

DRC 00291

IgE antibodies to insulin and related peptides, a result of insulin treatment?

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(Received 17 October 1988)

(Revision received 17 January 1989)

(Accepted 17 January 1989)

Summary

Anti-insulin immunoglobulin E (IgE) antibodies in sera of insulin-treated diabetic patients were characterized by affinity and concentration, and by binding to proinsulin and insulin A and B chains. The affinities of IgE to insulin scattered between $<10^7$ and 0.6×10^9 l/mol, the concentrations between 0.3 and 3.5 ng/ml. All positive sera of type 1 and few of type 2 patients recognized proinsulin, A chain, and B chain with comparable affinities and concentrations. Elevated concentrations of total serum IgE in 12 of the 16 anti-insulin IgE-positive sera from insulin-treated patients indicate that these patients are predisposed to allergies. The incidence of elevated levels of total serum IgE in type 1 (17.7%) and in type 2 (7.8%) diabetic patients did not differ from the general population.

Reagents to insulin and related antigens were also observed in sera of non-diabetic allergic persons without previous contact with exogenous insulin. The natural occurrence of insulin-specific reagents makes the use of IgE as a marker of antigenicity of insulin questionable. Discrepancies between the insulin-positive radioallergosorbent test (RAST) or skin test and clinical manifestations of insulin allergy exist, because IgE antibodies with low affinities require high concentrations of insulin for binding. Such amounts of insulin may occur at the injection site, but not in the circulation.

Key words: Anti-insulin IgE; Affinity; A chain; B chain; Proinsulin; Natural antibodies

Introduction

Clinical manifestations of insulin allergy are extremely rare though immunoglobulin E (IgE)

antibodies to insulin can be found in 5–10% of the sera from patients treated with insulin [1]. The initiation of an allergic reaction by bridging of specific IgE on mast cells [2,3] may not occur due to low affinity, low concentration, or low diversity of the reagents. These parameters have not yet been measured because the radioallergosorbent test (RAST) used for the detection of specific IgE

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produces score values [4] and cannot determine affinities and concentrations of IgE.

This work deals with the properties of anti-insulin IgE in sera from insulin-treated diabetic patients. We also investigated whether such antibodies naturally occur in non-diabetic allergic persons and in healthy subjects.

Materials and methods

Sera of diabetic patients were obtained from a previous German Multicenter Study on Semi-synthetic Human Insulin. A total of 297 diabetic patients (initial treatment: 79 type 1, 90 type 2; switched from animal to human insulin: 90 type 1, 38 type 2) were examined at 13 visits during 2 years [5,6]. Sera from 19 individuals were anti-insulin IgE-antibody positive ($> 3 \times$ background). As controls 30 sera from young healthy athletes, and 30 randomly collected sera from non-diabetic outpatients of our allergy clinic were analyzed. Informed consent was obtained.

Semisynthetic human insulin was supplied by Hoechst AG (Frankfurt, F.R.G.). Biosynthetic human proinsulin was a generous gift of Eli Lilly and Company (Indianapolis, IN, U.S.A.). Insulin A chain (SSO_3^-)₄ or (SS)₂ and B chain (SSO_3^-)₂ or (SS) were prepared by the Deutsches Wollforschungsinstitut (Aachen, F.R.G.). Insulin, proinsulin, and both forms of A and B chains and reduced chains (0.01 M mercaptoethanol, 20 min, 20°C) were coupled as antigens.

Cellulose disks were made from Whatman No. 54 filter paper (Whatman Ltd., Maidstone, U.K.), suspended in 50 ml ice water, and activated with 1 g CNBr in 5 ml dimethylformamide (20 min, pH 10–11). Five hundred disks were coated with 1 mg of each antigen in 10 ml of 0.1 M NaHCO_3 overnight. Excess active groups were blocked with 0.3 M ethanolamine, followed by 2% bovine albumin. Control disks without antigen were also prepared. An insulin radioimmunoassay (RIA; Pharmacia, Sweden) was used to measure residual insulin and cross-reacting proinsulin as well as A and B chains in the supernatants. The coupling efficiency was 83–87% for the different antigens.

Only a part of the antigen on the cellulose disks is available to the antibodies. This amount of active antigen was measured (Fig. 1). The competition for antibody binding of two antigen-coated disks to one another (fixed versus fixed antigen) was compared to the competition of one disk to increasing concentrations of soluble antigen (fixed versus soluble antigen). The soluble antigen is equivalent to the antigen on one disk if the amounts of antibody bound to the single disk at this antigen concentration and to each of the competing disks are identical (as assumed in Fig. 1). The antigenically active proportion of the material on the disks varied between 34 and 43%.

Antigen on a solid phase is concentrated in a small physical space. The distribution of such antigen in a solute is not homogeneous, so that the effective volume for antigen–antibody interaction needs consideration. The active antigen on the disks was equivalent to the same amount of soluble antigen at assay volumes between 50 and 300 μl . The concentrations of equivalent soluble antigen were thus used for calculations.

Total IgE was measured with Quanti Clone Kit (Kallestad, Austin, TX, U.S.A.). Antigen-specific IgE was determined in a similar procedure as described for active antigen: single antigen-coated disks were incubated overnight each with 50 μl of one serum. After washing with 3×2 ml of phosphate buffer 0.1 M, pH 8.0, two of these disks were incubated in one probe with ^{125}I -

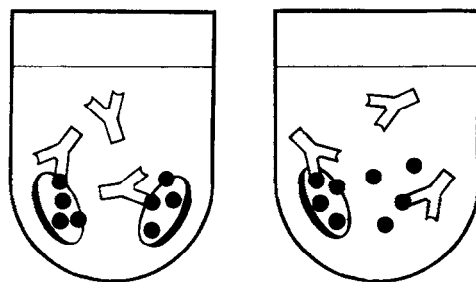


Fig. 1. Determination of active antigen (●) on a cellulose disk. Competition for antibody (Y-shaped symbols) binding between two antigen-coated disks (left) or one disk and soluble antigen (right).

labelled horse anti-IgE antibody (Kallestad, Austin, TX, U.S.A.), and adequately diluted to show good competition between the disks (fixed versus fixed IgE). The remaining disks were incubated with a dilution series of IgE standard (20–0.1 ng/ml) competing for the same amount of labelled anti-IgE antibody (fixed versus soluble IgE). After 4 h the disks were washed and counted. The amount of soluble standard IgE is equivalent to the amount of specific IgE bound to the disk ($\text{IgE}_{\text{bound}}$) when the counts of anti-IgE bound to a single disk and to each of the competing disks are identical. This procedure was applied to all sera.

Supernatant sera from the probes used to measure $\text{IgE}_{\text{bound}}$ were reincubated with new disks to obtain measurements of the residual specific IgE. This was repeated until background values were found. The amounts were added to form IgE_{free} . Losses of supernatant serum were compensated by starting the incubation series with surplus tubes.

The affinity constants were then calculated from the concentrations of active antigen and of antigen-specific IgE according to the law of mass action

$$K = \frac{[\text{IgE}_{\text{bound}}]}{[\text{IgE}_{\text{bound}} + \text{IgE}_{\text{free}}] \times [\text{Antigen}_{\text{active}}]}$$

TABLE 1

Affinities and concentrations of IgE antibodies binding to insulin, proinsulin and insulin A or B chain, and total serum IgE in sera of type 1 diabetic patients; initial treatment with human insulin

Patient code/ Serum from month of study	Total IgE in serum (ng/ml)	Specific IgE							
		Anti-insulin		Anti-proinsulin		Anti-A chain		Anti-B chain	
		affinity (l/mol)	conc. (ng/ml)	affinity (l/mol)	conc. (ng/ml)	affinity (l/mol)	conc. (ng/ml)	affinity (l/mol)	conc. (ng/ml)
3716/2	3737	0.8×10^8	2.6	0.8×10^8	1.3	0.8×10^8	3.0	0.7×10^8	3.7
7105/0	3379	0.7×10^8	1.2	$< 10^7$	+	0.1×10^9	0.5	$< 10^7$	+
2304/3	1606	0.6×10^8	1.4	$< 10^7$	+	$< 10^7$	+	0.4×10^8	2.5
3802/18	2400	0.4×10^8	2.7	0.1×10^9	0.7	0.1×10^9	0.3	$< 10^7$	+
3217/0.5	1478	0.2×10^8	1.2	0.2×10^8	1.2	0.2×10^8	0.4	0.2×10^8	0.8
8204/21	2585	$< 10^7$	+	$< 10^7$	+	$< 10^7$	+	$< 10^7$	+
8707/0	1054	$< 10^7$	+	0.7×10^8	0.8	$< 10^7$	+	$< 10^7$	+

+ indicates positive sera with low-affinity antibodies ($< 10^7$ l/mol), see also Methods.

The precision of the values decreases with the gradient between $\text{IgE}_{\text{bound}}$ and IgE_{free} producing a limit of 10^7 l/mol. Below this affinity no precise values for the concentrations are obtained because only a minor proportion of total specific IgE is bound to the disk (indicated by + in the tables).

Results

Reaginic antibodies to insulin were studied in insulin-treated diabetic patients, in allergic persons and in healthy subjects. The sera were also analyzed with regard to the binding of insulin-related antigens (proinsulin and three different chemical forms (SSO_3^- , SS, SH) of insulin A and B chains). The affinities and the concentrations of the specific reagents and total serum IgE were measured. The different chemical forms of each chain showed similar results, so that only the data obtained with the SSO_3^- form are given here.

At diagnosis 17.7% of type 1 and 7.8% of type 2 diabetic patients participating in the 2-year study had elevated total serum IgE concentrations.

Seven of 79 patients with type 1 diabetes and initial treatment with semisynthetic human insulin

TABLE 2

Affinities and concentrations of IgE antibodies binding to insulin, proinsulin and insulin A or B chain, and total serum IgE in sera of type 2 diabetic patients; initial treatment with human insulin

Patient code/ Serum from month of study	Total IgE in serum (ng/ml)	Specific IgE							
		Anti-insulin		Anti-proinsulin		Anti-A chain		Anti-B chain	
		affinity (l/mol)	conc. (ng/ml)	affinity (l/mol)	conc. (ng/ml)	affinity (l/mol)	conc. (ng/ml)	affinity (l/mol)	conc. (ng/ml)
2203/1	46	0.6×10^9	2.0	0.4×10^9	1.6	—	—	0.4×10^8	1.1
2316/1	166	0.3×10^9	1.0	0.3×10^9	0.9	—	—	—	—
1204/4	768	0.2×10^9	3.5	0.1×10^9	3.1	—	—	0.4×10^8	1.1
2311/15	941	0.2×10^9	0.5	0.1×10^9	0.5	—	—	0.1×10^9	0.5
1410/1	161	0.2×10^9	0.3	0.2×10^9	0.3	—	—	—	—
6209/2	367	0.1×10^9	1.1	$<10^7$	+	—	—	—	—
2505/6	2482	0.8×10^8	0.6	$<10^7$	+	0.5×10^8	0.7	0.4×10^8	2.8
3712/6	3578	$<10^7$	+	$<10^7$	+	0.2×10^9	0.6	0.4×10^8	6.0
3311/3	120	$<10^7$	+	$<10^7$	+	—	—	—	—

+ indicates positive sera with low-affinity antibodies ($<10^7$ l/mol), see also Methods.

showed IgE antibodies to insulin (Table 1). Two of them (7105 and 8707) were positive before receiving insulin therapy. All seven individuals had elevated total serum IgE concentrations (>300 ng/ml). Very low affinities to insulin of less than 10^7 l/mol were measured in two sera (8204 and 8707). In addition to insulin, all positive sera

recognized proinsulin, A chain and B chain with comparable affinities and concentrations.

Nine of 90 type 2 diabetic patients with initial human insulin treatment showed anti-insulin IgE antibodies (Table 2). Five of these individuals had elevated (>300 ng/ml) and four had normal total serum IgE concentrations. Six sera produced

TABLE 3

Affinities and concentrations of IgE antibodies binding to insulin, proinsulin and insulin A or B chain, and total serum IgE in sera of allergic persons without previous contact with exogenous insulin

Patient's initials	Total IgE in serum (ng/ml)	Specific IgE							
		Anti-insulin		Anti-proinsulin		Anti-A chain		Anti-B chain	
		affinity (l/mol)	conc. (ng/ml)	affinity (l/mol)	conc. (ng/ml)	affinity (l/mol)	conc. (ng/ml)	affinity (l/mol)	conc. (ng/ml)
O. B.	1836	0.2×10^9	0.8	0.1×10^9	0.6	$<10^7$	+	$<10^7$	+
M. F.	3267	$<10^7$	+	0.1×10^9	0.7	$<10^7$	+	$<10^7$	+
S. K., B. W.	3885, 2412	$<10^7$	+	$<10^7$	+	$<10^7$	+	$<10^7$	+
E. S.	879	—	—	0.2×10^9	1.1	—	—	—	—
W. E.	1672	—	—	—	—	0.8×10^8	1.8	0.1×10^9	1.5
L. G., F. K.	715, 956	—	—	—	—	$<10^7$	+	$<10^7$	+
J. D.	1145	—	—	—	—	$<10^7$	+	$<10^7$	+
H. F., A. S.	1063, 892	—	—	—	—	—	—	$<10^7$	+

+ indicates positive sera with low-affinity antibodies ($<10^7$ l/mol), see also Methods.

higher affinities to insulin than observed in type 1 patients. Affinities of less than 10^7 l/mol were measured in two sera (3712 and 3311). Five sera also recognized proinsulin, one was specific for insulin (6209). These six sera did not recognize insulin A chain, three recognized insulin B chain.

All 90 patients with type 1 diabetes switched from porcine or bovine to human insulin were negative. Three of 38 patients with type 2 diabetes switched to human insulin had IgE antibodies with low affinities to insulin. Sera from 30 athletes presenting for a routine check-up were negative except for that of one individual, which recognized proinsulin with low affinity.

The frequent observation of low-affinity IgE antibodies to insulin, proinsulin, and insulin A or B chain raised doubts regarding the specificity of these antibodies. The elevated concentrations of total serum IgE in many anti-insulin IgE-positive patients suggested that these patients were allergy-prone. We therefore tested 30 sera with total IgE concentrations of > 300 ng/ml from non-diabetic allergic persons (Table 3). Four sera recognized insulin and proinsulin with affinities comparable to those of insulin-treated type 1 diabetic patients. One serum selectively bound proinsulin with high affinity. A and/or B chains of insulin were bound in 10 cases.

Discussion

Allergic persons constitute about 15% of the general population [7]. They are characterized by an increased frequency of elevated total serum IgE levels [8]. The incidence decreases with age. The prevalence of elevated total serum IgE in this study was comparable to that in the general population considering the age distribution of each diabetes type. In contrast to others [1], most of our insulin-treated diabetic patients with IgE antibodies to insulin also had elevated total serum IgE concentrations, indicating that they were predisposed to allergies.

An immunizing process stimulates and selects those B cells which produce specific antibodies.

Repeated stimulation favors B cells synthesizing high-affinity antibodies [9,10]. The low-affinity anti-insulin IgE antibodies in insulin-treated patients are not the result of persistent selective pressure on IgE by immunization through insulin therapy. This and the following considerations raised the idea of the natural occurrence of reagins to insulin.

Proinsulin may be described as an A_1-B_{30} modification of insulin, where this part of the surface is covered by the C-peptide. Insulin-induced IgE antibodies should recognize less proinsulin than insulin, because the antibody-binding epitopes in the A_1-B_{30} region are covered. Anti-insulin IgE antibodies of many sera from type 1 and type 2 diabetics did not fulfill this criterion. It is thus questionable whether these reagins are induced by insulin therapy and cross-react with proinsulin.

Several sera from non-diabetic allergic patients and from one athlete also contained IgE antibodies binding insulin, proinsulin, A chain or B chain with affinities and concentrations similar to those of diabetic patients. This shows that insulin-specific reagins can occur naturally and are not necessarily induced by insulin treatment or an immune process underlying diabetes. The view is supported by the observation of anti-insulin IgE in patients prior to treatment here and elsewhere [1].

About 10% of both type 1 and type 2 diabetic patients with initial insulin therapy had anti-insulin IgE antibodies. The assumed mode of islet β cell loss related to an immune process in type 1 but not in type 2 diabetes is not reflected in the production of insulin-specific IgE antibodies. Previous long-term treatment with non-homologous insulin reduced the incidence of anti-insulin IgE, suggesting a desensitizing effect.

The minimum concentration of IgE antibody to produce a positive Prausnitz-Küstner reaction in humans was found to be as low as 0.25 ng of antibody protein per ml [11]. The concentrations of insulin-specific reagins in this study varied between 0.3 and 3.5 ng/ml, in both type 1 and type 2 diabetic patients. The scattering of the affinities

of IgE antibodies to insulin ($<10^7$ to 0.6×10^9 l/mol) may influence the clinical manifestation of allergy. According to the law of mass action the affinity is inversely proportional to the antigen concentration needed for complex formation. IgE antibodies with low affinities require high concentrations of insulin which may be present at the injection site but not in the circulation. This may explain the rarity of general allergic reactions, while minor irritation at the injection site may occur. Chain material as contaminant of non-purified insulin preparations or resulting from degradation at the injection site may also trigger an allergic reaction.

The concentrations of endogenous insulin in non-diabetic atopic patients with natural anti-insulin IgE are not adequate to produce allergic reactions. Inhalation of insulin might, however, produce high local concentrations with potential hazards in untreated but predisposed persons.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft. We wish to thank Dr. Schäfer and Dr. Hösl, Hoechst AG, Frankfurt, F.R.G., for the generous permission to re-examine sera of the German Multicenter Study on Semisynthetic Human Insulin. We thank Andreas Rynk for excellent technical assistance and Monika Pusch for typing the manuscript.

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