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Detection of IgE Insulin Antibody with Radioallergosorbent Test

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Summary. An in vitro method for detecting IgE insulin antibody using the principle of the radioallergosorbent test (RAST) is described. In six patients with insulin allergy, the RAST values were higher than in normal persons or insulin-treated diabetics without insulin-allergy. No differences were observed between normal persons and insulin-treated diabetics without insulin-allergy. Moreover, it was observed that in one patient treated with highly-purified insulin, there was a gradual decrease of RAST value parallel to the radioinsulin binding activity and clinical allergic symptoms. The RAST value of insulin is slightly inhibited by non-IgE antibodies and is, therefore, a semiquantitative value. However, the RAST is simple to perform and reproducible; It therefore is very useful in the detection of IgE insulin antibodies.

Key words: IgE insulin antibody, insulin allergy, radioallergosorbent test, highly purified insulin, IgE, monocomponent insulin.

hepatitis or other dangerous infections in the recipient.

In the in vitro test, Patterson et al [2] used radioimmunodiffusion (RID) and polystyrene tube immunoabsorbent assay (PTIA) to detect IgE insulin antibodies, but this RID method is time-consuming and insensitive to IgE antibodies and the preparations of high concentration of IgE and anti-IgE globulin solutions required for the PTIA is not feasible in many situations. It is therefore undesirable for routine use.

In 1967, Wide et al. [3] reported a method for detecting IgE antibody against pollen antigens with cellulose-coupled antigen and radioactive anti-IgE globulin (Radioallergosorbent Test, RAST).

This report describes a method for detecting IgE insulin antibody in serum by using the principle of the RAST and the application of this method for the diagnosis of insulin allergy.

Patients and Methods

Patients

The patients allergic to insulin used in this study were six diabetics who showed generalized or severe local dermal reactions within an hour after injection of insulin preparation. The type of the reactions and specificity of antigen were confirmed by skin tests with purified insulins from several sources.

As control, five diabetic patients treated with insulin without the reactions and five normal volunteers were used.

The clinical details of the patients and the volunteers are described in Table 1.

Test solutions for the skin test were prepared by dissolving the same purified insulins as those used

The "immediate type" of insulin allergy presents an obstacle to insulin therapy. The antibody causing this type of allergy was confirmed to be IgE class by Patterson et al. [1] in 1969. In the patient's serum there is only a low concentration of IgE antibodies in the presence of a high concentration of IgG antibodies; for this reason detection of the insulin specific IgE antibodies by direct binding of radioinsulin to the patient's serum is impracticable.

As a biological method for detecting reagenic antibodies, the passive cutaneous reaction on another person's skin (Prausnitz-Küstner Test) has been used, but this procedure can cause serum

Table 1. Details of patients and control subjects

<i>Allergic patients</i>						
	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age (years)	35	44	58	52	51	52
Sex	M	M	M	F	F	M
Duration of D. M. (years)	2	10	13	4	6	4
Type of D. M.	adult	adult	adult	adult	adult	adult
% of ideal body weight	101	113	98	113	111	90
Other drug used	no	no	no	no	no	no
Previous insulin therapy ^a	Lente (Novo) 6 m (2 y)	Lente (Novo) 50 d (9 y) 1 m (5 y) 2 m (2 y)	Lente (Novo) 20 d (10 y)	no	Lente (Novo) 2 m (2.5 y)	Lente (Novo) 5 m (4 y)
Allergy against	Lente (Novo)	Rapitard (Novo)	Rapitard (Novo)	Regular (Shimizu)	Regular (Shimizu)	Lente (Novo)
Insulin dose (U/d)	56	12	28	20	36	56
Insulin treatment before allergy	12 d	3 d	14 d	15 d	3 d	1 m
<i>Insulin-treated non-allergic patients</i>						
	Case 7	Case 8	Case 9	Case 10	Case 11	
Age (years)	66	59	54	58	55	
Sex	M	F	F	F	F	
Duration of D. M. (years)	18	19	7	4	7	
Type of D. M.	adult	adult	adult	adult	adult	
% of ideal body weight	88	110	81	92	100	
Previous insulin therapy	no	no	no	no	no	
Duration of insulin therapy (years)	4	11	1.8	4	7	
Insulin dose (U/d)	Lente 40 (Novo)	Rapitard 20 (Novo)	Lente 20 (Novo)	Lente 20 (Novo)	Lente 20 (Novo)	
<i>Normal subjects</i>						
	Case 12	Case 13	Case 14	Case 15	Case 16	
Age (years)	52	47	44	66	41	
Sex	F	F	M	F	M	
G. T. T.	normal	normal	normal	normal	normal	
Diseases	no	no	no	no	no	

^a Numbers out of parentheses show duration of previous treatment and numbers in parentheses show interval between previous treatment and present treatment

for the preparation of RAST discs in 9 g/l NaCl solution in the concentration of 0.06 U/ml. After filtering through sterilized Milipore filters, 0.05 ml of these insulin solutions were injected intradermally in the antecubital region. The extent of skin inflammation in the skin test was graded from 0 to 3. 0 indicates a non-specific inflammation (induration < 4 mm and/or erythema < 10 mm in diameter), 1 indicates slight inflammation (induration: 5–8 mm and/or erythema: 11–19 mm), 2 indicates moderate inflammation (induration: 9–14 mm and/or erythema: 20–39 mm), and 3 indicates severe inflammation (induration: > 15 mm and/or erythema

> 40 mm). The results of the skin tests are described in Table 2.

RAST

Filter paper discs (6 mm in diameter, Toyo Roshi Co., Tokyo, Japan) activated by a CNBr method [4] were coupled to insulin according to the method described below. The insulins used were purified bovine insulin (10 times recrystallized, Novo Research Institute, Copenhagen, Denmark), purified porcine insulin (10 times recrystallized, Novo), highly purified porcine insulin (Monocomponent insulin,

Table 2. Results of skin tests

<i>Allergic patients</i>						
Type of insulin allergy	Case 1 Immediate dermal local, severe	Case 2 Immediate dermal local, severe	Case 3 Immediate dermal generalized	Case 4 Immediate dermal local severe → generalized	Case 5 Immediate dermal generalized	Case 6 Immediate dermal
Skin test	Bovine	3	2	3	3	3
	Porcine	3	1	3	3	3
	MC-porcine	3	1	3	2	3
	Bonito	1	0	0	0	0
	9 g/l NaCl	0	0	0	0	0
	Bovine			2	1	
Fraunholz-Kustner test	Porcine			2	2	
	MC-porcine			2		
	Bonito				0	
	9 g/l NaCl			0	0	
	Interval from onset of symptoms to:					
	Skin test	42 d	4 d	50 d	6 d	14 d
Treatments in the interval	RAST	48 d	13 d	53 d	6 d	28 d
	Insulin	continued	discontinued next day	continued	continued	discontinued at 4th day
Antihistamine	D. P. H. ^a D. C. P. M. ^b		D. P. H. ^a	D. P. H. ^a		decrease in dose next day discontinued at 39th day
Radioinsulin-binding activity (B/F)	1.2	2.4	1.1	2.5	2.6	7.3
IgE level (U/ml)	103	4000			56	209
<i>Insulin-treated non-allergic patients</i>						
	Case 7	Case 8	Case 9	Case 10	Case 11	
Radioinsulin-binding activity (F/B)	0.6	1.3	1.8	10.3	1.6	
IgE level	173	140	221	54	135	
<i>Normal subjects</i>						
	Case 12	Case 13	Case 14	Case 15	Case 16	
Radioinsulin-binding activity (B/F)	0.00	0.01	0.00	0.00	0.00	
IgE level (U/ml)	130	117	380	163	193	

^a D. P. H.: Diphenhydramine 12 mg/d, p. o.

^b D. C. P. M.: Dextro-chlorpheniramine maleate 30 mg/d, s. c.

Novo) or bonito insulin (Shimizu Pharmaceutical Co., Shimizu, Japan).

One hundred activated paper discs were immersed in 10 ml of insulin solution (1 mg/ml of 0.1 mol/l NaHCO₃, pH 9.7) and kept under continuous rotation to ensure permeation of the filter paper discs by the insulin for 20 hours at 4° C.

The discs were then washed with 0.1 mol/l NaHCO₃ and treated with 1 mol/l ethanolamine for three hours at room temperature. Then the paper discs were washed three times with 0.1 mol/l borate buffer, pH 8.0 and 0.1 mol/l acetate buffer, pH 4.0 and kept in 0.05 mol/l phosphate buffer, pH 7.4

containing 2 g/l bovine albumin (Fraction V, Grade B, Calbiochem, La Jolla, California, USA) and 0.1 g/l sodium azide at 4° C.

One insulin-coupled paper disc was immersed in 0.1 ml of patient's serum in a small plastic tube and the mixture was incubated for 3 hours at room temperature. After washing three times with 9 g/l NaCl solution, the paper disc was immersed in 0.05 ml of ¹²⁵I-anti-IgE globulin solution (4.2 mCi/mg, 0.16 µg/ml, Pharmacia A. B., Uppsala, Sweden) and the mixture was incubated overnight at room temperature.

After washing three times with a portion of

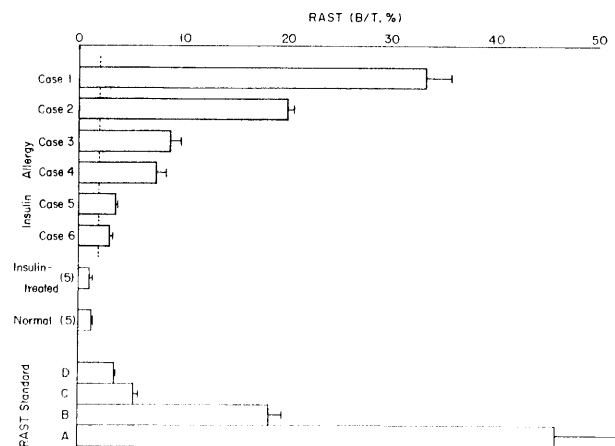


Fig. 1. RAST values of patients with insulin allergy, diabetics treated with insulin without allergy and normal subjects. Mean and standard deviation of triplicate determinations in allergic patients. The numbers in parentheses show the numbers of persons. — results are mean $\pm$ SD. A, B, C and D of RAST show the values of the reference discs reacted with reference sera (A, B, C and D) of Phadebas RAST (Pharmacia). The dotted line means the upper limit of control (Mean + 3 SD of insulin-treated non-allergic diabetics and normal persons)

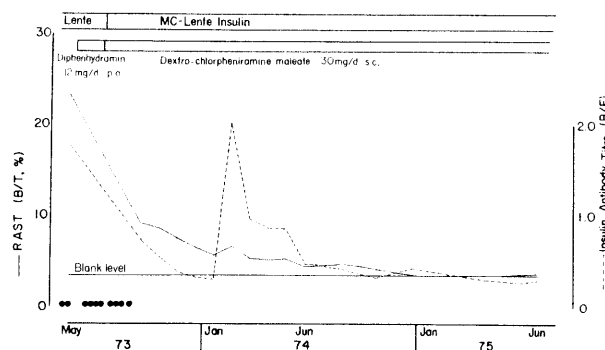


Fig. 2. Time course of serum RAST value, radioinsulin-binding titre and clinical symptoms in an allergic patient (Case 1, 35 year old, male) treated with highly-purified porcine insulin (Monocomponent insulin (Novo)). An abrupt increase in insulin antibody titre was due to an erroneous use of conventional insulin for a week. The black circles mean occurrence of urticaria

2.5 ml of 9 g/l NaCl solution, the radioactivity of the disc was counted with a well type scintillation counter (Nihon-Musen Manufacturing Co., Tokyo, Japan). The RAST value was expressed as the percentage of the count of disc to the count of the added ^{125}I -anti-IgE globulin solution (B/T).

The serum radioinsulin binding activity was measured by the polyethylene glycol method [5] with porcine ^{125}I -insulin (Abbott Laboratories, Oak Ridge, Tenn., USA, 270 mCi/mg, 100 pg/ml of incubation medium) and expressed as the ratio of free to bound insulin in the presence of 0.1 ml of serum in 1 ml of incubation medium (normal range: less than 0.02).

The serum IgE was determined using the

Phadebas IgE Test (Pharmacia A.B., Uppsala, Sweden).

Results

The results of RAST with the disc coupled with purified bovine insulin are illustrated in Figure 1. No significant differences were observed between the RAST values of normal persons and insulin-treated patients without insulin allergy. However, the RAST values of all patients with insulin allergy showed statistically significant higher values compared with the RAST values of normal persons and insulin-treated patients without insulin allergy. No

Table 3. Effect of concentrations of serum and ^{125}I -anti-IgE globulin on RAST values

Dilution of serum	RAST, c.p.m. (B/T, %) ^a		
	Concentration of ^{125}I -anti-IgE globulin ($\mu\text{g/ml}$)		
	0.16	0.08	0.04
2 $\times$	4041 (4.1)	2428 (5.0)	1476 (6.0)
8 $\times$	1893 (1.9)	1088 (2.2)	763 (3.1)
32 $\times$	687 (0.7)	571 (1.2)	307 (1.3)
128 $\times$	287 (0.3)	194 (0.4)	158 (0.6)

^a Numbers out of parentheses show the radioactivity of disc (c.p.m.) and numbers in parentheses show the RAST value (B/T)

correlation was observed between the RAST values and the severity of allergic symptoms or the size of area of inflammation in the skin test.

One patient with insulin allergy when taking a conventional insulin preparation was treated with a highly-purified insulin. He showed a gradual decrease of the RAST value and serum radioinsulin-binding activity (see Fig. 2).

The RAST values with the paper discs coupled with insulins of different species and purity are shown in Figure 3. A statistically significant difference between bonito and bovine insulin was observed in only one case.

The RAST values were similar with different lots of paper discs prepared under the same conditions. But when using out-dated ^{125}I -anti-IgE globulin solution, slightly low RAST and high blank values were observed.

A RAST-positive serum was diluted serially with pooled normal serum. The RAST values of these mixtures using three different concentrations of ^{125}I -anti-IgE globulin solution were determined. The RAST value fell with decrease in the concentration of the patient's serum at all three concentrations of ^{125}I -anti-IgE globulin. The RAST value increased and the radioactivity of the disc decreased with decrease of the ^{125}I -anti-IgE globulin concentrations (see Table 3.).

The inhibitory action of non-IgE antibody was assessed by measuring the RAST values of 1) a dilution of RAST-positive serum with RAST negative serum (both containing high titres of IgG antibodies) and 2) a dilution of RAST-positive serum with a normal pooled serum. The RAST values were slightly lower in the dilution of RAST-positive

Table 4. Inhibition of RAST by non-IgE antibody

Dilution of allergic serum	Dilution of allergic serum ^a with			
	normal serum ^b		serum from non-allergic insulin-treated patients	
	RAST (%)	Antibody (B/F) ^d	RAST (%)	Antibody (B/F) ^d
1 $\times$	14.7 $\pm$ 0.8	2.0	14.7 $\pm$ 0.8	2.0
2 $\times$	11.8 $\pm$ 1.0	1.2	9.5 $\pm$ 0.3	2.9
4 $\times$	7.9 $\pm$ 1.0	0.7	7.6 $\pm$ 0.3	3.2
8 $\times$	4.9 $\pm$ 0.4	0.4	4.8 $\pm$ 0.1	2.8
16 $\times$	4.5 $\pm$ 0.4	0.2	4.1 $\pm$ 0.2	2.3
Diluent	1.4 $\pm$ 0.1	0.0	1.5 $\pm$ 0.1	2.2

^a Pooled serum from allergic patients

^b Pooled serum from normal subjects

^c Pooled serum from non-allergic insulin-treated patients

^d Serum ^{125}I -insulin binding activity

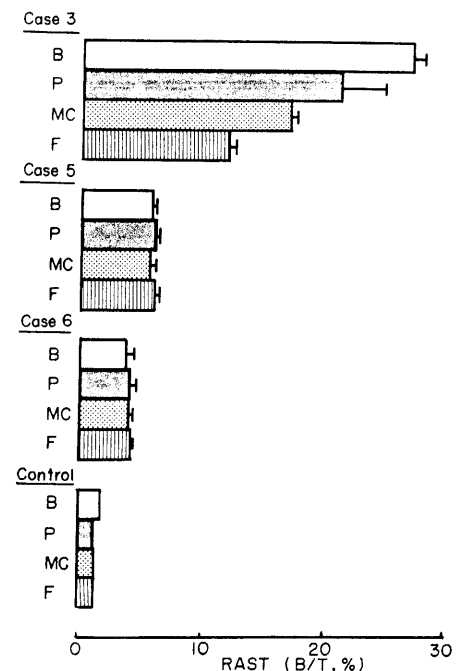


Fig. 3. RAST values with discs coupled with insulins from different sources. B: bovine insulin, P: porcine insulin, MC: highly-purified porcine insulin, F: bonito insulin (Mean and standard deviation of triplicate determinations)

serum with RAST-negative serum containing IgG insulin antibodies as compared to the dilution of the RAST-positive serum with a normal pooled serum (see Table 4).

Discussion

The clinical usefulness of the RAST in the diagnosis of antigens in pollen allergy has been confirmed by many investigators [6, 7].

However, in order to use RAST for detecting IgE insulin antibody in serum of patients with insulin allergy a theoretical problem still existed. In contrast to pollen allergy, many patients with insulin allergy have high titres of non-IgE insulin antibody in their sera and the non-IgE insulin antibodies could competitively inhibit the first reaction of the RAST.

In pollen allergy, Yuninger and Gleich [8] reported an inhibitory action of non-IgE antibody on the RAST in a dilution test and Fujita et al. [9] confirmed the inhibitory action of non-IgE antibody using purified IgE and non-IgE antibodies.

In the RAST value of insulin, as described above, though a slight inhibition of the RAST by non-IgE antibody was observed in the range of high concentration of IgE antibody, little inhibition was observed in the range of low concentration of IgE antibodies. Therefore, from the practical point of view, there is no obstacle for detecting IgE antibodies from non-IgE antibody containing serum.

The facts that the RAST value was only slightly inhibited by the presence of non-IgE antibodies and the use of different species of insulin might be explained by a high capacity of the disc-coupled antigen in insulin RAST.

Though the presence of reagenic insulin antibodies in the patient's serum was directly confirmed by the Prausnitz-Küstner test only in two out of the six allergic patients, judging from their clinical symptoms and results of direct skin tests with purified insulin from several sources, their presence was probable in the four other patients. The fact that all of the allergic patients had significantly higher values showed the usefulness of the RAST as an in vitro assay method for detecting IgE insulin antibody. This is supported by the fact that an allergic patient showed a gradual decrease after treatment with highly-purified porcine insulin corresponding to amelioration of the clinical allergic symptoms.

In this study, skin tests and the RAST determination could not be performed immediately after the onset of symptoms, so the discrepancy between RAST value and size of reaction in skin test and severity of clinical symptoms might be due to this lag time. However, factors such as 1) fixation of reagenic antibody to mast cells, 2) release of chemical mediators and 3) actions of the chemical mediators on the tissue, may affect the dermal reactions. The individual differences of these factors could affect the severity of dermal reaction in addition to levels of IgE insulin antibody.

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Oxidation of an Exogenous Glucose Load Using Naturally Labelled ^{13}C -Glucose

Effect of Butylbiguanide Therapy in Obese Mildly Diabetic Subjects

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Summary. The effect of a 14 day-administration of butylbiguanide was investigated in a group of 10 obese patients with mild-to-moderate glucose intolerance. Glucose tolerance was significantly improved, while fasting blood glucose and plasma levels of free fatty acids, insulin and glucagon remained unchanged. The estimation of the amount of the oral glucose load oxidized into CO_2 was performed by means of a recently described procedure using "naturally labelled ^{13}C -glucose" as tracer. The curves depicting the oxidation of the exogenous glucose load were similar in shape and magnitude before and after administration of the biguanide; in the latter case, however, slightly higher rates of oxidation of exogenous glucose were recorded during the 2nd, 3rd and 4th hours of the test. These data do not provide evidence that the biguanide-induced improvement in glucose tolerance in patients with mild-to-moderate glucose intolerance is associated with any inhibiting or delaying effect of this type of drug on intestinal absorption (and subsequent oxidation) of the exogenous glucose load. On the contrary, a slight, but statistically significant, increase in the oxidation of exogenous glucose has been observed after butylbiguanide.

Key words: Biguanide, butylbiguanide, diabetes, free fatty acids, glucagon, glucose, insulin, management, stable isotopes.

The aim of the present paper is to report the application of this new and innocuous procedure to the study of the mechanism of the blood glucose lowering action of butylbiguanide in human diabetes. Despite a wide therapeutic use [7], the site(s) and mode(s) of action of biguanides remain controversial [8]. Several recent observations suggest that the biguanides might slow or inhibit the intestinal absorption of glucose [9-12], while various clinical or experimental reports indicate that these drugs may act by decreasing liver gluconeogenesis [13] or increasing peripheral utilization of glucose [14, 15]. In the present study, we investigated the effect of a 14 day-administration of butylbiguanide on the oxidation of an exogenous glucose load, using naturally labelled ^{13}C -glucose, in a group of obese patients with mild-to-moderate glucose intolerance.

Methods

Study group

Ten obese patients (20 per cent or more in excess of their ideal body weight) served as experimental subjects (Table 1). All had normal liver and kidney function as evaluated by routine criteria. Four (n° 2, 3, 5, and 6) had moderate fasting hyperglycaemia (109 to 133 mg %) and all had glucose intolerance according to standard criteria [16]. All subjects were investigated before and 14 days after biguanide therapy (butylbiguanide or Silubin-retard[®], 100 mg twice daily, the last dose being given two hours before the beginning of the second test). During the study, and for at least 3 days before the first test, the subjects were fed on a normal diet providing a minimum of 250 g of carbohydrate per day and no other medication than biguanide was provided. The

We have recently reported a procedure which allows estimation of the amount of an oral glucose load oxidized into CO_2 in man [1-6]. The method includes the use of glucose "naturally labelled" [1] with the stable isotope, ^{13}C .