

SHORT REPORT

Specificity of IgE antibody against various insulins in a patient with anaphylaxis to beef–pork insulin but not to human (rDNA) insulin

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Introduction

Immediate-type, systemic hypersensitivity reactions to insulin are well recognized [1–3]. The pathogenesis involves a Type I, IgE-mediated mechanism. Several other immunologically mediated clinical reactions to insulin have been described [4,5]. The antigenicity of insulin preparations has been attributed to several of the components present in commercially available insulins, including the insulin molecule itself. The primary amino acid sequences of bovine and porcine insulin differ from that of human insulin by three and one amino acid, respectively. This greater dissimilarity between human and bovine insulin has been postulated to be the explanation for the greater antigenicity of bovine insulin as compared with porcine insulin [6,7]. Human (rDNA) insulin has also been reported to cause allergic reactions [8,9]. It is postulated that this occurs as a result of processing of human (rDNA) insulin which could cause alterations of tertiary structure. This was demonstrated to occur with native human insulin in a patient allergic to animal insulins who also reacted to his own insulin after extraction by an acid-alcohol procedure [10].

In each patient we have studied so far, with local or systemic reactions to insulin, we have found serum IgE directed against each of the insulins. In two patients with anaphylaxis to animal insulins we have reported that the binding of serum antibody to any insulin tested (bovine, porcine, or human (rDNA)) could be inhibited completely by any of the insulins [11,12].

In this report we describe one patient with anaphylaxis to beef–pork insulin and no local or systemic reactions to human (rDNA) insulin. Immunological studies were performed to define the specificity of the anti-insulin antibodies in this patient.

Subject and methods

Case report

A 60-year-old white female patient received an injection

of 40 units NPH mixed beef–pork insulin resulting in a local reaction at the injection site, urticaria, and pharyngeal oedema. One serum sample was obtained at the time of the reaction and another was obtained 2 days later. The patient had been treated for diabetes with insulin for approximately 7 years. Initially, she was treated with animal insulin but was switched to human (rDNA) insulin in 1984. She did not recall any adverse reaction to the animal insulin, nor had she had problems with human (rDNA) insulin. The patient received beef–pork insulin instead of her usual 40 units NPH human (rDNA) insulin because she was seen in the hospital out-patient department and did not have her usual insulin with her. Intradermal end-point titration was performed with serial 10-fold dilutions of pure porcine, bovine, and human (rDNA) insulin (Eli Lilly, Indianapolis, U.S.A.). The positive end-point was the most dilute solution producing a 5 mm weal and 10 mm flare.

ELISA

The ELISA for various insulins was performed according to previously published methods [13,14]. Pure, regular insulin at a concentration of 100 µg/ml in carbonate buffer, pH 9.6, was used to coat Immulon microELISA plates (Greiner and Sohne, Nürtingen, FRG). Appropriate washes with PBS-Tween (Sigma Chemical Co., St Louis, U.S.A.) were performed between each of the described steps. The sequence of reagent addition was as follows: patient serum in PBS-Tween; rabbit anti-human IgE, IgG, or IgA (Calbiochem-Behring, La Jolla, U.S.A.) diluted 1:1000 in PBS-Tween; alkaline phosphatase-conjugated goat anti-rabbit γ-globulin (Sigma Chemical Co.) diluted 1:1000 in PBS-Tween; and *p*-nitrophenyl phosphate (Sigma 104 phosphate, 1 mg/ml) in 10% diethanolamine buffer at pH 9.8. The optical density of each well was read at 405 nm on an ARTEK automated ELISA reader, Model 210, (ARTEK Systems, Farmingdale, U.S.A.).

A positive control serum was obtained from a non-atopic patient known to be allergic to insulin and to have

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elevated titres of IgE, IgG, and IgA against each insulin. The negative control sera were obtained from an atopic and a non-atopic individual, neither of whom had ever received insulin.

Each assay was performed in duplicate on two different days and the results were averaged. The ELISA titre end-point was defined as the concentration of the most dilute serum solution that had an optical density reading at least twice the mean of the negative control sera; this value is the upper limit of 95% confidence above the mean of the negative control sera.

Inhibition of specific IgE binding

In order to demonstrate inhibition of IgE binding by various insulins, assays similar to the ELISA described above were performed. A 1:10 dilution of the patient's serum was pre-incubated with various concentrations of a single insulin (pork, beef, or human (rDNA)) or ragweed antigen E (AgE, National Institutes of Health, Bethesda, U.S.A.).

Results

Immunological studies

The intradermal end-point titres of the patient to bovine, porcine and human (rDNA) insulin were 10^{-2} , 10^0 and $> 10^1$ unit/ml, respectively. The patient had demonstrable IgG against each insulin tested (Fig. 1). The IgG end-point titres to bovine, porcine and human (rDNA) insulin were 1:5000, 1:1000 and 1:500, respectively.

The patient only had demonstrable IgE to bovine insulin (Fig. 2). The IgE end-point titres to bovine, porcine and human (rDNA) insulin were $> 1:80$, $< 1:10$ and $< 1:10$, respectively.

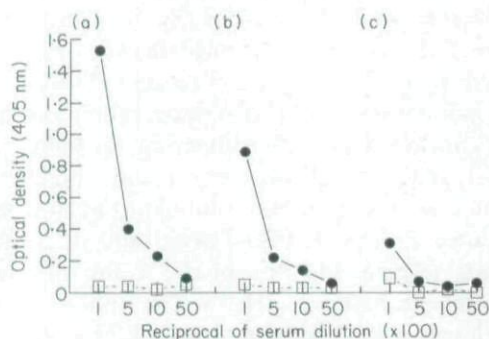


Fig. 1. IgG against (a) bovine, (b) porcine, and (c) human (rDNA) insulin in a patient (●—●) with systemic allergy to animal insulin but not to human (rDNA) insulin. Mean values of two negative control sera are shown (□—□).

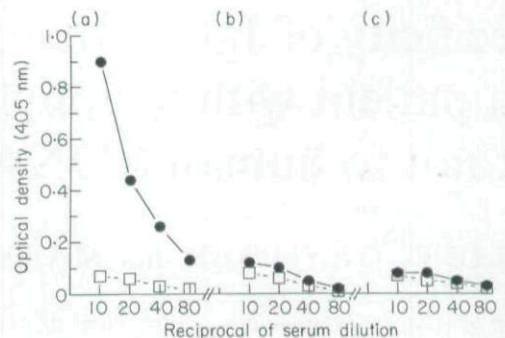


Fig. 2. IgE against (a) bovine, (b) porcine, and (c) human (rDNA) insulin in the same patient (●—●). Mean values of two negative control sera are shown (□—□).

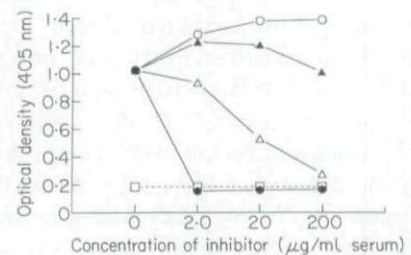


Fig. 3. IgE against bovine insulin in the same patient with inhibition by bovine (●—●) porcine (Δ—Δ) and human (rDNA) insulin (▲—▲), and by AgE (○—○). The mean values of negative control sera are also shown (□—□). The values are for serum which has been diluted 1:10.

The only insulin against which the patient had IgA was bovine (results not shown). The IgA end-point titres to bovine, porcine and human (rDNA) insulin were the same as for IgE.

Inhibition of specific IgE binding

Results are shown in Fig. 3 for inhibition of the patient's IgE binding to bovine insulin. Pre-incubation with bovine insulin caused total inhibition at a concentration of 2 $\mu\text{g/ml}$ while porcine insulin caused total inhibition at 200 $\mu\text{g/ml}$. Neither human (rDNA) insulin nor AgE, an antigen unrelated to insulin, appeared to cause any inhibition.

Discussion

The patient on whose sera we performed immunological studies tolerated human (rDNA) insulin but had a systemic allergic reaction to beef-pork insulin to which she had previously been sensitized. Each of the other patients we have studied who have had systemic reactions

to animal insulin had some measurable IgE to each insulin tested. Moreover, in two patients there was significant cross-inhibition by each insulin [11].

In contrast, in this patient there was no demonstrable IgE to human (rDNA) or porcine insulin by ELISA and there was no demonstrable immediate-type cutaneous reactivity to human (rDNA) insulin. Moreover, cross-inhibition studies of IgE binding to bovine insulin demonstrated that bovine insulin resulted in complete inhibition, porcine insulin in complete inhibition at a 100-fold higher concentration, and human (rDNA) insulin in essentially no inhibition. This is consistent with the anti-insulin IgE recognizing a determinant that is present on bovine insulin and not on human (rDNA) insulin. A possible explanation would be that this patient's IgE antibody is directed against a determinant resulting solely from the altered primary amino acid sequence of bovine insulin as compared to human (rDNA) insulin.

Neither IgG nor IgA are pathogenic in this patient's clinical presentation of systemic anaphylaxis. However, it is interesting to note that this patient's IgA only bound bovine insulin while the IgG bound bovine, porcine and human (rDNA) insulin; in short, different isotypes have different specificities.

There are two clinically significant aspects of this patient's immunological findings. First, a patient may be highly sensitive to one insulin and have absolutely no reactivity to another. It is therefore useful to titrate insulin allergic patients to determine whether there is an insulin to which the patient is not reactive. Secondly, it cannot be assumed that a patient who is tolerating one species of insulin will tolerate another, i.e. insulin species changes should only be made with due consideration of possible immunological consequences.

Acknowledgments

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