Relationship of Insulin Autoantibodies to Presentation and Early Course of IDDM in Children

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Insulin antibodies have been documented before (insulin autoantibodies [IAAs]) and after (insulin antibodies) insulin administration in children with new-onset insulin-dependent diabetes mellitus (IDDM). Whereas the relationship of IAA to various factors at presentation has been studied in some detail, little is known about their relationship to events during the 1st yr after diagnosis. Furthermore, the course and factors affecting insulin-antibody response to human insulin administration in children with newly diagnosed IDDM remain poorly defined. To study these questions, we measured serum glucose, pH, bicarbonate, C-peptide, and IAA at diagnosis in 84 children between 0.5 and 18 yr of age. Basal and peak C-peptide responses to Sustacal ingestion, glycosylated hemoglobin (HbA_{1c}), and IAA were then measured in 33 of these patients at 10 days and 1, 3, 6, and 12 mo after diagnosis. At presentation, IAAs were absent (binding below the mean + 3SD of the binding of control serums) in 51 patients (61%) and present (binding above the mean + 3SD) in 33 patients (39%). Multiple regression analysis showed a significant nonlinear relationship between IAAs and both age ($P < .005$) and blood glucose ($P < .05$) at onset. There was a stepwise increase in median insulin-antibody binding throughout the 1st yr. This increase was most marked during the 1st mo of insulin therapy and showed a statistically significant difference between successive measurements only during this period. Analysis of variance showed no effect of the factors measured at diagnosis (age, blood glucose, C-peptide, pH, bicarbonate, IAA) or those measured during the 1st yr (blood glucose, insulin dose, C-peptide, HbA_{1c}) on the antibody response to insulin. Furthermore, IAA did not correlate with either insulin antibodies or C-peptide at any time during the 1st yr. Because IAAs do not appear to be related to residual insulin secretion and do not predict the insulin-antibody response to human insulin administration, the data failed to define a role for these antibodies in the pathogenesis of this disorder. This suggests that IAAs may be markers of the immune confrontation with the β-cell rather than active participants in its destruction. Furthermore, insulin-antibody response after human insulin administration appears to have no effect on residual insulin secretion, insulin requirement, or metabolic control during the 1st yr after diagnosis. ***Diabetes Care*** **12:517–23, 1989**

Several groups have recently found insulin autoantibodies (IAAs) in 30–40% of children with newly diagnosed insulin-dependent diabetes mellitus (IDDM) before therapy with insulin was begun (1–3). The presence of these antibodies provides further circumstantial evidence for the incrimination of the immune system in the pathogenesis of IDDM. Their absence in ~60% of patients at diagnosis supports the concept of etiologic heterogeneity (4). Many studies have examined the relationship between these antibodies and various clinical, immunological, and biochemical features at presentation. Whereas most studies have found a negative correlation with age at onset of diabetes and IAAs (2,5), dispute about their relationship to islet cell antibodies (ICAs) remains (6,7). Furthermore, Eisenbarth (4) and Atkinson et al. (8) have suggested that IAAs may have a predictive role in the high-risk nondiabetic population.

Use of human insulin from diagnosis has been shown

Glucose 1 mM = 18 mg/dl

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to result in lower titers and a decreased frequency of insulin antibodies compared with the use of insulin from animal sources (9). The implication of these observations with regard to residual insulin secretion, metabolic control, and insulin dose requirement remains undetermined. Also, factors that may influence or predict the antibody response to human insulin, e.g., HLA type or IAA, need to be more clearly delineated.

As part of an ongoing study of residual insulin secretion after diagnosis of IDDM, we have collected data on IAA, insulin-antibody response to human insulin, HbA_{1c}, and insulin dose in a cohort of children with IDDM. In this study, *IAA* refers to insulin antibodies present before the initiation of insulin therapy in children with newly diagnosed IDDM. *Insulin antibodies* refers to antibodies found after the initiation of insulin therapy. These data have allowed us to examine the following issues relating to insulin antibodies both before and after therapy with human insulin: *1*) the relationship of IAA to clinical and biochemical factors at diagnosis of IDDM, i.e., does the presence of IAA help predict any specific characteristics at IDDM onset? *2*) insulin-antibody response to the administration of human insulin; *3*) relationship of IAA to the insulin-antibody response to insulin therapy during the 1st yr after diagnosis; and *4*) influence of insulin antibodies on residual insulin secretion, metabolic control, and insulin requirement.

RESEARCH DESIGN AND METHODS

At diagnosis of IDDM and before starting insulin therapy, blood was obtained for measurement of serum glucose, pH, bicarbonate, C-peptide, and IAA from 84 children between 0.5 and 18 yr of age. Basal and peak C-peptide responses to Sustacal ingestion (Mead Johnson, Belleville, Ontario, Canada), HbA_{1c}, and insulin antibodies were then measured in 33 patients at 10 days and 1, 3, 6, and 12 mo after diagnosis (10). The protocol was approved by the Human Experimentation Committee and consent obtained from all participants or their parents.

After hospital discharge, our patients were followed by a multidisciplinary team on an outpatient basis approximately monthly for the first 3 mo and then every 3 mo. Either biosynthetic (Humulin N and Humulin R, Lilly, Indianapolis, IN) or semisynthetic (Novolin NPH/lente and Novolin regular, Connaught Novo, Toronto) human insulin was used for all patients from the time of diagnosis. In general, our patients were given a single injection of intermediate- and short-acting insulin in the morning. Insulin adjustments and the introduction of an evening injection were determined by blood glucose results obtained initially in the hospital and then at home with the aid of self-monitoring of blood glucose.

Serum glucose was measured by a glucose oxidase method (Ektachem). HbA_{1c} was measured by a highly specific high-performance liquid chromatography method, which incorporated an acetate buffer incubation step for removal of the labile fraction of HbA_{1c} (12). C-peptide was measured by radioimmunoassay with antiserum M 1230 and ^{125}I-labeled C-peptide obtained from Novo (Copenhagen) (13). The lower limit of detectability of this assay is 0.025 pM. Intra- and interassay coefficients of variation (C.V.) were 3.5 and 9.8%, respectively (10).

IAAs and insulin antibodies were measured as the percent binding of radiolabeled insulin after removal of bound insulin by acid-charcoal extraction (1,5,14). The method depends on the percentage of radiolabeled insulin bound to precipitated proteins at a fixed plasma dilution in the absence of added antibody or nonradiolabeled insulin. Results are thus expressed as percent binding. To perform the assay, an aliquot of the acid-charcoal–extracted plasma to be tested was incubated at 4°C for 24 h with ^{125}I-labeled pork insulin of high specific activity (~20,000 cpm/tube) and 0.04 M phosphate buffer (pH 7.5). Bound tracer was precipitated by the addition of 15% polyethylene glycol (PEG) with 1% Tween 20 and centrifuged at 2500 rpm at 4°C for 30 min. The pellet was washed once with 12.5% PEG, centrifuged again, and counted. A low level of nonspecific binding was present when buffer was mixed with ^{125}I; this was subtracted from that found in both healthy control and diabetic plasma. The intra- and interassay C.V.s for this technique are <8%, and the results are unaffected by the glucose concentration in the specimen (1,5,14). IAAs were considered positive if binding exceeded the mean + 3SD of the binding of control serum. Control subjects were 50 children, aged 6–18 yr, without evidence of IDDM or other autoimmune disease. IAA levels in the subjects ranged from 0 to 0.70% binding.

Statistical analysis. A normal distribution was obtained after logarithmic transformation ($\log_e$) of data with a nonnormal distribution (IAA, insulin antibody, C-peptide). These transformed data were used in all statistical calculations. Multiple regression analysis was used to evaluate the effect of the measured biochemical, immunological, and clinical factors on IAA at diagnosis. Differences between groups were determined with Student's *t* test for unpaired means. A repeated-measures analysis of covariance was used to assess the significance of insulin-antibody changes over time. Comparisons between periods were made by means of *t* tests with the significance level corrected (Bonferroni) for multiplicity. The factors affecting insulin antibodies during the 1st yr were evaluated by multiple regression analysis with a repeated-measures design. Normally distributed data are expressed as means ± SD and nonnormally distributed data as median and interquartile range (IQR). The IQR is the distance between upper and lower quartiles when results are expressed with a box plot (i.e., range that encompasses the middle 50% of observations).

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TABLE 1
Clinical and biochemical characteristics of 84 children with insulin-dependent diabetes mellitus at presentation

	Mean ± SD	Range
Age at onset (yr)	9.2 ± 4.1	0.5–18.0
Duration of symptoms (wk)	5.3 ± 8.7	1–52
Blood glucose (mM)	28.3 ± 11.8	14.7–77
pH	7.30 ± 0.11	6.97–7.45
Serum bicarbonate (mM)	19.9 ± 7.6	2.2–30
C-peptide (pM)	0.05 ± 0.08	0.01–0.56
Insulin autoantibodies (%)	1.14 ± 1.40	0–12.5

RESULTS

The clinical and biochemical characteristics of the 84 patients evaluated at diagnosis are presented in Table 1. The patients ranged in age from 0.5 to 18 yr (means ± SD 9.2 ± 4.1 yr); there were 47 males. Biochemical features at presentation varied widely: plasma glucose, 14.7–77 mM (mean 28.3 ± 11.8 mM); serum HCO_3, 2.2–30 mM (19.9 ± 7.6); serum pH, 6.97–7.45 (7.33 ± 0.11); and C-peptide <0.025–0.56 pM (median 0.03, IQR 0.05).

The mean IAA level was significantly higher in patients than in controls (1.14 ± 1.4 vs. 0.25 ± 0.2%; $P < .05$). IAAs were absent (binding below the mean + 3SD of the binding of control serums) in 51 patients (61%) and present (binding above the mean + 3SD) in 33 patients (39%). Sixteen (19%) IAA^+ patients had binding above the mean + 3SD but below the mean + 5SD, and 17 (20%) had binding above the mean + 5SD of the control serums.

There were no significant differences between IAA^+ and IAA^- patients with respect to any of the variables tested at diagnosis. However, multiple regression analysis with log IAA as the dependent variable and age, blood glucose, C-peptide, bicarbonate, and pH at diagnosis as the independent variables showed a significant nonlinear relationship with both age ($P < .005$) and blood glucose ($P < .05$). When allowance was made for the effect of blood glucose, the nonlinear relationship between IAA and age could be resolved into two separate linear regression equations (i.e., a negative equation up to the age of 14 yr and a positive equation after this age; Fig. 1). The relationship between IAA and

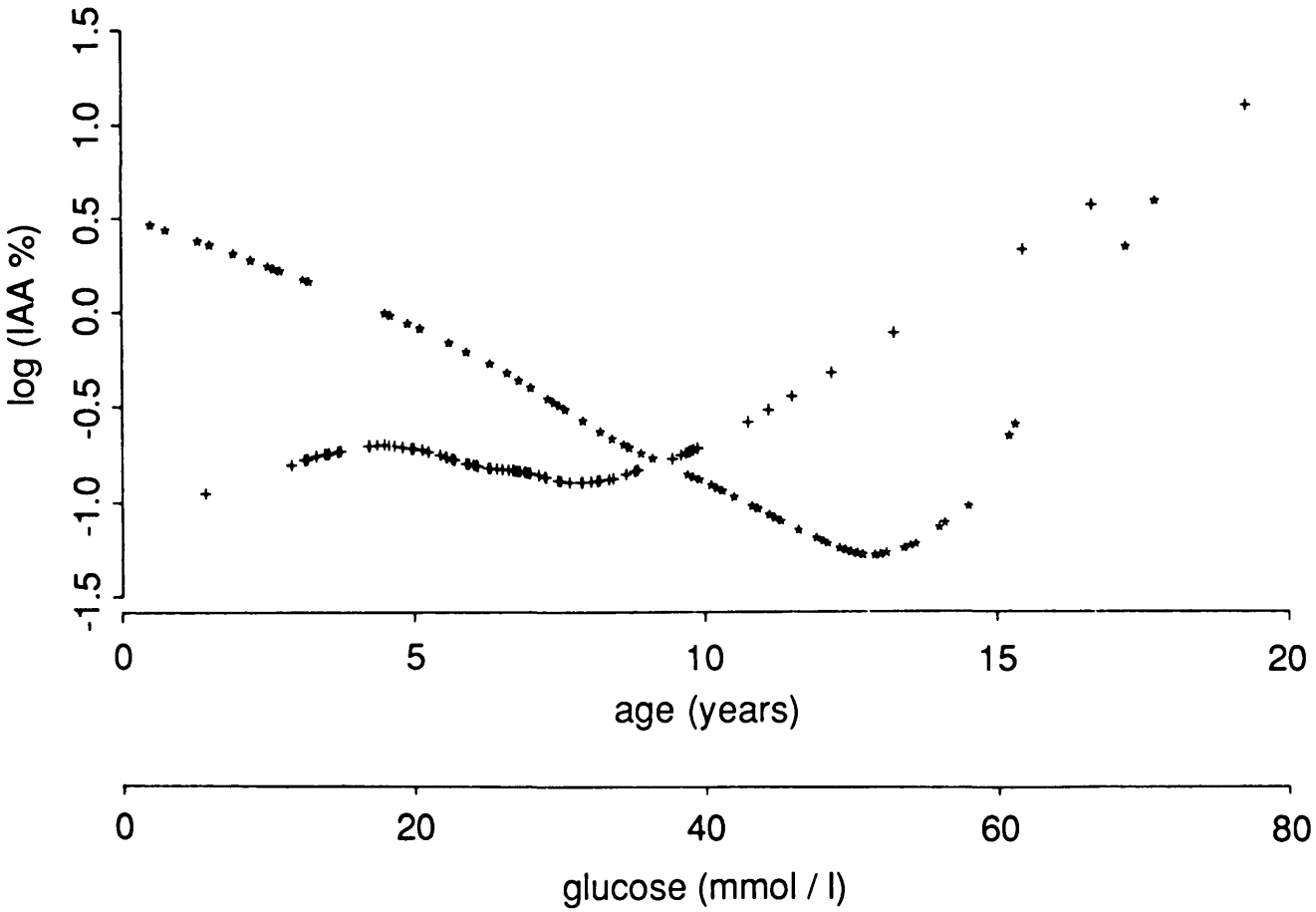

FIG. 1. Relationship between insulin autoantibodies (IAAs) and age (⋆) and blood glucose (+) concentration at insulin-dependent diabetes mellitus onset in 84 children. IAA%, percent binding of radiolabeled ^{125}I.

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blood glucose (allowance having been made for age) could be best described as quadratic. There was no difference in IAA between male and female patients.

Insulin-antibody response to human insulin. Figure 2 depicts the course of insulin antibodies, residual insulin secretion (basal and peak C-peptide response to Sustacal ingestion), and HbA_{1c} for the 33 children with newly diagnosed IDDM who were tested repeatedly during the 1st yr of their illness.

There was a stepwise increase in the median insulin-antibody binding (median, IQR: 10 days, 1.93, 1.3%; 1 mo, 8.01, 5.5%; 3 mo, 10.55, 12.9%; 6 mo, 10.55, 12.9%; 12 mo, 14.3, 19.2%) throughout the 1st yr. This increase was most marked during the 1st mo of insulin therapy and showed a statistically significant difference between successive measurements only during this period ($P < .05$). Ten days after diagnosis, both basal and peak C-peptide concentrations (median and IQR for basal and peak, respectively, 0.11, 0.12, and 0.25, 0.16 pmol/ml) were significantly higher than the C-peptide concentrations at diagnosis (0.03 and 0.045 pmol/ml, $P < .05$). Basal and peak C-peptide concentrations reached a maximum at 1–3 mo (median, IQR: 1 mo, basal 0.23, 0.16 pmol/ml, peak 0.48, 0.40 pmol/ml; 3 mo, basal 0.18, 0.18 pmol/ml, peak 0.43, 0.44 pmol/ml) and then declined to 0.08, 0.15, and 0.16, 0.36 pmol/ml, respectively, at 1 yr. Mean HbA_{1c} level decreased significantly between 10 days and 1 mo (11.4 ± 1.2 to 7.9 ± 1.2%, $P < .01$). The nadir occurred at 3 mo, corresponding to the highest basal and peak C-peptide concentrations. Between 3 and 12 mo there was a gradual, although nonsignificant, increase in HbA_{1c}. Repeated-measures analysis of covariance showed no effect of the factors outlined at diagnosis (age, blood glucose, C-peptide, pH, bicarbonate, IAA) or those measured during the 1st yr (blood glucose, insulin dose, C-peptide, and HbA_{1c}) on the antibody response to insulin during this period. Note, IAA levels did not correlate with insulin antibodies, C-peptide, or HbA_{1c} at any time during the 1st yr.

Furthermore, individuals who tested positive for IAA at diagnosis did not differ from subjects who tested negative with respect to any of the parameters followed during the 1st yr. Specifically, there was no relationship

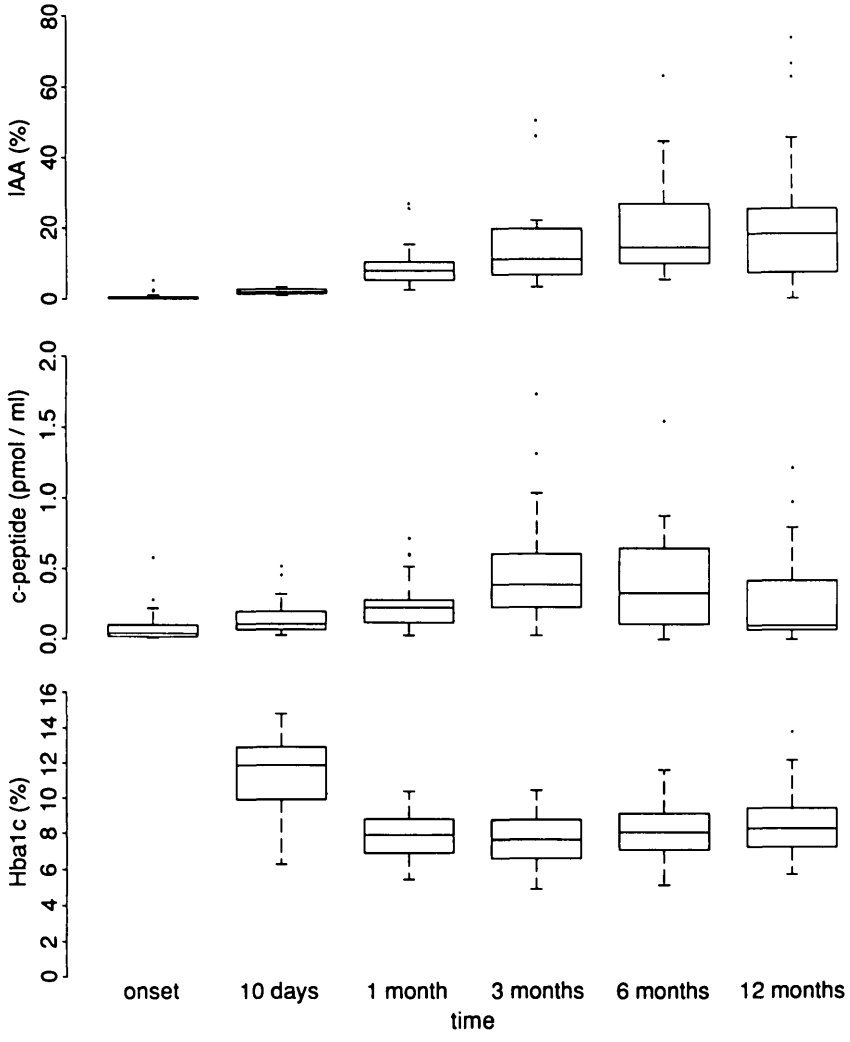

FIG. 2. Insulin-antibody binding (*top*), peak C-peptide concentrations (*middle*), and HbA_{1c} levels (*bottom*) in 33 children with insulin-dependent diabetes mellitus followed prospectively. Results are expressed with box plots. *Horizontal line* within each *box* locates median, whereas *upper* and *lower ends* of box mark 75th and 25th percentiles, respectively. Interquartile range is defined by height of box. *Bars* indicate upper and lower adjacent values. Insulin autoantibody refers to percent binding of radiolabeled ^{125}I. From 10 days onward, this reflects insulin-antibody binding in response to exogenous insulin administration.

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found between either IAA at diagnosis or insulin-antibody binding thereafter and the concentrations of C-peptide during the 1st yr of IDDM.

DISCUSSION

The finding of IAA in a significant proportion of children with newly diagnosed IDDM before exposure to exogenous insulin is consistent with earlier studies (1–3). These studies note that higher IAA titers exist in younger patients and have ascribed this to a more vigorous immune response in the young. When the effect of blood glucose was taken into account, our data was found to be similar but only to the age of 14 yr, after which a positive correlation with age was noted. Whereas the number of patients >14 yr old is admittedly small, the latter observation suggests that the immune response to endogenous insulin may be determined by age-associated factors rather than by the effect of age itself.

Note that blood glucose levels >40 mM at presentation were positively correlated with IAA. An explanation for this is not available. Glucose concentration is not known to interfere with the insulin-antibody assay used in this study, making a technical factor seem unlikely. Therefore, at higher titers, insulin antibodies may have a direct effect on blood glucose concentration (i.e., through the binding of free insulin). An alternative explanation that high antibody titers reflect severity of β-cell destruction is not supported because we were unable to find a correlation between IAA and residual insulin secretion. It is also highly unlikely that the degree of dehydration present in those with severe hyperglycemia (>40 nM) is sufficient to account for this relationship between IAA and blood glucose concentrations.

Many questions posed by Palmer et al. (1) in their original description of these antibodies have been only partially answered; specifically, the relationship of IAA to residual β-cell function and the antibody response to human insulin therapy.

We and Arslanian et al. (5) have been unable to find a correlation between IAA and C-peptide concentration at diagnosis. In most of our patients, C-peptide levels were very low at diagnosis and then increased within 10–14 days of starting insulin therapy. We have suggested that the rapid improvement in C-peptide secretion may be due to reversal of a functional component and, furthermore, that the C-peptide level at 10–14 days probably more accurately reflects residual β-cell reserve at presentation than the concentration at diagnosis (15). We did not, however, find a correlation between IAA and C-peptide concentration at this time. Furthermore, IAA did not correlate with C-peptide at any time during the 1st yr. The absence of a relationship between IAA and residual insulin secretion either at diagnosis or during the 1st yr is evidence against a causal link between IAA and β-cell destruction. Whereas the data do not preclude a role for these antibodies in the pathogenesis of IDDM, they do suggest such a role would be minor.

Previous studies, such as that by Schernthaner et al. (9) in which IgG insulin antibodies were compared at 6 mo in patients treated from diagnosis with human (Novo) or monocomponent pork insulin, found that 14% of the human-insulin–treated group had these antibodies. Iavicole et al. (17) showed significantly lower insulin antibodies 12 mo after diagnosis in the group treated with human insulin compared with groups treated with monocomponent pork and conventional insulins, respectively. The insulin-antibody response to human insulin in both these studies was significantly less than the response to either monocomponent pork insulin (intermediate response) or beef/pork insulin (highest response). During the 1st yr, the insulin-antibody response in our patients can best be described as quadratic (i.e., a sharp increase over the 1st mo), with no further statistical change during the remainder of the year. The apparent discrepancy between our and previous observations is explained by the differences in sensitivities of the assays used (insulin-antibody binding 1–3 μU/ml insulin vs. IgG insulin antibodies 30–40 μU/ml insulin). Many of our results are within a range below the level of detectability with Christiansen's method (18) of IgG insulin-antibody determination.

Fineberg et al. (19) reported a higher probability of insulin antibodies (% binding) developing in both IDDM and non-insulin-dependent diabetes mellitus (NIDDM) patients treated de novo with either purified pork, mixed beef/pork, or human insulin in patients without residual insulin secretion compared with those retaining some insulin secretion. In addition, Ludvigsson (20) showed that the maximum C-peptide response 9 mo after diagnosis was significantly lower in IDDM children in whom detectable insulin antibodies developed. He proposed that insulin antibodies may have a deleterious effect on residual insulin secretion. However, an alternative interpretation is that increased endogenous insulin secretion may have a dampening effect on the immune system's response to exogenous insulin.

In our cohort of patients followed during the 1st yr, we were unable to document a relationship between the antibody response to human insulin and residual insulin secretion. However, our observations are not strictly comparable to those made by Ludvigsson (20) or Fineberg et al. (19). In the former study, animal-based insulins and a less sensitive insulin-antibody assay were used. In the latter, adult patients with both IDDM and NIDDM were investigated. Our data therefore suggest that in children treated de novo with human insulin, insulin antibodies do not influence residual insulin secretion during the 1st yr after diagnosis.

Whereas Gonen et al. (21) and Asplin et al. (22) found no correlation between insulin antibodies and metabolic control in IDDM patients beyond the remission phase, a negative association has been reported by Ludvigsson et al. (23). Likewise, some investigators have found a positive correlation between insulin antibodies and in-

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sulin dose (24,25), whereas others have not (26). There are no data on the effect of insulin antibodies on metabolic control during the 1st yr after diagnosis. The lack of correlation between insulin antibodies and either HbA_{1c} or insulin dose noted in our study during the 1st yr suggests little if any effect of insulin antibodies after human insulin administration on metabolic control. The insulin-antibody levels reported in this study are probably at levels too low to influence metabolic control adversely.

In summary, our data do not suggest a role for IAAs in the destruction of the β-cell prediagnosis, and they are not useful in predicting either residual insulin secretion or the antibody response to human insulin administration in the 1st yr after diagnosis. We have previously shown that the presence or absence of ICAs or islet cell surface antibodies at diagnosis does not predict residual insulin secretion at diagnosis or during the 1st yr (27). Thus, evidence is mounting that immune phenomena, such as IAAs and islet antibodies, although serving as markers of an immune confrontation with the β-cell, are not likely to be active participants in this battle.

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