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Case Report

Circulating immune complexes containing bovine insulin in a patient with systemic allergic manifestations

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Summary

A woman treated for 15 days with bovine insulin for gestational diabetes presented with severe urticaria of the chest and back, distant from the injection site. She had neither local reaction nor general manifestations. Replacement of bovine NPH insulin by biosynthetic human NPH was followed by regression of urticaria. We isolated the circulating immune complex (CIC), mainly of IgG class, from the patient's serum. It disappeared when bovine insulin administration had been ceased for 48 h. There were no specific IgE-insulin-antibodies. The IgG-CIC were dissociated. Insulin was identified by RIA in the CIC. Insulin characterization was carried out by high-performance liquid chromatography (HPLC), which showed that the insulin in the complexes was injected bovine insulin.

Introduction

Subcutaneous injection of insulin can occasionally give rise to local or systemic hypersensitivity phenomena in man (for review see [1]). Such reactions have been seen after injection of various types of bovine, porcine or human insulin. Histological data suggestive of the Arthus phenomenon have also been seen in some insulin-treated patients [2]. Furthermore, circulating insulin immune complexes (CIC) with IgA, IgG or IgE antibodies have been

found in insulin-treated diabetics in the absence of allergic manifestations [3,4]. We report a case of hypersensitivity to bovine insulin. We were able to isolate the immunogenic compound related to the allergic phenomena from CIC, as well as to characterize the antigen (bovine insulin) and the antibody (principally IgG). To the best of our knowledge, this is the first unequivocal demonstration of this phenomenon.

Case study

Mrs. B., aged 43 years, developed gestational diabetes during the 21 weeks of her 6th pregnancy.

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Treatment started with NPH bovine insulin in the morning and evening; she had not previously been treated with insulin. Two weeks after the first injections, she presented with an episode of pruritus beginning 20 min after injection, followed half an hour later by urticaria lasting 45 min. These eruptions developed principally over the abdomen and the thorax; the site of injection of insulin was always the arm or the upper part of the thigh. After 4 weeks, urticaria had become permanent with recrudescence following each insulin injection. (The patient had no fever or joint pains.) Urticaria was resistant to antihistamines and promethazine. Blood glucose control was achieved throughout the 24-h cycle, glycosylated haemoglobin was 1.68 nmol 5-hydroxy-methyl furfural/mg haemoglobin ($N = 1.62 \pm 0.11$). Replacement of biosynthetic human insulin (BHI) with bovine NPH insulin 8 weeks after the start of therapy brought about a regression of urticaria and pruritus within 48 h. The patient's child was delivered at term, weighing 3.8 kg, and presenting no disturbances in glucose tolerance, or any malformations.

The patient had no personal or family history of allergic autoimmune disorders, but her father was obese with noninsulin-dependent diabetes; her HLA phenotype was A3-9, B7-15, DR2-X. No islet cells or other organ and non-organ specific autoantibodies were detected in her serum.

Since this patient no longer required insulin after delivery of the child, reintroduction as a challenge was precluded for ethical reasons. Four months after the insulin treatment was completed, we carried out an intradermal test with bovine, porcine and biosynthetic human NPH insulin, as well as with various insulin solution components: phenol, glycerol, zinc and NaCl. At this time all tests were negative.

Materials and methods

Serum samples were obtained when the patient was treated with bovine insulin (and displayed an urticaria rash), then when she had been treated with human insulin for 2 days and after delivery when she did not receive insulin.

Specific porcine and bovine anti-insulin IgE were assayed using the Phadebas-Rast, a radioimmunoassay kit manufactured by Pharmacia Diagnostics. Sensitivity was below 0.35 PRU/ml.

Preliminary screening for circulating immune complexes (CIC) was carried out by precipitation with 2% polyethyleneglycol 6000 (PEG) (Merck), dissolved in 0.1 M borate buffer, pH 8.3, and mixed with an aliquot of the serum. The precipitated IgG, IgA and IgM in the CIC were assayed by laser nephelometry (Behring).

A CIC-enriched fraction was prepared by adding 4% PEG to the remaining serum. Precipitation was encouraged by chilling the solution on ice for 2 h, and the precipitate thus formed was spun down and redissolved in borate buffer at roughly 4 times the original concentration. This solution was then applied to a protein A Sepharose 6B column (Pharmacia), and the unbound fraction was thoroughly washed with phosphate-buffered saline (PBS). IgG was eluted with glycine HCl, pH 2.8, onto 0.1 g of solid Tris, and dialyzed overnight against PBS. Glycine-HCl was then added to dissociate the still complete immune complexes from antibody. The mixture was passed through an x AC 54 column (LKB) and the second peak was recovered onto solid Tris to neutralize the low pH, dialysed for 2 days at 4°C with 3 changes of 1000 ml PBS, concentrated to approximately 2 ml, and dialyzed again in the same way. Insulin was tested in the resulting solution.

Insulin was assayed with a RIA-gnost-insulin kit (Behring) in the second column chromatography peak.

Characterization of the type of insulin in the CIC was carried out as follows: 8 ml effluent was homogenized, acidified to pH 2.5 and supplemented with 0.1 M sodium acetate and 0.1 M sodium chloride, adjusted to pH 5.4 and centrifuged. The pellet was dissolved in 0.01 M HCl and 25 μ l was introduced into a high-pressure liquid chromatograph (HPLC) with the following operating conditions: H.P. Waters model 660/481 chromatograph, Novapac C18 column heated to 45°C, solvent buffer NaH_2PO_4 , 2 H_2O 0.5 M + TMAHO 0.25 M with solvent A: 10% in acetonitrile; solvent B: 40% in acetonitrile — gradient 7 — solvent B: 36–60% in 35 min, flow rate 0.6 ml/min, detector 220 μ m.

TABLE 1
LONGITUDINAL STUDY OF IMMUNO-ALLERGIC PARAMETERS

	Treated with bovine insulin	Treated with BH-NPH insulin for 48 h	Untreated	Normal values
Total white cells	11.7×10^3	9.8×10^2	6.2×10^3	4×10^3 – 10^4
% of eosinophils	4	1	1	< 10
Total globulins (g/l)	11	10	8	7–15
Total IgE (U/ml)	37	46	28	< 120
Anti-insulin IgE (PRU/ml)	0.20	0.18	< 0.35	< 0.35
IgG-CIC (mg/ml)	1.5	0.7	0.4	< 0.5
IgA-CIC (mg/ml)	0.4	0.4	0.4	< 0.2
IgM-CIC (mg/ml)	0.6	0.4	0.3	< 0.3

CIC = circulating immune complexes.

Results

Results for allergy are listed in Table 1. We noted that total IgE and anti-insulin IgE levels were normal throughout this study, while CIC levels were fairly high (1.5 mg/ml); these were predominantly IgG (1.5 mg/ml).

Insulin was demonstrated by RIA in the second column chromatography peak (see Materials and methods).

HPLC (Fig. 1) showed the presence of bovine insulin with a retention time concordant with that of a bovine insulin standard (24.50 min). Analyzing a mixture of effluent + standard, the peaks were superimposable. Chromatograms are shown in Fig. 1.

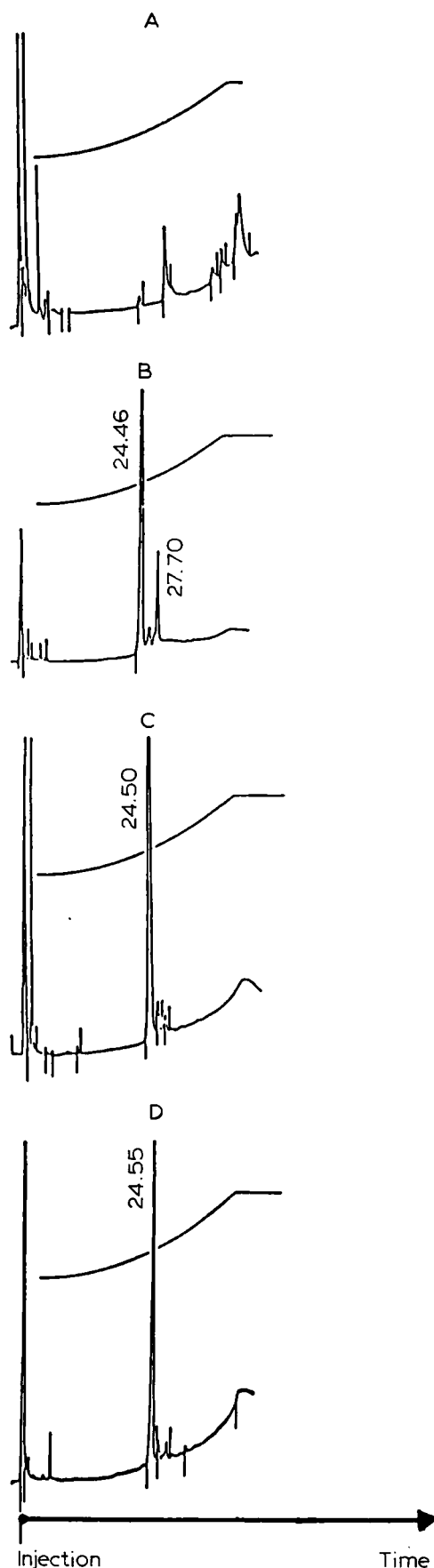
Discussion

Our patient presented cutaneous allergic manifestations which were completely resolved upon 48 h after replacement of bovine NPH insulin with BHI. These two insulin preparations contain exactly the same solvents (metacresol, protamine sulfate, sodium phosphate, phenol, glycerol, hydrochloric acid and water) in equivalent proportions. Since cutaneous manifestations occurred distal to the injection site, this constituted general hypersensitivity. It was not accompanied by any rise in total IgE

or raised eosinophils, and we did not find any specific anti-insulin IgE [5–7] by RIA.

Immunological reactions of our patient differed from patients treated with bovine insulin which developed CIC or insulin-antibodies. These patients developed CIC within 2–4 months of starting the treatment with bovine insulin [8] and our patient within 10 days [15]. When subjects switched from bovine to BHI, IgG-insulin-antibodies decreased in sera after 2–4 months and had not disappeared within 2 days, as in our patient.

The clinical history, in particular the 10-day lag between the first insulin injection and the beginning of the urticaria, along with the large quantity of CIC (principally type IgG) suggest a phenomenon similar to serum sickness. However, we did not evaluate the eventual consumption of complement. We did not obtain biopsies of the hives lesion, and were thus unable to know if immune complexes would be shown in the lesion. Surprisingly, in these CIC, the antigen here was exogenous bovine insulin, demonstrated by HPLC which failed to show any endogenous insulin in the CIC. Clinical manifestations and CIC resolved upon replacement of bovine insulin with BHI insulin (48 h later), and have not recurred since this patient no longer requires insulin treatment. It should be stressed that all CIC investigations have been negative since then (2 years of follow-up). The immune reaction thus appears to be a function of differences in the pri-



mary structure of biosynthetic human insulin and bovine insulin. Regardless of the origin of insulin, between 0.1 and 0.2% of treated patients show hypersensitivity to the hormone [1] and patients of HLA B7-DR3 appear to present the greatest risk [9]. As far as we know, there have been no previous reports of CIC-containing injected insulin causing urticaria. While such complexes have been described in insulin-treated patients in previous similar reports, CIC were not associated with general clinical allergy. It is known that patients rapidly develop antibodies directed against exogenous insulin shortly after the beginning of insulin therapy. Antigenicity of insulin could be a function of its origin; despite the existence of antibodies against BHI [10–13], it appears to be the least immunogenic of the insulin preparations [14,15]. The antibodies can be of IgA, IgG or IgM types; Subjects with HLA DR3 [16] or B15 [17] (like our patient) would present a greater risk of developing such antibodies. Spontaneous autoantibodies against endogenous insulin have also been detected in newly diagnosed untreated diabetic patients [18]. No particular HLA association has been described with their presence so far.

Our patient presented insulin intolerance with urticaria and CIC-containing bovine insulin. These CIC may be related to the origin of the cutaneous phenomena. It is thus probable that the CIC found in this patient differ in terms of toxicity from those found in insulin-treated patients. It will be important to study the mechanisms of immune reactivity to exogenous insulin; in view of the present report, several mechanisms are undoubtedly involved. Finally, the patient's HLA status and absence of islet cell antibodies make development of classical type I insulin-dependent diabetes unlikely.

Fig. 1. High-performance liquid chromatography patterns. (A) Human IgG solution (25 μ l). (B) Bovine insulin (10 μ l); first peak (24.46) = bovine insulin; second peak (26.13) = mono-desamido-insulin; third peak (27.70) = di-desamido-insulin. (C) Patient's immune complexes dissociated at pH 2.5 (25 μ l). (D) Patients immune complexes dissociated at pH 2.5 (25 μ l) in solution with bovine insulin (1.1 μ g).

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