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Immediate-Type Allergy Against Insulin Itself: Clinical and Immunologic Studies on a Diabetic Patient with Insulin Intolerance

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In this report clinical and immunologic data of a female diabetic patient with immediate-type allergy against insulin preparations of different species, including pancreatic human insulin, biosynthetic human insulin, and semisynthetic human insulin are described. The determination of insulin-specific antibodies showed relatively high IgE-antibody titers in contrast to low IgG-antibody concentrations, which is in accordance with the assumption that the ratio of IgE- to IgG-insulin antibodies is important in the outcome of clinically manifest allergic reactions. Negative results for cutaneous delayed-type insulin hypersensitivity and for stimulation of lymphocyte blastogenesis exclude type IV immunopathogenic reaction in this insulin-allergic patient. Preliminary data suggest that inherited individual factors—such as DR2, DR3 positivity in this diabetic individual—might predispose diabetic patients to insulin allergy.

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The development of allergic reactions is a common finding during the initial phase of therapy with conventional insulin preparations.^{1–5} Several factors^{6,7} may be involved in this immunologic process (Table 1). Additives in insulin preparations—for instance, protamine⁸ or surfen,⁹ “contaminants” such as desamido-insulin,¹⁰ preservatives such as parabens, or the insulin molecule itself (beef, pork, or human)—are the agents most probably causing allergic side effects following conventional insulin therapy. Some cases of “insulin” allergy may be due to the zinc component¹¹ of the insulin preparation, and this possibility should be considered in patients not responding to conventional measures. Insulin allergy manifests itself either locally or systemically as urticaria or anaphylactic reactions.^{1–8} Systemic allergy and local allergy of the immediate type are suggested to be IgE-mediated.^{12–18} In patients treated from the very beginning of insulin therapy with highly purified pork insulin preparations, insulin allergy has so far not been observed.^{6,19,20} We report here immunologic and clinical observations of an unusual case of immediate-type allergy against insulin preparations, including pancreatic human insulin, biosynthetic human insulin (BHI), and semisynthetic human insulin.

PATIENT HISTORY

A female patient (K. Th.), 69 years of age, developed non-insulin-dependent diabetes in 1963. The patient was placed

on a diet and on oral hypoglycemic agents (5–15 mg glibenclamide/day) between 1963 and 1980. Deterioration of metabolic control and continuous weight loss made insulin therapy necessary in February 1980. An extended localized allergic reaction occurred at the site of injection 3 days after onset of treatment with a highly purified insulin (Novo Lente MC insulin). The patient was placed on other forms of highly purified insulins; however, due to the increasing severity of the immediate-type allergy, insulin treatment had to be discontinued after 10 days. The patient denied having received any treatment with insulin between 1963 and February 1980. Although the patient consulted various physicians and clinics during this extended period of time, contact with a not highly purified insulin cannot be excluded with absolute certainty, but after extensive review of the files of her physicians seems highly unlikely. In June 1980, the patient was admitted to our department for detailed immunologic examinations. At that time, all in vitro tests (IgE- and IgG-insulin antibodies, insulin-induced lymphocyte transformation) as well as skin testing were performed.

METHODS

Skin testing. As a measure of sensitivity of the patient to insulin itself and to the different additives and “contaminants” in insulin preparations, detailed skin testing was undertaken. Intradermal tests were performed with various insulin preparations of animal origin including commercial beef and pork

TABLE 1
Factors possibly involved in “insulin allergy”

Contaminants
Additives
Preservatives
Zinc
Mode of administration
Species of origin
Insulin molecule itself
Genetic predisposition (Irr genes?) to allergy

insulins and highly purified pork insulins. In addition, human pancreatic insulin, BHI, and semisynthetic human insulin were used. Insulin was diluted to 5 U/ml and 0.05 ml was injected intradermally on the anterior aspect of the forearm. Reactions were graded according to the size of the wheal (read after 20 min) and for the degree of induration (checked after 24 h). The size of a wheal comparable to the histamine reaction (0.1 mg/ml) was considered to be positive (+++). An induration 5 mm in diameter was considered positive.

IgE-insulin antibodies. Insulin was covalently coupled to sepharose and incubated in the patient serum. Thereby all insulin antibodies, including insulin-specific IgE, were bound. After washing procedures, ¹²⁵I-anti-human IgE (Pharmacia, Uppsala, Sweden), which forms complexes with insulin-bound patients' anti-insulin IgE (IgE_I), was added. The radioactivity was measured in a gamma-scintillation counter. Results were expressed as insulin-specific IgE antibodies in international units of IgE/ml calculated from a standard curve. The nonspecific binding to sepharose without insulin was found to be 0.2 ± 0.3 IU/ml, for which results were corrected.²¹

In insulin-dependent-diabetic (IDD) patients, daily treated with 4–5 times crystallized insulin for 1 year, IgE-insulin antibodies were 1.2 ± 0.2 IU/ml,²¹ whereas IDD patients exclusively treated with MC insulins for 2 years showed IgE-insulin-antibody levels of 0.4 ± 0.06 IU/ml.²¹

IgG-insulin antibodies. IgG-insulin antibodies were measured by radioimmune electrophoresis according to Christensen.²² Details of our testing procedure were previously reported.²³ Nonspecific binding of insulin to IgG was less than 0.05 U/L in 75 nondiabetic subjects.

Insulin-induced lymphocyte transformation. All lymphocyte stimulation studies were performed concomitantly on the patient and six age-matched controls, using microtechniques for (1) isolated mononuclear cells and (2) whole blood. For the former technique, mononuclear cells were isolated by density centrifugation (Ficoll-Hypaque, Nysgaard), washed twice, and suspended in RPMI 1640 (Gibco, Glasgow) supplemented with 10% fetal calf serum. Thereafter aliquots of 0.2 ml cell suspension containing 2×10^6 cells were placed into microtiter wells. For the whole blood method, heparinized blood was diluted 1:4 with RPMI 1640 and aliquots of 0.2 ml placed into wells of U-shaped microtiter plates (Nunc, Norway). Then 20μ l antigen solution (or medium

only) was added to each well. After 6 days of incubation at 37°C in a humidified atmosphere, the cultures were labeled with ³H-thymidine (specific activity 5 Ci/mmol, dose per culture 0.2μ Ci) and terminated 18 h later by harvesting them for scintillation counting in an automatic beta-counter. All experiments were performed in quadruplicates. The results were expressed as counts per minute (cpm), and the effect of antigen in stimulating blastogenesis was expressed as

$$\text{transformation index} = \frac{\text{cpm of cultures with antigen}}{\text{cpm of cultures without antigen}}$$

Insulin preparations. The following insulin preparations were used for in vivo testing: CR (Hoechst), CS (Hoechst), Depot-Klar CR (Hoechst), Comb CS (Hoechst), Actrapid MC (Novo), Monotard MC (Novo), Insulin Novo Lente MC (Novo), Rapitard MC (Novo), Semilente MC (Novo), Ultralente MC (Novo), Neutral R.I. (Leo), Mixtard R.I. (Leo), Retard R.I. (Leo), Des- β^1 -Phenylalanine OIS (Hoechst), Des- β^1 -Phenylalanine O3S (Hoechst), biosynthetic human (BHI, Lilly), semisynthetic human (Novo), and pancreatic human (kindly supplied by the Novo Research Institute, Copenhagen). For in vitro testing the following insulins were used in three concentrations (4μ U/ml, 40μ U/ml, 500μ U/ml): biosynthetic human (Lilly), pancreatic human, semisynthetic human (Novo), Actrapid MC (Novo), Novo Lente MC (Novo), and CR (Hoechst).

HLA typing. All known antigens of the A-, B-, C-, and DR-locus were determined by the NIH-lymphocytotoxicity technique (ABC) and by the two-color fluorescence technique (DR) according to van Rood et al.²⁴

RESULTS

Skin testing with various insulins—regardless of mode of preparation, physical state, purity, and/or species—resulted in strong immediate allergic reactions (Table 2). Positive tests were also noted after highly purified insulins, such as monocomponent (MC) or rare immunogenic (R.I.) insulins, were used. Furthermore, human insulin preparations (BHI and semisynthetic human insulin) also induced local allergic skin reactions 10–20 min following insulin administration. At the first skin testing, pancreatic human insulin produced a flare, but no wheal reaction. After retesting 7 days later, an unequivocal wheal reaction developed. BHI, not available at the first time of testing, was included in the second test series and also produced an unequivocal wheal reaction. Control testing with saline, human albumin, or other hormones (glucagon, calcitonin, parathormone) did not result in allergic reactions at the injection site.

IgG-insulin antibodies were found in very low concentrations of only 0.22 U/L. The titer of insulin-specific IgE was 1.0 U/ml (Table 3).

None of the several insulin preparations used for testing cellular hypersensitivity to various insulins produced a significant stimulation of lymphocyte blastogenesis in the patient

TABLE 2

Results of intradermal skin testing in patient K.Th.; 0.05 ml of various insulin solutions (5 U/ml) were injected intradermally. Reactions were read after 20 min and results expressed in millimeters of diameter

Histamine (0.1 mg/ml) NaCl	(positive control) (negative control)	14 × 17	14 × 17
Insulin CR (beef)	—	9 × 10	ND*
Insulin CS (pork)	—	6 × 9	ND
Depot-Klar CR (beef)	—	9 × 8	ND
Comb-CS (pork)	—	9 × 7	ND
Actrapid MC (pork)	—	8 × 6	12 × 11
Monotard MC (pork)	—	6 × 6	ND
Insulin Novo Lente MC (beef/pork)	—	6 × 5	ND
Rapitard MC (beef/pork)	—	8 × 9	ND
Semilente MC (pork)	—	8 × 7	ND
Ultralente MC (beef)	—	8 × 7	ND
Insulin Leo Neutral R.I. (pork)	—	8 × 7	ND
Insulin Leo Mixtard R.I. (pork)	—	8 × 9	ND
Insulin Leo Retard R.I. (pork)	—	8 × 8	ND
Des-β ¹ -Phenylalanine-insulin 01S (pork)	—	9 × 10	ND
Des-β ¹ -Phenylalanine-insulin 03S (pork)	—	8 × 10	ND
Biosynthetic human insulin	—	ND	10 × 11
Semisynthetic human insulin	—	9 × 7	10 × 11
Pancreatic human insulin	—	†	10 × 11

* ND = not done.

† Flare but not wheal reaction seen.

or controls when test systems for either whole blood (Table 4) or isolated mononuclear cells (Table 5) were applied.

The HLA phenotyping showed the following antigens: A3, B12, B40, DR2, DR4.

DISCUSSION

Immunologic response to insulin therapy occurs in almost all diabetic patients treated with conventional insulins, resulting in the production of insulin antibodies²⁵ and in the emer-

TABLE 3

Anti-insulin antibody titers in patient K.Th. with immediate-type allergy against insulin itself

IgE-insulin antibodies	1.00 U/ml*
IgG-insulin antibodies	0.23 U/L†

* 0.6 ± 0.1 U/ml in 25 nondiabetic patients.²¹

† <0.05 U/L in 75 nondiabetic patients.²⁵

gence of cellular hypersensitivity. Fortunately, the immune response remains clinically insignificant in most insulin-treated patients.⁵ Clinically, immune responsiveness to insulin usually manifests itself as a local cutaneous hypersensitivity reaction, and only rarely as urticaria or as generalized anaphylaxis. These side effects of insulin therapy were recognized within 2 years of the introduction of insulin for the treatment of insulin-deficient diabetic patients.¹ During the last 10 years evidence has accumulated^{6,7,19,26} that the purification of insulin has significantly reduced the intensity of local pain and swelling at the site of insulin injection, suggesting that a high number of suspected "insulin" allergic reactions are not directed against the insulin molecule itself, but are most probably induced due to "contaminants," additives, or other non-insulin components. Insulin allergy has so far not been reported in patients treated with highly purified preparations from the very beginning of insulin therapy.^{19,27} However, in presensitized patients, even highly purified insulins^{10,27} or human insulin preparations^{28,29} may cause local allergic reactions.

We observed a female diabetic patient, who presented with immediate-type (type I) allergy at the site of insulin injection following the first administration of Novo Lente MC insulin. Detailed skin testing of this patient showed immediate cutaneous hypersensitivity against all available insulin preparations, including human biosynthetic and human semisynthetic insulin. The latter finding suggests that in exceptional cases^{26,30}, as in our patient, allergy against the in-

TABLE 5

Lymphocyte stimulation with various insulin preparations in patient K.Th. and in six healthy control subjects. Micromethod using isolated mononuclear cells suspended in RPMI 1640

Insulin		4 μU/ml		40 μU/ml		500 μU/ml	
		cpm*	TI†	cpm	TI	cpm	TI
Human biosynthetic insulin	K.Th.	1548 ± 97	1.0	1716 ± 313	1.1	1380 ± 230	0.9
	Controls	1431 ± 112	1.0	1390 ± 210	1.0	1487 ± 188	1.0
Human pancreatic insulin	K.Th.	1453 ± 252	0.9	1183 ± 141	0.8	1321 ± 394	0.8
	Controls	1391 ± 211	1.0	1412 ± 192	1.0	1382 ± 241	1.0
Human semisynthetic insulin	K.Th.	1107 ± 170	0.7	1047 ± 146	0.7	950 ± 157	0.6
	Controls	1423 ± 109	1.0	1410 ± 91	1.0	1476 ± 219	1.0
Actrapid pork MC	K.Th.	1084 ± 343	0.7	936 ± 164	0.6	1274 ± 262	0.8
	Controls	1318 ± 72	0.9	1378 ± 188	1.0	1397 ± 152	1.0
Novo Lente beef/pork MC	K.Th.	1120 ± 180	0.7	1055 ± 150	0.7	1260 ± 160	0.8
	Controls	1410 ± 120	1.0	1385 ± 160	1.0	1350 ± 198	1.0
Hoechst beef CR	K.Th.	1301 ± 306	0.8	1037 ± 98	0.7	1388 ± 472	0.9
	Controls	1481 ± 301	1.0	1337 ± 167	0.9	1401 ± 251	1.0

* cpm obtained in medium only (without insulin): 1356 ± 208 in K.Th.; 1412 ± 206 in controls (N = 6).

† TI = transformation index.

sulin molecule itself might develop in the course of treatment.

Reaginic antibodies to insulin have been demonstrated previously by the Prausnitz-Küstner reaction and by radioimmunosorbent assay in insulin-allergic patients.^{12-18,21} The anti-insulin-IgE titers were considerably higher in insulin-allergic diabetic patients compared with insulin-treated patients without insulin allergy.^{15,17,18} Furthermore, it has been shown that the ratio of ¹²⁵I-insulin bound by IgE and IgG was significantly higher in the insulin-allergic group.¹⁵ The determination of insulin antibodies in our patient showed relatively high IgE-insulin antibodies (1.00 U/L) considering the fact that the serum sample was withdrawn 4 months after the severe local insulin-allergic reaction. IgG-insulin antibodies were also detectable, however, in low titers only. The concomitant presence of IgE- and IgG-insulin antibodies probably accounts for the slightly weaker insulin-induced wheal and flare reaction when compared to the histamine control. This is also in accordance with the hypothesis that the ratio of IgE- to IgG-insulin antibodies may play an important role in the outcome of clinically manifest allergic reactions.

Recent studies in both human beings³¹⁻³⁵ and animals³⁶⁻³⁸ suggest that the immune response to insulin is under genetic control. It has been reported that strong humoral immune response to insulin, even against monocomponent insulin preparations,³⁹ is associated with the risk axis HLA DR4-B18-Cw3, whereas HLA DR3-B8, as well as the risk axis HLA DR2-B7, is associated with a weak immune response to insulin.³¹⁻³⁵ In a recent study,⁴⁰ HLA B7, which is negatively associated with insulin-dependent diabetes mellitus,⁴¹ was found to be significantly increased in diabetic patients with insulin allergy. The increase of the genetic marker DR2-B7 and the well-documented¹²⁻¹⁸ elevation of IgE-insulin antibodies in insulin-allergic patients suggest a genetic control in the production of reaginic insulin antibodies.

Therefore, inherited individual factors might predispose diabetic individuals to insulin allergy. This might also apply to our patient, who was found to be DR2-positive. To what extent immunogenetic factors influence the ratio of IgE- to IgG-insulin antibody formation should be investigated in further studies.

When peripheral mononuclear cells or whole blood were cultured in the presence of varying concentrations of several insulin preparations, no significant stimulation of lymphocyte blastogenesis was observed. These findings, as well as the negative test results for cutaneous delayed-type insulin hypersensitivity, virtually exclude type IV allergy to any of the insulins used. Only a few authors reported insulin-induced lymphocyte transformation in diabetic patients with or without insulin allergy.⁴²⁻⁴⁶ MacCuish et al.⁴⁴ described blastogenesis of lymphocytes to bovine or pork insulins in diabetic patients without insulin allergy. Limited and controversial data have been reported for diabetic patients with insulin allergy—both the immediate and the delayed type.^{29,42,43,45,46} In a recent study,⁴⁶ about 50% of patients with insulin allergy showed an increased mitogenic response to insulin or proinsulin, and 20% of patients with insulin allergy and insulin antibodies had lymphocytes that failed to respond to insulin when tested in vitro.⁴⁶ The latter findings confirm that a considerable number of diabetic individuals with insulin allergy lack significant cellular immune responsiveness.

In summary, evidence for the existence of immediate-type allergy against insulin itself was obtained in a diabetic patient. This was substantiated by the demonstration of insulin-specific IgE antibodies and by immediate-type skin reactions to various insulin preparations, including biosynthetic, semisynthetic, and pancreatic human insulins. The short interval between the onset of insulin therapy and the first manifestation of immediate-type allergy suggests presensitization,

TABLE 4

Lymphocyte stimulation with various insulin preparations in patient K.Th. and in six healthy control subjects. Whole blood micromethod

Insulin		4 μU/ml		40 μU/ml		500 μU/ml	
		cpm*	TI†	cpm	TI	cpm	TI
Human biosynthetic insulin	K.Th.	142 ± 28	1.3	140 ± 4	1.3	110 ± 10	1.0
	Controls	132 ± 27	1.1	135 ± 12	1.1	130 ± 16	1.0
Human pancreatic insulin	K.Th.	138 ± 14	1.3	139 ± 13	1.3	110 ± 14	1.0
	Controls	122 ± 12	1.0	128 ± 12	1.0	180 ± 15	1.0
Human semisynthetic insulin	K.Th.	132 ± 11	1.2	138 ± 22	1.3	127 ± 33	1.2
	Controls	129 ± 9	1.0	131 ± 6	1.1	180 ± 11	1.0
Actrapid pork MC	K.Th.	117 ± 17	1.1	122 ± 9	1.1	127 ± 58	1.2
	Controls	131 ± 12	1.1	128 ± 8	1.0	129 ± 22	1.0
Novo Lente beef/pork MC	K.Th.	130 ± 10	1.0	122 ± 8	1.1	110 ± 15	1.0
	Controls	129 ± 11	1.0	131 ± 10	1.0	130 ± 14	1.0
Hoechst beef CR	K.Th.	111 ± 12	1.0	111 ± 52	1.0	116 ± 15	1.1
	Controls	132 ± 8	1.1	124 ± 9	1.0	127 ± 18	1.0

* cpm obtained in medium only (without insulin): 110 ± 11 in K.Th.; 129 ± 8 in controls (N = 6).

† TI = transformation index.

although there was no proof of this. Since pure and native human insulin is unlikely to cause IgE-mediated allergic reactions in patients not presensitized, it is hoped that treatment with human insulin from the very beginning of the disease will prevent the occurrence of type I allergic reactions in the future.

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