin were prepared by coupling of 10 μ l humanized insulin (Actrapid Human Insulin, 40 IE/ml, Novo, Denmark) and 10 μ l human insulin of recombinant DNA type (Humulin Regular, 40 IE/ml, kindly provided by Dr. Berglund, Kabi-Vitrum AB, Stockholm, Sweden) per CNBr-activated (1) filter paper discs, respectively. The non-

specific trapping of IgE was, for all four types of insulin discs, not significant for IgE-concentrations below 3000 kU/l.

Allergen cross-reactions were studied by RAST inhibition (6). One volume of serum was incubated with two volumes of insulin neat and serially diluted in a 0.05 M phosphate buffer pH 7.5 containing 0.15 M NaCl, 0.2% bovine serum albumin, 0.1% Tween 20 and 0.02% NaN₃. The result obtained with serum and buffer alone was used as the reference for 100% binding.

IgG antibodies to insulin were determined by the Sepharose protein-A technique (8) using ¹²⁵I labelled insulin (kindly provided by Professor H. Löw, Karolinska Hospital, Stockholm, Sweden). The results were expressed in per cent binding.

RESULTS

Case report

A 28-year-old housewife acquired gestational diabetes during her third pregnancy. Hypurin Isophane insulin (highly purified beef) 8–10 units daily was required from 30 weeks until delivery. She was then treated with diet alone and remained well until she presented 18 months later with thirst, polyuria and a blood glucose of 29.1 mmol/l. She had a past history of childhood urticaria and penicillin allergy.

She was treated with Hypurin Neutral insulin (highly purified beef) 6 units twice daily and, 12 days later, developed a generalized urticarial rash 15 min after an injection. This reaction increased in severity with each dose and Velosulin (highly purified porcine) caused a similar

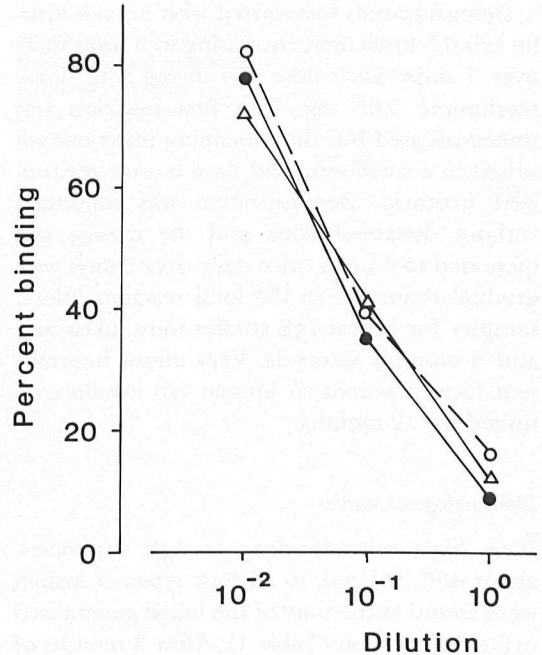


Fig. 1. Porcine insulin was allowed to inhibit the IgE-antibody reaction in RAST with discs coated with porcine (●—●), bovine (○---○) and humanized porcine (△—△) insulins.

reaction. Humanized porcine insulin, human insulin emp (Actrapid Human Insulin, Novo, Denmark) 6 units resulted in an even more severe reaction with widespread urticaria and swelling of hands and feet.

A blood sample was taken for IgE antibody studies, and skin-prick testing, not skin-patch testing, as incorrectly stated in a preliminary report (4), was performed with Hypurin Neutral, Velosulin, Soluble Insulin, Neusilin, Actrapid, Human Insulin emp, Human Insulin crb (Humulin S), with a specially prepared solution of M-Cresol 0.16% v/v + Phenol 0.065% w/v (the preservative used in Hypurin insulins) and with a solution of zinc sulphate 0.0006% w/v as negative control. A positive weal and flare reaction with a weal of at least 5 mm was obtained with all the insulins, but, subjectively, there was less irritation with Human insulin crb. No reaction was obtained with M-Cresol/Phenol or Zinc.

Table 1
Results in RAST (PRU)/ml

Insulin allergen	Presentation	3 months	6 months
Bovine	360	3.2	2.4
Porcine	390	3.1	1.9
Human (emp)	410	4.1	2.2
Human (crb)	420	3.4	2.2

Desensitization was started with human insulin crb 0.5 units daily increasing to 3 units daily over 7 days. Each dose was mixed with dexamethasone 0.05 mg. The first injection was uncomplicated but the remaining injections resulted in a small weal and flare but no generalized urticaria. Desensitization was continued without dexamethasone and the dosage was increased to 6 units twice daily over 5 days with gradual reduction in the local reaction. Blood samples for repeat IgE studies were taken at 3 and 6 monthly intervals. Very minor intermittent local reactions to human crb insulin continued for 12 months.

Immunological studies

Very high concentrations of IgE antibodies, about 400 PRU/ml, to all four types of insulin were found at the time of the initial generalized urticarial reaction (Table 1). After 3 months of treatment the IgE antibody levels dropped 100-fold but no further reduction occurred. A 16-fold increase in IgG antibody concentration measured as per cent binding of ^{125}I labelled insulin accompanied the initial IgE antibody drop.

The specificities of the IgE antibodies were tested in RAST cross-inhibition experiments (Fig. 1). Superimposable dose-response inhibition curves were obtained when the different types of insulin were tested.

DISCUSSION

Our patient developed a generalized allergic reaction with purified bovine, purified porcine and human emp insulin. Sensitization to zinc or a perservative can be discounted in this case in view of the results of skin-prick testing and the high levels of IgE antibody to insulin. The IgE antibody specificity was directed to antigenic determinants of insulin common to all four preparations used and tested. This lends support to the suggestion by Ross et al. (9) that the bovine insulin can initiate a hapten-specific hapten-carried mediated IgE response. Treatment with human insulin is analogous to the administration of the hapten alone which is known to

induce immunologic tolerance. A generalized reaction to human insulin without prior exposure to bovine or porcine insulin has not been reported.

Desensitization using human insulin crb was successful and was associated with a dramatic fall in IgE antibody. We used small doses of insulin mixed with dexamethasone as did Wiles et al. (11), but the addition of dexamethasone may not be necessary (2).

We recommend in view of our experience with this patient that human insulin be used in desensitization of insulin allergy. The use of human insulin in all newly diagnosed insulin-dependent diabetics with a history of allergy might reduce the risk for the appearance of allergic reaction to insulin.

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