Affinity of IgG-Insulin Antibodies to Human (recombinant DNA) Insulin and Porcine Insulin in Insulin-treated Diabetic Individuals With and Without Insulin Resistance

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The affinity of human (recombinant DNA) insulin and porcine insulin to preformed IgG-insulin antibodies of insulin-treated diabetic individuals was studied in 29 insulin-treated diabetic patients. The binding of ^{125}I-human insulin and ^{125}I-porcine insulin with antibodies was similar in the group of patients without insulin resistance (N = 25). The sera of insulin-resistant diabetic patients (N = 4), containing very high IgG-insulin immunoglobulins, showed a significantly lower affinity for human insulin compared with porcine insulin ($P < 0.01$). All four patients with insulin-antibody–mediated insulin resistance were positive for HLA-DR4, but negative for -DR3, supporting the concept of an immunogenetically transferred anti-insulin immune response in insulin-treated diabetic individuals. Based on the reduced binding of human insulin to IgG-antibodies of very high titers in patients with insulin resistance, a potential therapeutic advantage of human insulin therapy can be expected in such infrequent cases of immunologic insulin resistance. DIABETES CARE 5 (SUPPL. 2): 114–118, 1982.

The presence of insulin-binding immunoglobulins in the sera of diabetic patients treated with insulin was reported first by Berson and co-workers in 1956.[1] It was soon established that the majority of diabetic individuals develop circulating antibodies to the administration of heterologous insulin obtained by acid-ethanol extraction of animal pancreas. All five major classes of immunoglobulins (G, M, A, E, and D) have been found in insulin-treated diabetic patients;[2] quantitatively, the most significant antibodies are those of the IgG-class. Consecutive studies[3–10] have shown that the magnitude of insulin antibody formation shows considerable variations in different patients and depends on several factors: species source,[3,4] purity,[3,5,6] and pharmaceutical form[2,3] of insulin; pattern of insulin therapy[7–9] and mode of insulin administration,[6–10] as well as the immunogenetic background (HLA-DR–associated genetic mechanisms) of the diabetic patients.[4,11] In 1959 Berson and Yalow[12] characterized the anti-insulin antibodies as nonprecipitating insulin-binding immunoglobulins from which the insulin is freely dissociable. In further studies they reported[13] that different sera showed marked variations in affinity for insulin from different animal species.

Since 1981 human insulin, prepared by recombinant DNA technology using *E. coli*[14] or by semisynthesis from porcine insulins, is available for clinical long-term use in insulin-dependent diabetic patients. Based on the availability of human insulin, it is expected that in the future a high number of diabetic individuals will substitute human insulin for their animal insulin preparations. Thus, it was of particular interest to study the affinity of insulin-antibody–containing sera for human insulin (recombinant DNA origin) and porcine insulin. Sera from insulin-resistant and nonresistant insulin-treated diabetic individuals were tested.

PATIENTS, MATERIALS, AND METHODS

The sera for the comparative affinity studies of insulin antibodies for human insulin and porcine insulin were taken from 29 insulin-treated diabetic subjects, in whom IgG-insulin antibodies were determined previously. The sera of 25 nonresistant diabetic individuals (group A) were divided into three groups according to the titers of insulin antibodies measured with ^{125}I-human insulin (low IgG-insulin antibodies below 1.0 mU/ml; moderate titers between 1.01 and 5.0 mU/ml; high titers above 5.0 mU/ml). The duration of diabetes of these patients ranged from 1–17 yr; the mean daily insulin requirement was 0.6 U/kg body wt., ranging from 0.3 to 0.9 U/kg. In addition, four sera of insulin-dependent diabetic patients suffering from insulin-antibody–mediated insulin resistance (group B) could be included in the insulin affinity studies. The daily insulin requirement for satisfactory metabolic control (HbA_1 concentrations about 10%) was con-

stantly high (above 1.5 U/kg body wt.) in these patients (A.K., W.R., B.G., and H.J.). All diabetic subjects in this group (B) had very high IgG-insulin antibodies and the increased daily insulin requirement was not caused by (1) diabetic ketoacidosis; (2) significant infection; (3) significant dietary indiscretion (obesity); (4) lipoatrophic diabetes; (5) complicating endocrine disease; or (6) insulin receptor defects. The detailed clinical data of the insulin-resistant patients are given in Table 1.

The IgG-insulin antibodies were measured by radioimmunoelectrophoresis according to Christiansen.[16] For the comparative insulin affinity studies ^{125}I-human insulin (Eli Lilly Laboratories, Indianapolis, Indiana) and ^{125}I-porcine insulin (Eli Lilly Laboratories) with almost identical specific activities were used (monoiod-125 human insulin, Lot No. J 84-02N-166, specific activity 361.2 μCi/μg: 16.3 μCi on 42.5 ng of human insulin; monoiod-125 porcine insulin, Lot No. J 84-02N-162, specific activity 362.9 μCi/μg: 16.1 μCi on 44.4 ng of porcine insulin). HLA-DR antigens were determined using the two-color fluorescence technique according to van Rood and co-workers.[17] Serum C-peptide was measured according to Heding.[18]

RESULTS

The binding of human insulin and porcine insulin to 25 sera with preformed IgG-insulin antibodies of different titers taken from nonresistant insulin-treated diabetic persons is shown in Table 2. No significant difference in the affinity of the two insulins was noted when the total number of sera was analyzed. However, in some cases human insulin was bound in a higher concentration than porcine insulin and in other cases reverse bindings were noted. The affinity of human insulin and porcine insulin to IgG-insulin antibodies was not dependent on the titers (not different to sera with low, moderate, or high insulin-antibody titers) of the anti-insulin immunoglobulins in the patients without insulin resistance (group A). In four patients with a constant high daily insulin requirement based on insulin-antibody–mediated immunologic insulin resistance (group B), the binding of human insulin to the preformed antibodies was significantly lower ($P < 0.01$) compared with porcine insulin (Table 1).

DISCUSSION

In the present investigation, sera of insulin-treated diabetic individuals without insulin resistance showed no significantly different binding of porcine and human insulin, whether the preformed IgG-antibodies had low, moderate, or high titers. Earlier investigations on sera of porcine insulin-treated nonresistant diabetic individuals performed by Berson and Yalow[19] also showed comparable affinities to human and porcine insulins, findings that were confirmed recently.[20] By contrast, Kumar[21] reported a significantly lower binding of ^{125}I-human insulin compared with ^{125}I-porcine insulin in 6 of 16 sera with insulin-binding capacities above 80 μg/L. These sera were obtained from insulin-treated diabetic persons who were classified as nonresistant. Based on the present findings, it can be concluded that the administration of human insulin instead of porcine insulin will not significantly reduce the daily insulin requirement in the majority of insulin-treated patients not suffering from insulin resistance. However, it cannot be excluded that long-term treatment with human insulin will decrease the amount of insulin antibodies and, consequently, the daily insulin dose in patients possessing high anti-insulin antibodies as a consequence of immunization with heterologous dirty insulins. In subjects never treated with insulin, who developed insulin-binding antibodies due to up-to-now unidentified mechanisms, the affinity of the antibodies for human insulin tends to be the same as the affinity for porcine insulin and to be greater than the affinity for beef insulin.[22] By contrast, in insulin-treated patients[3,20,23] the greatest affinity of preformed insulin antibodies has been noted to beef insulin, which might be of clinical relevance at least in diabetic patients with high insulin antibodies.

The administration of antigenic heterologous insulin preparations to diabetic patients may be associated with two types of insulin-antibody–mediated clinical manifestations. Insulin allergy with local and/or systemic reactions of the immediate hypersensitivity is mediated by IgE-antibodies directed against the insulin.[24–27] Immunologic insulin resistance is mainly caused

TABLE 1
Clinical data, C-peptide concentrations, HLA-DR antigens, and IgG-insulin-antibody–binding concentrations (mU/ml) for human insulin and porcine insulin of four insulin-resistant diabetic patients treated with dirty insulin preparations

Patient	Age (yr)	Duration of diabetes (yr)	Insulin requirement (U/kg body wt.)	C-peptide (pmol/ml)	HLA-DR antigens	IgG-insulin antibodies	
						^{125}I-human insulin (recombinant DNA) (mU/ml)	^{125}I-porcine insulin (mU/ml)
A.K.	36	4	1.7	0.03	DR4	8.1	12.2
W.R.	44	21	1.9	0.12	DR4, DR7	7.8	14.8
B.G.	45	10	1.8	0.11	DR4	8.9	13.7
H.J.	39	11	1.6	0.03	DR4, DR1	8.9	14.1
Mean						8.4	13.7
SEM						0.3	0.6

TABLE 2
IgG-binding concentrations (mU/ml) for human insulin and porcine insulin in the sera of 25 non-insulin-resistant diabetic patients pretreated with bovine and/or porcine insulin preparations

Titers	Patient	Age (yr)	Duration of disease (yr)	IgG-insulin antibodies	
				^{125}I-human insulin (recombinant DNA) (mU/ml)	^{125}I-porcine insulin (mU/ml)
Low anti-insulin	N.E.	20	1	0.05	0.04
IgG-antibodies	F.P.	17	2	0.09	0.08
(<1.0 mU/ml)	A.J.	16	1	0.18	0.18
	W.L.	21	3	0.28	0.28
	B.A.	31	1	0.30	0.28
	G.J.	19	2	0.46	0.54
	G.W.	24	1	0.55	0.65
	D.A.	15	4	0.96	0.75
Moderate anti-insulin	M.S.	16	2	1.10	1.20
IgG-antibodies	R.S.	21	2	1.86	1.54
(1.01–5.0 mU/ml)	S.W.	17	1	1.86	1.54
	S.L.	14	3	2.97	2.88
	P.A.	24	2	2.90	3.13
	L.U.	32	12	3.10	3.40
	W.D.	29	5	3.26	3.17
	P.B.	27	7	3.60	3.55
	T.E.	25	6	3.80	2.70
	H.C.	18	14	3.87	3.65
	S.J.	42	15	4.07	4.52
High anti-insulin	R.P.	16	6	5.5	4.2
IgG-antibodies	S.W.	24	8	6.4	4.0
(>5.0 mU/ml)	P.A.	28	10	5.1	5.4
	D.E.	17	12	8.4	8.2
	B.A.	32	14	13.1	13.7
	S.W.	31	17	19.5	20.3
Mean				3.72	3.64
SEM				0.91	0.94
Number				25	25

by high titers of circulating anti-insulin IgG-antibodies;[9,25,28] only extremely rare cases might be related to the production of antibodies reactive with the insulin receptor.[29] Previously, insulin resistance was defined as a state in nonketoacidotic, insulin-treated persons whose daily dose of insulin had to exceed 200 U to produce a clinical effect.[30] This definition is out-of-date now since the mean daily dose of insulin decreased to around 36 U (about 0.5 U/kg body wt.) by using highly purified insulin preparations.[31] There is an increasing tendency to apply the term immunologic insulin resistance to all cases of insulin-deficient diabetic patients with high insulin antibodies in which the daily insulin dose exceeds 1.5 U/kg body weight. With the increasing use of highly purified insulin preparations, immunologically conditioned insulin resistance is now very rare in Austria; therefore, only four cases could be selected out of a very high number of insulin-treated diabetic subjects for the insulin affinity studies. Interestingly, all four patients have been primarily treated with antigenic insulin preparations, and all of them have a history of insulin allergy and of interrupted insulin therapy.

These clinical features are in good agreement with previous reports on patients with immunologic insulin resistance.[2,9,21,25,28,30] However, many diabetic individuals have a similar history in their clinical course and do not develop immunologic insulin resistance. Thus, other factors, i.e., constitutional variations in the individual patient, could be involved in the development of antibody-mediated insulin resistance. In this respect, it is of interest that the antibody response to exogenous insulin treatment is associated with the HLA. In diabetic individuals HLA-DR typing has shown that higher IgG-insulin antibody titers tend to occur in -DR4, with lower titers in HLA-DR3-positive patients.[4,11] All four patients with immunologic insulin resistance were positive for HLA-DR4, but negative for -DR3 (Table 1), supporting the hypothesis that immunogenetically transferred immune mechanisms could play a role in this uncommon side effect of insulin therapy. In the future, it is expected that with the increasing use of highly purified (especially with human) insulin preparations problems caused by insulin-binding antibodies will become even less frequent than they

are today. A recent report[4] on 102 newly diagnosed type I diabetic individuals suggests that insulin antibody formation after human MC insulin therapy is significantly lower in incidence and titers compared with the use of the corresponding porcine MC insulin preparations. In diabetic persons, who already suffer from insulin-antibody–mediated insulin resistance, the lower affinity of the preformed antibodies to human insulin compared with the porcine insulin seems to be of clinical relevance. Interestingly, one insulin-resistant patient (B.G.) treated with semisynthetic human insulin (kindly supplied by the Novo Research Institute, Copenhagen, Denmark) showed a dramatic reduction in insulin requirement from 1.8 to 0.7 U/kg body wt. In accordance with the present findings, Kumar[21] reported a binding of ^{125}I-human insulin of 14 ± 4% compared with 37.6 ± 9% of ^{125}I-porcine insulin to four sera of insulin-resistant diabetic patients.

In summary, these observations indicate that in the majority of insulin-treated patients the affinity of human insulin and porcine insulin to preformed IgG-insulin antibodies is almost identical. The sera of diabetic individuals possessing very high IgG-insulin antibodies and suffering from immunologic insulin resistance appear to recognize and discriminate the therapeutic porcine insulin molecule from the human insulin molecule. A particular susceptibility constellation could also be involved, in addition to other well-known factors that contribute to the development of immunologic insulin resistance, although this has to be confirmed in further studies with a higher number of insulin-resistant diabetic individuals.

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REFERENCES

[1] Berson, S. A., Yalow, R. S., Bauman, A., Rothschild, M. A., and Newerly, K.: Insulin I^{131} metabolism in human subjects. Demonstration of insulin binding globulin in circulation of insulin treated subjects. J. Clin. Invest. *35*: 170–90, 1956.

[2] Kahn, R. C., and Rosenthal, A. S.: Immunologic reactions to insulin: insulin allergy, insulin resistance, and the autoimmune insulin syndrome. Diabetes Care *2*: 283–95, 1979.

[3] Chance, R. E., Root, M. A., and Galloway, J. A.: The immunogenicity of insulin preparations. Acta Endocrinol. *83* (Suppl. 205): 185–98, 1976.

[4] Schernthaner, G., Borkenstein, M., Fink, M., Mayr, W. R., Menzel, J., and Schober, E.: Immunogenicity of human monocomponent insulin or pork monocomponent insulin in HLA-DR typed insulin dependent diabetics. Am. J. Med. In press.

[5] Schlichtkrull, J., Brange, J., Christiansen, Aa. H., Hallund, O., Heding, L. G., and Jørgensen, K. H.: Clinical aspects of insulin-antigenicity. Diabetes *22*: 649, 1972.

[6] Andersen, O. O.: Insulin antibody formation: II The influence of species difference and method of administration. Acta Endocrinol. *72*: 33, 1973.

[7] Hanauer, L., and Batson, J. M.: Anaphylactic shock following insulin injection. Diabetes *10*: 105, 1961.

[8] Lamkin, N., Lieberman, P., Hashimoto, K., Morohashi, M., and Sullivan, P.: Allergic reactions to insulin. J. Allergy Clin. Immunol. *58*: 213, 1976.

[9] Davidson, J. K., and DeBra, D. W.: Immunologic insulin resistance. Diabetes *27*: 307, 1978.

[10] Charles, M. A., Ong, K., Slater, L., Golden, M., and Arquilla, E. R.: Immunologic implications of insulin delivery and the role of immune complexes in diabetic sequelae. Diabetes Care *5* (Suppl. 1): 88–92, 1982.

[11] Schernthaner, G., Ludwig, H., and Mayr, W. R.: Immunoglobulin G-insulin antibodies and immune region-associated alloantigens in insulin-dependent diabetes mellitus. J. Clin. Endocrinol. Metab. *48*: 403–407, 1979.

[12] Berson, S. A., and Yalow, R. S.: Quantitative aspects of the reaction between insulin and insulin-binding antibody. J. Clin. Invest. *38*: 1996–2016, 1959.

[13] Berson, S. A., and Yalow, R. S.: Species-specificity of human anti-beef, pork insulin serum. J. Clin. Invest. *38*: 2017–2025, 1959.

[14] Chance, R. E., Kroeff, E. P., and Hoffmann, J. A.: Chemical, physical and biological properties of recombinant human insulin. *In* FDA Conference on Insulin and Growth Hormone. Washington, D.C., NIH, 1980.

[15] Markussen, J., Damgaard, U., Pingel, M., Snel, L., Sørensen, A. R., and Sørensen, E.: Human monocomponent insulin: chemistry and characteristics. Am. J. Med. In press.

[16] Christiansen, Aa. H.: Radioimmunoelectrophoresis in the determination of insulin binding to IgG. Methodological studies. Horm. Metab. Res. *5*: 147–54, 1973.

[17] Van Rood, J. J., Leeuwen, A., and Ploem, J. S.: Simultaneous detection of two cell populations by two-colour fluorescence and application to the recognition of B-cell determinants. Nature *262*: 795, 1976.

[18] Heding, L. G.: Radioimmunological determination of human C-peptide in serum. Diabetologia *11*: 541–48, 1975.

[19] Berson, S. A., and Yalow, R. S.: Antigens in insulin determinants of specificity of porcine insulin in man. Science *139*: 844, 1963.

[20] Federlin, K., Laube, H., and Velcovsky, H. G.: Biologic and immunologic in vivo and in vitro studies with biosynthetic human insulin. Diabetes Care *4*: 170–74, 1981.

[21] Kumar, D.: Immunoreactivity of insulin antibodies in insulin-treated diabetics: significance of the beta-chain carboxy-terminal amino acid (B-30) of insulin. Diabetes *28*: 994–1000, 1979.

[22] Hirata, Y., Tominaga, M., Ito, J. I., and Noguchi, A.: Spontaneous hypoglycaemia with insulin autoimmunity in Graves' disease. Ann. Intern. Med. *81*: 214–18, 1974.

[23] Kurtz, A. B., Matthews, J. A., and Nabarro, J. D. N.: Insulin-binding antibody: reaction differences with bovine and porcine insulins. Diabetologia *15*: 19–22, 1978.

[24] Patterson, R., Mellies, C. J., and Roberts, M.: Immunologic reactions against insulin. II. IgE anti-insulin, insulin allergy and combined IgE and IgG immunologic insulin resistance. J. Immunol. *110*: 1135, 1973.

[25] Witters, L. A., Ohman, J. L., Weir, G. C., Raymond, L. W., and Lowell, F. C.: Insulin antibodies in the pathogenesis of insulin allergy and resistance. Am. J. Med. *63*: 703–709, 1977.

[26] Schernthaner, G., Ludwig, H., Jarisch, R., and Bruneder, H.: Immediate-type allergy against insulin itself: clinical and immunological studies on a diabetic patient with insulin intolerance. Diabetes Care *4*: 196–201, 1981.

[27] Falholt, K.: Determination of insulin specific IgE in serum of diabetic patients by solid-phase radioimmunoassay. Diabetologia *22*: 254–57, 1982.

[28] Patterson, R., O'Rourke, J., Robert, M., and Suszko, I.: Immunologic reactions against insulin. I. IgG anti-insulin and insulin resistance. J. Immunol. *110*: 1126–34, 1973.

[29] Flier, S., Kahn, C. R., Jarret, D. B., and Roth, J.: Characterization of antibodies to the insulin receptor: a cause of insulin-resistance diabetes in man. J. Clin. Invest. 58: 1442–49, 1976.

[30] Martin, W. P., Martin, H. E., Lyster, R. W., and Strouse, S.: Insulin resistance. J. Clin. Endocrinol. Metab. *1*: 387, 1941.

[31] Poulsen, J. E., and Deckert, T.: Insulin preparations and the clinical use of insulin. III. Side effects of insulin therapy. Acta Med. Scand. [Suppl.] *601*: 227–45, 1976.