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Autoantibodies to the insulin receptor in juvenile onset insulin-dependent diabetes

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Insulin-dependent diabetes mellitus (IDDM) usually begins in childhood or early adulthood, and its aetiology is thought to involve autoimmune damage to the islet cells that secrete insulin[1]. To investigate an additional target of autoimmunity in IDDM we examined sera for antibodies to insulin receptors. Such antibodies were defined by their ability to compete with insulin for binding to insulin receptors and by their capacity to behave like insulin in activating lipogenesis in adipocytes. We now report the occurrence of anti-insulin receptor antibodies of the IgM class in the sera of 10 of 22 IDDM patients obtained before their treatment with exogenous insulin. Furthermore, two of five IDDM patients who were initially negative developed anti-insulin receptor antibodies during treatment with human or pork insulin. These findings suggest that autoimmunity to the insulin receptor may contribute to the pathophysiology of IDDM.

The population of IDDM patients we examined comprised children who were referred consecutively to the Department of Endocrinology of the Sophia Children's Hospital. Sera were obtained from 22 patients before treatment and from 5 of them also after they began to receive insulin. Control sera were obtained from healthy volunteer blood donors at the Blood Bank of the Leiden University Hospital and from the investigators. We surveyed the sera by assaying 0.5–5 μl for insulin-like activity in stimulating lipogenesis in rat adipocytes measured by incorporation of radiolabelled glucose into lipid[2,3].

Table 1 shows that the insulin-like activity in a representative serum could be identified as anti-insulin receptor antibody of the IgM class by fractionating the serum and testing the various fractions for lipogenic activity. For example, 75% of the lipogenic activity found in the whole serum could be eluted from an anti-μ column that specifically binds IgM. The effluent which contained the non-IgM material had relatively little lipogenic activity. Filtration through Sephacryl 300 to separate IgM from IgG confirmed that the lipogenic activity was a property of the fraction containing IgM, while the fraction containing IgG was inactive. Dissociation of possible insulin–insulin antibody complexes by acidification and separation of serum fractions on Sephadex G-100 showed that the lipogenic activity remained intact and resided in the insulin-free, high-molecular weight fraction[2]. The low-molecular weight fraction did not contain sufficient insulin to activate lipogenesis. The lipogenic activity of the positive sera was not inhibited by adding antibodies to insulin, illustrating by another method that the lipogenic activity was not due to insulin itself. In contrast, goat antibodies to human IgM inhibited ~95% of the lipogenic activity in the positive sera. Lipogenic IgM did not bind to immobilized insulin (not shown). These results taken together indicate that the lipogenic activity could be assigned to IgM molecules and not to free insulin or insulin–insulin antibody complexes. The lipogenic IgM was identified as anti-receptor antibody as it specifically competed with radiolabelled insulin for binding to insulin receptors. Table 2 illustrates that increasing amounts of IgM purified on an anti-μ column increasingly inhibited the binding of insulin to the insulin receptors of adipocytes. Control IgM isolated from the serum of an IDDM patient without lipogenic activity had no effect on the binding of insulin to its receptor.

Table 1 Anti-insulin receptor antibodies in serum of IDDM patient are IgM

Treatment of serum	Serum fraction	% Relative lipogenesis of adipocytes
None	Whole	100
Anti-μ column	Effluent (not IgM)	15
	Eluate (IgM)	75
Sephacryl 300	Large (IgM)	100
	Small (IgG)	0
Dissociation of complexes, Sephadex G-100	Large (Ig)	90
	Small (insulin)	0
Antibodies to insulin*	Whole	100
Antibodies to IgM†	Whole	5

Sera were fractionated[2] using columns of goat antibodies to human μ-chain (Dako Immunoglobulins), bound to Sepharose (Pharmacia[11]), Sephacryl 300 (Pharmacia[12]) or Sephadex G-100 (Pharmacia). Antigen–antibody complexes were dissociated by acidification (0.01 M HCl, *p*H 2.7) of serum[2] before gel filtration. Control serum or fractions for each procedure were obtained from healthy donors and from an IDDM patient whose whole serum was negative for anti-receptor antibody activity. Lipogenic activity was computed relative to that found in 1 μl of the unfractionated serum (100%). Adipocytes were obtained from the epididymal fat pad of male Wistar rats (90–120 g) and the incorporation of D[U-^{14}C]glucose (4–7 mCi mol^{-1}; NEN) into lipid was measured as described previously[2,3]. Maximal lipogenesis (100%) was equivalent to that produced by incubation of the adipocytes with insulin (10 ng ml^{-1}) and was 300% of control lipogenesis obtained without added insulin.

* Guinea pig anti-insulin antiserum (Miles-Yeda; titre 10^{-5}) was added (2 μl) to the lipogenic assay.

† Goat antiserum to IgM (Miles-Yeda) was added (30 μl) to the lipogenic assay.

Normal human IgG stimulates lipogenesis in rat adipocytes *in vitro*[4]. Although the IgM anti-receptor antibodies described here also caused lipogenesis, the two lipogenic effects differ with regard to both ligand and receptor. The stimulatory effect of IgG is exerted through the non-variable Fc portion of the molecule[4,5] and normal IgG does not compete with insulin for binding to the insulin receptor (P. Dandona, personal communication).

Table 3 documents the presence of anti-insulin receptor antibodies in the sera of IDDM patients before and after treatment with exogenous insulin. Ten out of 22 patients had these antibodies in their sera at the time they first presented,

Table 2 Purified IgM receptor antibody competes with insulin for binding to insulin receptors on rat adipocytes

Affinity-purified IgM (μg)	% Inhibition of insulin binding to adipocytes	
	Origin of IgM	
	Test serum	Control serum
5	29	9
10	48	4
20	72	0

Affinity-purified IgM eluted from an anti-μ column (see Table 1) was added in the indicated amounts to rat adipocytes (10^5 cells) in plastic tissue culture tubes containing 0.35 ml KRB buffer (*p*H 7.4)–0.3% bovine serum albumin and ^{125}I-insulin (35,000 c.p.m.). The tubes were incubated at 25 °C for 40 min and the adipocytes were then separated from unbound insulin on a Millipore filter (EGWP, 0.2 μm), washed with ice-cold buffer and counted for radioactive content[13]. Extent of binding was 1.7 fmol per 10^5 adipocytes of which 70% was specific (displaced by 1 μM cold insulin). Per cent inhibition was computed relative to the binding obtained in the absence of added IgM.

before they had been treated with exogenous insulin. We have had the opportunity to examine serial bleedings obtained after treatment of five patients who had been negative at the outset. Two of these patients became positive within 4 months of receiving treatment with exogenous insulin, one having been given human and the other porcine insulin.

The investigation described here was prompted by the observation that mice developed anti-insulin receptor antibodies spontaneously after immunization to insulin[2]. These receptor antibodies were identified as anti-idiotypes to insulin antibodies, suggesting that they might have arisen as components of an idiotype–anti-idiotype network[6]. The anti-idiotypes probably functioned as receptor antibodies by mimicking the conformation of the antigen insulin[7]. We reasoned that humans might possibly develop similar anti-idiotypic insulin receptor antibodies in response to their own insulin antibodies produced by accidental immunization to exogenous insulin used for treatment. However, a large number of the pretreatment sera which we had believed would serve as negative controls were found to be positive for anti-receptor antibodies (Table 3). Therefore, we must conclude that autoimmunity to insulin receptors, rather than resulting merely from an iatrogenic accident, may be generated during the pathological processes intrinsic to IDDM. Moreover, the prevalence of these antibodies in an unselected group of patients suggests that their presence is neither sporadic nor infrequent (Table 2). We have no evidence to indicate whether or not the anti-insulin receptor antibodies in the IDDM patients are anti-idiotypes to insulin antibodies.

Table 3 Anti-insulin receptor antibodies in sera of IDDM patients before and during treatment with exogenous insulin

Serum donors	Anti-insulin receptor antibodies
Normal controls	0/20
IDDM patients	
Before treatment	10/22
After treatment	2/5

Anti-receptor antibodies in each serum were identified by two or more of the assays described in Table 1. Sera of 22 patients were obtained before they were treated with injections of insulin. Sera were obtained serially from five patients treated with injections of insulin after being negative for receptor antibodies before treatment. Two patients became positive for anti-receptor antibodies during 4 months of observation.

Insulin receptor antibodies have been found in a few dozens of patients with acanthosis nigricans and severe diabetes[8]. It seems, however, that IDDM and the acanthosis nigricans diabetic syndrome are diverse entities with distinct anti-receptor antibodies. Unlike IDDM, the anti-receptor antibodies in the acanthosis nigricans patients are mostly IgG rather than IgM[8], the disease is extremely rare, the resistance to treatment with insulin is marked and the patients seem to have a primary structural defect of their insulin receptors[9].

Patients with IDDM, with or without IgM receptor antibodies, do not have the degree of insulin resistance characteristic of the acanthosis nigricans syndrome. The disparate clinical entities associated with these anti-receptor antibodies may be attributed to the diverse biological effects of IgG and IgM receptor antibodies, to differences in the fine specificities of the receptor antibodies, to the intrinsic state of the insulin receptors and/or to the presence or absence of additional pathological processes.

Regardless of the mechanism of insulin receptor antibody generation, their presence indicates that autoimmunity in IDDM is not limited to islet cells[1]. The clinical consequences and theoretical implications of these anti-receptor antibodies may be important. What is their role, alone or together with viral infection and islet cell antibodies, in the pathogenesis of IDDM? Do they influence the response to treatment or the development of late complications? Can they be used to identify degrees of risk or immune-response genes? Are they a factor in the subclinical insulin resistance that is a prominent feature of IDDM[10]?

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