Insulin and Insulin-Receptor Autoantibodies in Children With Newly Diagnosed IDDM Before Insulin Therapy

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SUMMARY

Twenty-nine children, aged 1–15 yr, with newly diagnosed insulin-dependent diabetes mellitus (IDDM) had sera taken before insulin therapy to be examined for the presence of insulin-receptor antibodies by measuring the inhibition of binding of radiolabeled insulin to IM-9 lymphocytes in both whole serum and purified IgG fractions. Groups of children with long-standing IDDM and autoimmune endocrine disease as well as a normal control group were studied. A positive result, defined as binding ≥2 SD below the mean zero standard, was found in 3 (10.3%) of the 29 newly diagnosed diabetic patients. As a group, they showed significantly greater binding inhibition than the normal control group for both whole serum and purified IgG (one-tailed *t* test, $P < .05$ and $P < .002$, respectively). Insulin autoantibodies were also measured by a sensitive radioimmunoassay technique. A positive result, defined as binding >3 SD above the normal control pooled sera, was found in 9 (37.5%) of 24 of the newly diagnosed IDDM group tested. All 3 subjects positive for insulin-receptor antibodies were also positive for insulin autoantibodies, whereas 6 of the 21 receptor-antibody–negative subjects were positive for insulin autoantibodies (Fisher's exact test, $P = .0415$). This suggests the possibility that the presence of insulin autoantibodies is a prerequisite for the development of insulin-receptor antibodies, i.e., as an anti-idiotypic response. Insulin-receptor antibodies and insulin autoantibodies may play a currently undefined pathophysiologic role in the development of IDDM. Conversely, these may represent epiphenomena of the disease process itself or may indicate a predilection for development of the disease. Taken together with other markers associated with IDDM, e.g., HLA haplotype and islet cell antibodies, it may prove possible in the future to define a select population at risk for the development of the disease and/or to predict the clinical course in individual cases. *Diabetes* 36:420–25, 1987

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Insulin-dependent diabetes mellitus (IDDM) is believed to be primarily an autoimmune disease (1). Recent interest has centered on describing various immunological markers associated with this disorder. Islet cell antibodies and HLA-DR3 and -DR4 haplotypes have been proposed as specific serum markers. Although the occurrence of both is significant in the prediction of either presence or risk of disease, the search for more, perhaps better, markers continues (2–4).

Insulin-receptor autoantibodies inhibit binding of insulin to its receptor and are known to cause clinical syndromes characterized by severe insulin resistance (5–8). Insulin-receptor antibodies that are receptor agonists have also been described and can result in hypoglycemia (9,10). Successful immunosuppressive therapy emphasizes the underlying immunological disturbance in these syndromes (11).

We have directed our efforts toward the insulin-receptor antibody to further define its role in the immunological abnormalities of IDDM and to see whether it might serve as another serum marker for this disease. To pursue this avenue, we examined the unfractionated sera and the purified IgG fractions from patients with IDDM before insulin treatment for the presence of insulin-receptor antibodies by means of the IM-9 lymphocyte assay. We also examined the same population for the presence of insulin autoantibodies.

MATERIALS AND METHODS

Patient population. Twenty-nine Caucasian children, aged 1–15 yr, newly diagnosed with IDDM were included in this study. Sera were also obtained from children with long-standing IDDM treated with heterologous insulin and autoimmune thyroid disease and from normal children aged 5–12 yr who were undergoing investigation for problems unrelated

to diabetes and who had no family history of either diabetes or of any autoimmune disease.

Sera from patients with known insulin resistance due to insulin-receptor antibodies were kindly donated by Dr. Barry Posner (McGill Univ., Montreal, Canada) and by Dr. Simeon Taylor (NIH, Bethesda, MD) to serve as positive controls.

Cell culture. Human lymphoblastoid cells (IM-9) (12,13) were maintained in continuous culture in RPMI-1640 medium supplemented with 25 mM HEPES, 0.2% $NaHCO_3$, 10% fetal calf serum, penicillin, and streptomycin at 37°C in 5% CO_2. Cells were split 3 times weekly, and fresh medium was added. Viability of the cells was monitored by erythrosin B dye exclusion and assessed before each assay to be >90%.

Insulin-receptor–antibody assay. The binding inhibition assay for antireceptor activity was based on the method of Flier et al. (6). Briefly, IM-9 cells harvested in the late log growth phase were preincubated at a concentration of 10^7 cells/ml with 0.1 M HEPES buffer with 1% BSA (0.2 ml) and an equal volume of either serum or purified IgG, in duplicate, at 4°C for 60 min. After washing and a final incubation with ^{125}I-labeled insulin (sp act 200 μCi/μg; New England Nuclear, Boston, MA) at 15°C for 90 min, the cells were spun, and the radioactivity of the cell pellet was determined. Maximum specific binding was 19.7 ± 6.0% (mean ± SD). Overall, intra-assay coefficient of variation (C.V.) was 6.1%, and interassay C.V. was 13.0%. A positive result was defined as a binding inhibition value ≥2 SD (derived from the overall C.V. of 6.1%) below the mean zero standard (defined as 100%) for the particular assay. Therefore, binding ≤87.7% was considered to be positive. No difference was found between buffer and serum standards after repeated testing.

IgG fractionation. IgG was fractionated from serum with protein A–Sepharose (Pharmacia, Uppsala, Sweden) chromatography according to the method of Ey et al. (14) as modified for human IgG by Duhamel et al. (15). In the final step the IgG fraction was brought down to volume by ultrafiltration to approximate the original serum concentration, taking into consideration an overall recovery of 70% of the IgG present in the original serum sample as gauged by nephelometry. Thus, the IgG fraction was purified but not concentrated. We were unable to test paired serum and IgG fractions from all patients because of the small quantities of

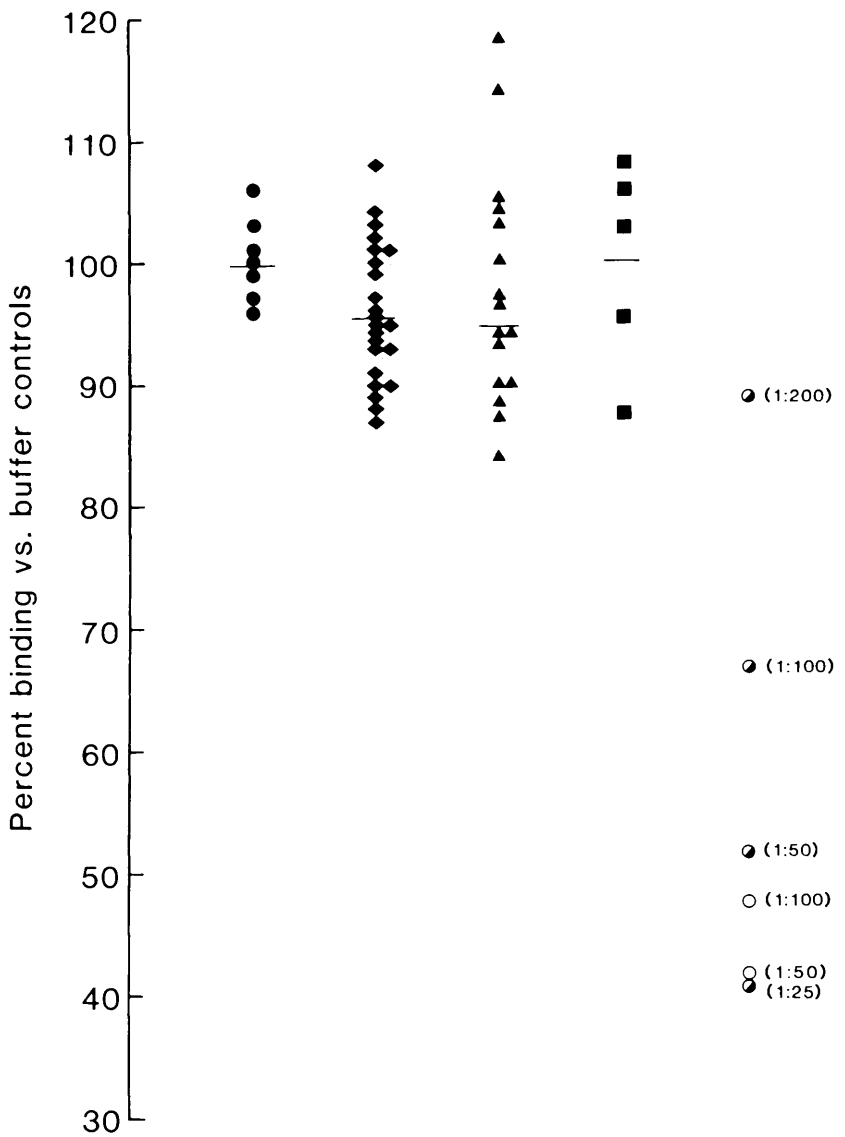

FIG. 1. Percentage binding of radiolabeled insulin to IM-9 lymphocytes, expressed relative to 100% binding in buffer controls, in whole-serum samples from patients with newly diagnosed IDDM (♦), long-standing IDDM (▲), autoimmune endocrine diseases (■), normal controls (●), and positive controls (○, ◕).

serum available from most of the children. Thirteen of 29 samples from subjects with newly diagnosed IDDM were purified for the IgG fraction. Six of these 13 were tested by use of IgG alone. In seven samples, where sufficient serum was available, each serum or purified IgG specimen was tested at least twice in different assays. The results from repeated testing were in agreement. Purified IgG fractions were similarly obtained from the other patient groups.

Insulin-autoantibody assay. Serum samples were tested for their ability to bind insulin by the method of Palmer et al. (16) with radiolabeled porcine insulin (New England Nuclear) at 50 pg/tube as ligand. The mean binding of labeled insulin by 20 μl of serum from the nondiabetic normal control pooled serum was 1.4 ± 0.4% (mean ± SD). A positive result was defined as a value >3 SD above the mean binding of the normal pool (17), i.e., >2.6%. Sufficient serum was available to test 24 of the 29 newly diagnosed diabetic patients.

RESULTS

Binding-inhibition studies. Two (8.7%) of 23 subjects with newly diagnosed IDDM tested before insulin therapy were positive with whole serum. Results with purified IgG fractions derived from seven of these serum specimens were concordant. One confirmed a positive serum result and six confirmed negative results. In addition, one of six specimens not previously examined with whole serum was also positive. Thus, overall, 3 (10.3%) of 29 subjects were positive for insulin-receptor antibodies. For comparison, 5 (20.8%) of 24 of the individuals with long-standing IDDM were positive. The relative frequency of insulin-receptor antibodies in the two diabetic groups was not significantly different ($\chi^2 = 0.530$, $P = .47$).

Figure 1 shows results with whole serum for the four population groups and the positive control group. Also shown is the dose-related binding inhibition of the two positive controls. Mean binding was 99.7% for the normal control group, 95.4% for the newly diagnosed IDDM group, 94.7% for the long-standing IDDM group, and 100.0% for the autoimmune endocrine disease group. There was no significant difference between the means of the two diabetic groups and between the normal control and the autoimmune disease groups. In contrast, comparison of the newly diagnosed

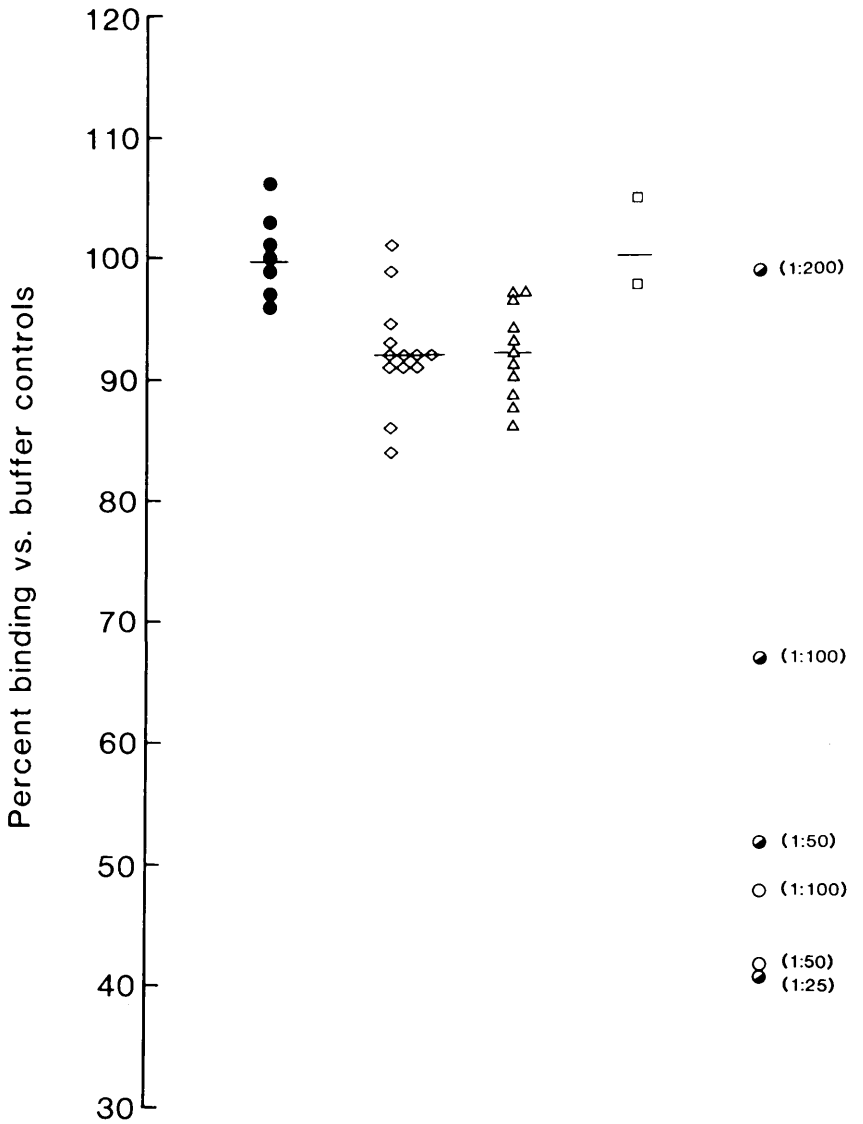

FIG. 2. Percentage binding of radiolabeled insulin to IM-9 lymphocytes, expressed relative to 100% binding in buffer controls, in purified, unconcentrated IgG fractions from patients with newly diagnosed IDDM (◇), long-standing IDDM (△), autoimmune endocrine diseases (□), normal controls (●), and positive controls (○, ◕).

IDDM group with the normal control group by one-tailed *t* test analysis indicated a significant difference ($t = 1.86$, $P < .05$).

Figure 2 shows results with purified IgG fractions. Mean binding values were 92.2% for the newly diagnosed IDDM group, 92.1% for the long-standing IDDM group, and 101.5% for the autoimmune endocrine disease group. One-tailed *t* test analysis showed a highly significant difference between the newly diagnosed IDDM group and the normal control group ($t = 3.493$, $P < .002$).

Insulin-autoantibody studies. Figure 3 shows the overall results of these studies. Nine (37.5%) of 24 patients with newly diagnosed IDDM were positive for insulin autoantibodies. Mean binding for this autoantibody-positive group was 3.5% (range 2.7–5.2%). For comparison, most of the long-standing diabetic group (23 of 25, or 92%) possessed antibodies. Mean binding for this autoantibody-positive group was 17.2%.

A correlation between the presence or absence of insulin-receptor antibodies with insulin autoantibodies in the newly diagnosed IDDM group revealed that all three subjects positive for insulin-receptor antibodies were also positive for insulin autoantibodies, whereas of the 21 receptor-negative subjects, 6 were positive for insulin autoantibodies (Fisher's exact test, $P = .0415$). However, there was no significant correlation between binding inhibition and percent ^{125}I-insulin binding ($r = .071$, $P = .683$). A trend, although not statistically significant, toward a negative correlation between age and level of insulin autoantibody ($r = -.28$, $P > .4$, $n = 24$) was also observed in this group. This result along with the overall prevalence of insulin autoantibodies in 37.5% of the newly diagnosed IDDM group is comparable with other reports (17,18).

DISCUSSION

According to the immunological surveillance network theory proposed by Jerne (19), insulin autoantibodies could occur spontaneously as natural idiotypes. An idiotype is defined as a collection of antigenic determinants on the variable region of the antibody molecule (19,20). In Jerne's proposal,

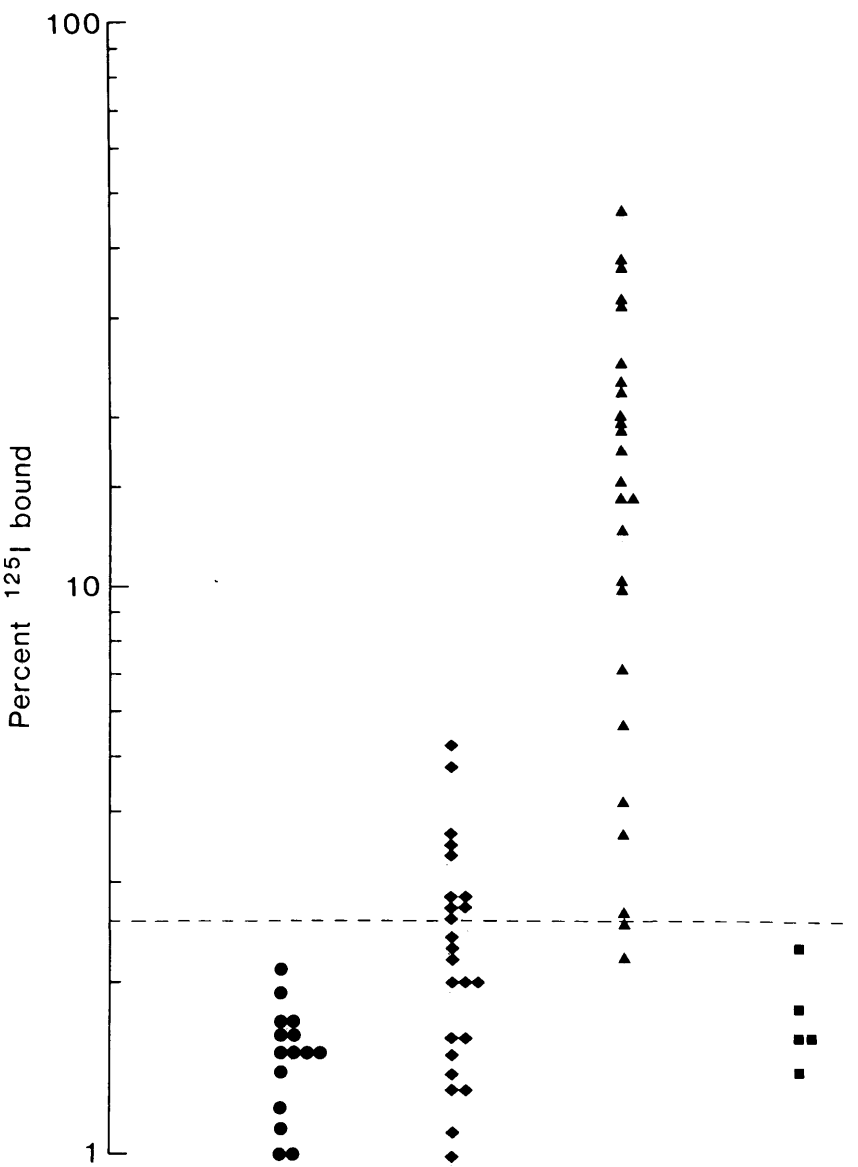

FIG. 3. ^{125}I-labeled insulin binding to serum from patients with newly diagnosed IDDM (♦), long-standing IDDM (▲), autoimmune endocrine diseases (■), and normal controls (●). *Dotted line* represents 3 SD above mean of pooled normal control sera. Any value above this line, e.g., 2.6%, is considered positive for presence of insulin autoantibodies.

antibodies to the idiotypes (anti-idiotypes) should also arise spontaneously and affect immunoregulation through a feedback system on the original antibody. Shechter et al. (20) demonstrated with a mouse model that insulin-receptor antibodies of the IgG class develop spontaneously in animals immunized with insulin. The subsequent finding of Maron et al. (21) of insulin-receptor antibodies (of the IgM class) in a group of newly diagnosed IDDM patients suggested a role for these antibodies in a disturbance of normal immunoregulation and, perhaps, an additional role in the development of IDDM.

We demonstrate the presence of insulin-receptor antibodies in newly diagnosed diabetic subjects with whole sera or purified, unconcentrated IgG fractions. Although only 10.3% of the individuals were positive by strict criteria, when considered as a group they were statistically different from the normal group. This difference not only confirms the presence of insulin-receptor antibodies in this group but also suggests the presence of low-titer antibodies in a higher proportion of these patients, as previously suggested (21). Further delineation of a more accurate prevalence might be done by study of purified IgG and/or IgM concentrations in larger groups of patients.

Our study and the recent observations in a case of anti-receptor antibodies in a long-standing diabetic patient (22) do not prove the anti-idiotypic nature of these receptor antibodies. Proof requires the demonstration that purified receptor antibodies are capable of recognizing the insulin–anti-insulin antibody complex. However, our finding that all three receptor-antibody–positive subjects were also insulin-autoantibody positive, whereas only 6 of 21 receptor-antibody–negative subjects were positive for the other, may bear on this question. Indeed, this finding suggests that the acquisition of insulin-receptor antibodies may require the antecedent presence of insulin antibodies, in keeping with Jerne's hypothesis (19). Certainly, the development of insulin autoantibodies does not guarantee the subsequent development of anti-idiotypic receptor antibodies. In fact, the lack of correlation between binding inhibition in the receptor assay and insulin-antibody binding suggests that insulin-autoantibody titer alone is not a decisive factor for receptor-antibody development.

If these anti-receptor antibodies are indeed anti-idiotypic, the similarity in mean receptor binding, both in serum and IgG, between the long-standing and the newly diagnosed diabetic groups could be interpreted as either persistence of the pretreatment immunologic state, maintenance of the pretreatment state by exogenous insulin, or a restimulation of antibody formation by insulin treatment after an initial decline. Longitudinal studies of insulin-receptor antibody status in the newly diagnosed IDDM group might help differentiate among these possibilities.

The ultimate importance of insulin-receptor antibodies in newly diagnosed IDDM is still undetermined. There is precedent in both endocrine and nonendocrine autoimmune diseases for the development of receptor antibodies and deranged receptor function. Myasthenia gravis (23,24), pernicious anemia (25), and Graves' disease (26) are such examples. A similar disorder of immunoregulation may be operative in IDDM, as reflected by our findings. On the other hand, the abnormalities described may be secondary to the disease process and may just be markers of the condition. There has been a wide variety of immunologic markers associated with IDDM (27,28), and our findings add to this collection. As suggested by Srikanta et al. (17) and Soeldner et al. (27), perhaps high-risk individuals could be defined by the array of autoimmune markers present. We believe it is within this context that insulin-receptor antibodies may prove to be clinically useful.

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