Value of Antibodies to Islet Protein Tyrosine Phosphatase–Like Molecule in Predicting Type 1 Diabetes

Mohammed Hawa, Rachael Rowe, Michael S. Lan, Abner L. Notkins, Paolo Pozzilli, Michael R. Christie, and R. David G. Leslie

Islet antigens associated with type 1 diabetes include a recently identified protein tyrosine phosphatase–like molecule IA-2, which contains the intracellular fragment IA-2ic. To determine whether combinations of antibodies including those to IA-2 characterize and predict type 1 diabetes, we studied antibodies to IA-2, IA-2ic, glutamic acid decarboxylase (GAD_{65}), and islet cell antibodies (ICAs) in 1) 60 newly diagnosed type 1 diabetic patients followed for 1 year, 2) 31 monozygotic twin pairs discordant for type 1 diabetes followed up to 12 years (11 twins developed diabetes), 3) 18 dizygotic twin pairs discordant for type 1 diabetes, and 4) normal healthy control subjects. Newly diagnosed type 1 diabetic patients frequently had antibodies to IA-2 (62%), IA-2ic (67%), GAD_{65} (77%), and ICAs (85%). The intracellular fragment of IA-2 probably contains the immunodominant epitope as 137 of 143 samples with IA-2 antibodies from type 1 diabetic patients also had IA-2ic antibodies. Monozygotic twins were usually discordant for antibody specificities. Concordance was higher in monozygotic than matched dizygotic twins for both antibody combinations (33 vs. 6%, $P < 0.05$) and the development of diabetes (33 vs. 0%, $P < 0.01$). In monozygotic twins, all the antibodies were highly predictive of type 1 diabetes (positive predictive values all >87%), although antibodies were also detected in twins at low risk of disease. In summary, IA-2 emerges as a major antigen associated with type 1 diabetes and distinct from GAD_{65}. Type 1 diabetes–associated autoimmunity, which is probably induced by environmental factors, does not necessarily herald progression to the disease. However, genetic factors may influence the development of combinations of disease-associated antibodies and the progression to type 1 diabetes. *Diabetes* 46:1270–1275, 1997

Type 1 diabetes is caused by the destruction of the insulin secreting islet cells of the pancreas, mediated by T-cells that probably recognize islet β-cell–specific antigens (1). Immune changes, including the production of antibodies against islet cells and islet cell proteins, can be detected many months or years before the onset of diabetes. Multiple islet antigens that have been identified include insulin, the 65-kDa isoform of glutamic acid decarboxylase (GAD_{65}), the 37- and 40-kDa trypsinized fragments of a 64-kDa molecule, and ICA512, a fragment of a tyrosine phosphatase–like molecule (2–5). The presence of combinations of antibodies to GAD_{65} and ICAs, for example, in an individual increases his or her risk of developing type 1 diabetes (4,6). Preliminary studies suggest that tyrosine phosphatase–like molecules might be an additional important antigen in type 1 diabetes (5,7–9). It has been found that the antigenic 37- and 40-kDa proteolytic fragments are derived from the proteins IA-2β and IA-2, respectively, which are members of the receptor-linked family of protein tyrosine phosphatases whose mRNA is primarily expressed in islet and brain tissue (9–11). Moreover, the intracellular fragment of IA-2 has sequence homology with ICA512 (amino acids 643–960) (5,11,12). In a preliminary cross-sectional study of nondiabetic identical twins, we identified antibodies to the 37- and 40-kDa fragments as predictive of type 1 diabetes (4). The realization that antibodies to these fragments and to GAD are distinct and the development of more sensitive radioimmunoassays using recombinant GAD and a fragment of IA-2 have allowed us to consider for the first time both the features and the predictive value of combinations of antibodies to distinct islet antigens in a twin cohort followed prospectively. The aim of the present study is to determine whether the presence of combinations of antibodies to this tyrosine phosphatase–like molecule and other antibodies characterizes type 1 diabetes and predicts the disease, using unique cohorts of monozygotic and dizygotic type 1 diabetic patients and their nondiabetic twins followed prospectively.

RESEARCH DESIGN AND METHODS

We studied four groups of subjects *1*) newly diagnosed type 1 diabetic patients, *2*) a cohort of type 1 diabetic twins and their nondiabetic monozygotic twins, *3*) a cohort of type 1 diabetic twins and their nondiabetic dizygotic twins, and *4*) normal healthy control subjects.

Newly diagnosed type 1 diabetic patients. A consecutive population-based series of 60 patients of European origin who presented with type 1 diabetes between 1989 and 1993 in Lazio, a region of Italy, were followed for 1 year in a col-

laborative study. The patients were selected because 1) they had type 1 diabetes defined according to standard guidelines (13), 2) their age at diagnosis was <35 years (mean age, 15.5 ± 7.5 years; range, 5–31 years; 37 males), 3) their duration of clinical disease was <4 weeks, and 4) they had no other autoimmune disease or chronic illness.

Type 1 diabetic twins. We followed a consecutive series of type 1 diabetic twins of European origin from the U.K. and 1) their 31 nondiabetic monozygotic twins referred within 4 years of the diagnosis of the index twin and 2) their 22 nondiabetic dizygotic twins. Both sets of twins were tested between June 1982 and February 1996. Twins were ascertained from June 1982 to June 1994. Monozygosity was established in the monozygotic twin pairs as previously described (14,15). Nondiabetic twins had oral glucose tolerance tests (glucose was given as 75 g or 1.75 g/kg, whichever was the least) to confirm that they were not diabetic both initially and at intervals thereafter; diagnosis of type 1 diabetes was made according to standard guidelines (13). Blood was taken initially from both the index type 1 diabetic twin and the nondiabetic twin for comparison in 30 of 31 monozygotic and all of the dizygotic pairs. The 30 type 1 diabetic monozygotic twins were 16.2 ± 7.3 years of age at sampling, when sufficient sera was available for assay of all antibodies; their median duration of diabetes at the time of sampling used in this study was 1.9 years (range, 0–10 years).

Comparison of monozygotic and dizygotic twins. From the pool of 22 dizygotic twin pairs discordant for type 1 diabetes, we were able to match 18 pairs of both monozygotic and dizygotic twins for age (13.3 ± 6.4 and 14.4 ± 6.3 years, respectively) and disease duration in the index twin (3.2 years [range, 4–29 years] and 3.4 years [range, 0.2–34.0 years], respectively). Of these twins, 6 of 18 monozygotic but 0 of 18 dizygotic twins developed type 1 diabetes subsequently (follow-up, 5.3 ± 3.7 and 3.2 ± 2.8 years, respectively).

Prospective study of monozygotic twins. In the prospective study of the 31 monozygotic twin pairs, the nondiabetic twins were tested for oral or intravenous glucose tolerance, if possible, at least annually and more often if they showed immune or metabolic changes. Eleven twins developed type 1 diabetes (age at entry, 13.4 ± 3.6 years; 6 male subjects) and 20 remain nondiabetic (age on entry, 16.8 ± 8.4 years; 11 male subjects). The 11 prediabetic twins were followed for a median of 24 months (range, 5–132 months), and sufficient sample for the assays in this study was obtained on 56 occasions before they developed diabetes. The 20 twins who remain nondiabetic were followed for a median of 71 months (range, 19–153 months), and sufficient sample was obtained from them on 69 occasions.

Normal healthy control subjects. To validate the antibody assays, 100 normal healthy control subjects were tested (mean age, 25 years; range, 16–71 years). These subjects were negative in all the antibody tests. A negative test was defined as >3 SD above their mean control level, except for ICAs and whole IA-2 (see below). All the antibody specificities were then tested in an additional 38 normal control subjects (mean age, 22 ± years; range, 7–42 years; 20 male subjects), ascertained from the community in Britain and not attached to a hospital, who were followed during the same period as the twins. These normal control subjects were selected to achieve a similar distribution for age and sex as the twins. They had no family history of diabetes, were taking no drugs, and had no clinical signs of illness. Limited sera were available and only the initial sample was tested for this study in these control subjects. The subjects or their parents gave informed consent, and the study was approved by the ethical committees at St. Bartholomew's Hospital, Westminster Hospital, King's College Hospital, and University of Rome, La Sapienza.

Sera were stored at –20°C and all samples were analyzed when sufficient volume was available for each antibody assay. Analysis was performed on batched samples by observers blinded to the clinical status of the subjects.

IA-2, IA-2ic, and GAD$_{65}$ assays. The radioimmunoprecipitation assays for IA-2 whole molecule (amino acids 1–979), IA-2ic (amino acids 603–979), the putative intracellular fragment, and GAD$_{65}$ all employ in vitro transcription and translation systems (Promega Madison, WI). Human full-length IA-2 cDNA in pCR11 vector (Invitrogen, San Diego, CA) (11,12) and IA-2ic cDNA in pGEM-4Z (10) were in vitro transcribed and translated. Human islet GAD$_{65}$ cDNA in the vector pB 1882 (gift of Dr. Thomas Dyrberg, Novo Nordisk, Denmark) was used according to the manufacturer's instructions (Promega, Madison, WI). For all three antibody assays between 0.8 and 1.0 µg, DNA was transcribed and translated with SP6 (for IA-2 and IA-2ic) and T7 (for GAD$_{65}$) RNA polymerase in a TNT coupled rabbit reticulocyte lysate system (Promega, Madison, WI) in the presence of ^{35}S methionine (0.8 mCi/ml) (Amersham, U.K.). Incorporated radioactivity was determined by precipitation with 10% trichloroacetic acid and scintillation counting. For the immunoprecipitation in each assay, 50-µl aliquots of antigen labeled with ^{35}S-methionine (50,000–75,000 cpm) were incubated overnight with 5 µl serum (final dilution 1:10) in Tris-buffered saline. The immunocomplexes were isolated by adding 1 mg protein A-Sepharose and counted on a multiwell Wallac counter. For the determination of IA-2, the immunocomplexes were boiled in sodium dodecyl sulfate sample buffer before separation on 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and autoradiography; IA-2 bands (~106 kDa) were

identified by two independent investigators. All samples were tested in duplicate including positive and negative control standard sera. Each assay for IA-2ic and GAD$_{65}$ antibodies included serially diluted sera from a stiff-man syndrome patient and a prediabetic individual (a twin who subsequently developed type 1 diabetes but was not part of this study) to further evaluate the cutoff level for positivity for GAD$_{65}$ and IA-2ic. Except for IA-2, values >3 SD above the control population (values greater than 1:8,000 dilution of both stiff-man syndrome and prediabetic sera) were taken as positive. In the latest IA-2ic (M.H., R.D.G.L., unpublished observations) and GAD$_{65}$ antibody proficiency workshops, our assays had a sensitivity, specificity, validity, and consistency of 100% (16).

Islet cell antibody determination. Undiluted sera were screened for ICAs. The presence of ICAs was established by testing serum with indirect immunofluorescence on a fresh group O cryofixed human pancreas (4). Positive samples were then titrated by doubling dilutions in phosphate-buffered saline and end-point titers converted to Juvenile Diabetes Foundation units (JDF U) with a threshold of detection of 4 JDF U. The results are presented as positive or negative, with positive tests being ≥4 JDF U. The ICA assay assessed in the European Nicotinamide in Diabetes Intervention Trial workshop (M. Shattock, G.F. Bottazzo, unpublished observations) had a sensitivity and specificity of 100%.

Statistical analysis. Results are expressed as means ± SD or median (range), where appropriate. The proportions of subjects in each group positive for each antibody, antibody combinations, or progression to type 1 diabetes were compared using χ^2, with Fisher's exact test. Standard Kaplan-Meier actuarial life-table methods were used to calculate the cumulative risk of twins remaining free of type 1 diabetes from the initial sample. We estimated 1) the positive predictive value by calculating the number of twins with antibodies who later developed type 1 diabetes as a percentage of the overall number of twins with the antibodies, 2) the sensitivity by dividing the number of twins with antibodies who developed diabetes by the overall number of twins who developed diabetes, and 3) the specificity by dividing the number of twins without an antibody who did not develop type 1 diabetes by the total number of twins who did not develop diabetes. As immune changes may not be static, the calculated values were determined on the first available sample taken. Changes in the study were considered significant at $P < 0.05$.

RESULTS

Newly diagnosed type 1 diabetic patients. Of 60 newly diagnosed type 1 diabetic patients, antibodies were found to IA-2 in 37 (62%), IA-2ic in 40 (67%), GAD$_{65}$ in 46 (77%), and ICAs in 51 (85%) (Table 1) (for all antibodies $P < 0.0001$, compared with 100 control subjects). Four (7%) patients had none of the antibodies. Antibodies to both IA-2 and GAD$_{65}$ and IA-2ic and GAD$_{65}$ were detected in 32 (53%) and 34 (57%), respectively, and to either antibody in 52 (87%) for both combinations (Fig. 1). Only three patients with ICAs did not have IA-2, IA-2ic, or GAD$_{65}$ antibodies. Both IA-2 and IA-2ic antibodies were more prevalent in patients <15 years of age (23 and 25 of 30 positive, respectively) than those >15 years of age (13 and 15 of 30 positive, respectively; $P < 0.02$ for both); antibody positivity was not related to sex. In general, antibody positivity at diagnosis persisted at 1 year after diagnosis and did not differ significantly; antibodies at 1 year were found to IA-2 in 36 (60%), IA-2ic in 34 (57%), GAD$_{65}$ in 45 (75%), and ICAs in 50 (83%). Patients who were antibody-positive close to diagnosis tended to remain so at 1 year postdiagnosis (Table 1), although some became negative at 1 year: for IA-2 in only 1 case (2%), IA-2ic in 6 (10%), GAD$_{65}$ in 1 (2%), and ICAs in 4 (7%). In one patient, antibodies not detected at diagnosis were detected at 1 year to IA-2ic, IA-2, and ICAs but not to GAD. Of 143 samples from type 1 diabetic patients (twins and singletons) and prediabetic twins who were IA-2 antibody-positive, 137 were also IA-2ic antibody-positive.

Type 1 diabetes and nondiabetic twins
Cross-sectional study. We studied 1) 31 pairs of monozygotic twins discordant for type 1 diabetes and 2) 18 pairs of monozygotic and 18 pairs of dizygotic twins matched for age and disease duration in the type 1 diabetic index twin.

Of the 31 type 1 diabetic monozygotic twins, 30 were tested initially at the same time as the nondiabetic twins,

TABLE 1
Antibodies to IA-2 and IA-2ic in *1*) 60 patients at diagnosis of type 1 diabetes and *2*) at follow-up after 1 year, *3*) 30 monozygotic type 1 diabetic twins, and *4*) 38 normal healthy control subjects

Subjects	Number of tests	IA-2$^+$	IA-2ic$^+$	GAD$_{65}$$^+$	ICAs >4 JDF U
Type 1 diabetic patients (at diagnosis)	60	37 (62)	40 (67)	46 (77)	51 (85)
Type 1 diabetic patients (at follow-up)	60	36 (60)	34 (57)	46 (75)	50 (83)
Type 1 diabetic monozygotic twins	30	20 (67)	24 (80)	26 (87)	23 (76)
Control subjects	38	1 (3)	0	0	0

Data are *n* (%).

and in them antibodies were found to IA-2 in 20 (67%), IA-2ic in 24 (80%), GAD$_{65}$ in 26 (87%), and ICAs in 23 (76%) (for all antibodies $P < 0.0001$, compared with 38 control subjects). Only one twin (3%) with ICAs did not have IA-2, IA-2ic, or GAD$_{65}$ antibodies (see Fig. 2), and only one (3%) had no antibodies. The antibody frequencies in these twins did not differ significantly from those in the newly diagnosed type 1 diabetic patients (Table 1; Fig. 2). In the 31 nondiabetic monozygotic twins, antibodies were found initially to IA-2 in 11 (35%), IA-2ic in 10 (32%), GAD$_{65}$ in 8 (26%), and ICAs in 11 (35%); 18 (58%) twins had no antibodies.

Comparison of monozygotic and dizygotic twins. In 12 of 18 monozygotic twin pairs, one twin had at least one of the antibodies, while the other twin did not. Similarities for the presence or absence of antibodies were detected in only 6 of 18 monozygotic pairs (in these six pairs, both twins had at least one antibody) and 4 of 18 dizygotic pairs (in two pairs, both twins had at least one antibody, and in two pairs, neither twins had any antibody). In the 18 nondiabetic monozygotic and 18 nondiabetic dizygotic twins, antibodies were detected to IA-2 (in five and two pairs, respectively), GAD$_{65}$ (in four and two pairs, respectively), and ICAs (in five and four pairs, respectively) and to any one antibody in 6 of 18 monozygotic and 6 of 18 dizygotic twins (Table 2). More monozygotic than dizygotic twin pairs were concordant for combinations of more than one antibody: IA-2, GAD$_{65}$, or ICAs (6 of 18 vs. 1 of 18, *P* < 0.05). The six nondiabetic monozygotic twins, all with a combination of more than one antibody, developed type 1 diabetes, but none of the dizygotic twins have done so (*P* < 0.01).
Prospective study of monozygotic twins. Of the 31 nondiabetic monozygotic twins tested prospectively, 11 developed type 1 diabetes and were therefore prediabetic during the study period.
Prediabetic monozygotic twins. Of 11 prediabetic twins tested, antibodies were found initially to IA-2 in 10 (91%), IA-2ic in 10 (91%), GAD$_{65}$ in 7 (64%), and ICAs in 11 (100%) (for all antibodies *P* < 0.001, compared with antibodies in initial sample in 20 twins remaining nondiabetic). Antibodies to IA-2, IA-2ic, and GAD$_{65}$ were all detected in the initial sample in six cases (55%) and to any one antibody in 11 (100%). On follow-up, 56 samples were taken from the 11 prediabetic twins; antibodies were found to IA-2 in 51 of 56 (91%), IA-2ic in 48 of 56 (85%), GAD$_{65}$ in 30 of 56 (53%), and ICAs in 49 of 56 (88%) (Table 3). Antibodies to IA-2 and GAD$_{65}$ or IA-2ic and GAD$_{65}$ were detected in 25 (45%) and 22 (39%), respectively, and to either in 56 (100%) of the 56 samples.
Nondiabetic monozygotic twins. Of 20 nondiabetic twins tested, antibodies were found initially to IA-2 in one and, in a different twin, to GAD$_{65}$ in one, but none of the twins had

antibodies to IA-2ic or ICAs. On follow-up, 69 samples were taken from the 20 twins remaining nondiabetic; antibodies were found to IA-2 in 1 of 69 (1%), GAD$_{65}$ in 4 of 69 (6%), and ICAs in two other samples (3%), but none of the 69 samples had antibodies to IA-2ic.
Normal healthy control subjects. 100 controls were, by definition, negative for IA-2ic and GAD$_{65}$ antibodies and also for IA-2 antibodies. Of the 38 controls followed during the same period as the twins, antibodies were found to IA-2 in one subject (3%), but none of these control subjects had antibodies to IA-2ic, GAD$_{65}$, or ICAs (Table 1).
Predictive values. The cumulative risk of twins developing type 1 diabetes within 12 years of the index twins' diagnosis by life-table analysis was 43% (95% CI 30–59). In this cohort, the positive predictive value, specificity, and sensitivity for IA-2 antibodies in the initial sample were 91, 95, and 91%, respectively; for IA-2ic antibodies, 100, 100, and 91%, respectively; for GAD$_{65}$ antibodies, 88, 83, and 64%, respectively; and for ICAs, 100, 100, and 100%, respectively. The positive predictive value for each one of the antibodies when positive on two consecutive occasions from the initial sample was 100%, except for GAD$_{65}$, which was 83%. The positive predictive value, speci-
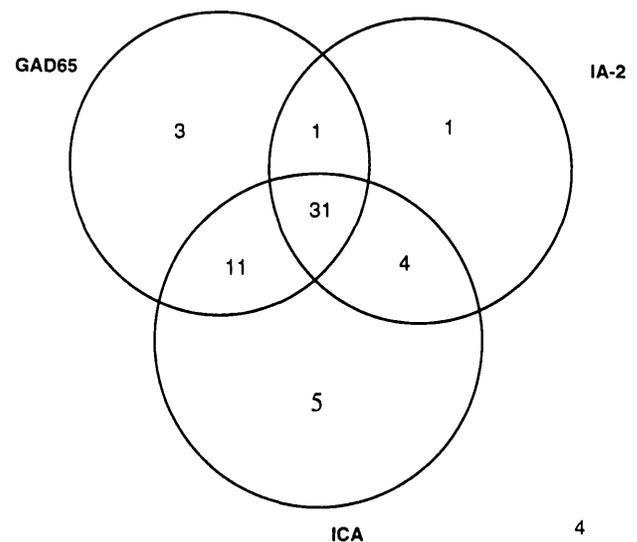

FIG. 1. The number of 60 newly diagnosed type 1 diabetic subjects positive for autoantibodies to GAD, IA-2, and ICAs. Intersecting regions indicate the number of patients positive for different combinations of autoantibodies. Four subjects did not have GAD, IA-2, IA-2ic, or ICA antibodies.
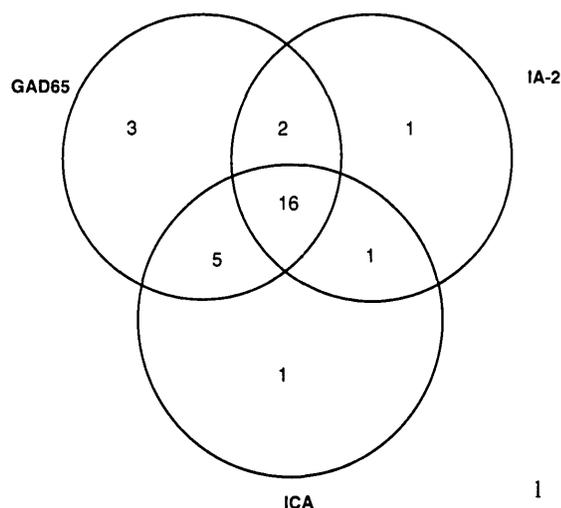
FIG. 2. The number of 30 type 1 diabetic monozygotic twins positive for autoantibodies to GAD, IA-2, and ICAs. Intersecting regions indicate the number of patients positive for different combinations of autoantibodies. One twin did not have GAD, IA-2, IA-2ic, or ICA antibodies.

ficity, and sensitivity for the combination of either IA-2 and GAD_{65} antibodies or IA-2ic and GAD_{65} antibodies in the initial sample was the same for both: 100, 81, and 55%, respectively.

DISCUSSION

These observations indicate that a protein tyrosine phosphatase–like molecule is a major antigen in type 1 diabetes; antibodies to it were a feature of newly diagnosed type 1 diabetic patients and were sensitive and specific predictors of type 1 diabetes in monozygotic twins. The frequency and predictive value of antibodies to this tyrosine phosphatase–like molecule are similar to those for antibodies to GAD_{65}, suggesting that it has comparable significance to GAD_{65} as an type 1 diabetes–associated antigen (17). Antibodies to either IA-2 (and its intracellular fragment) or GAD_{65} were detected in 87% of newly diagnosed type 1 diabetic patients and in all 56 prediabetic samples from 11 twins. Even though our present study is the only population-based study of IA-2ic antibodies in newly diagnosed type 1 diabetic patients, there was a remarkable consistency in the frequency of IA-2ic and GAD antibodies in type 1 diabetic patients from Europe, irrespective of their country of origin. Thus, antibody frequencies of IA-2ic were similar, not only in the Italian (67%) and British (80%) type 1 diabetic patients we studied, but also in other studies of recent onset Italian (61%), Spanish (65%), and Belgian (58%) patients (18–20). Factors associated with an increase in IA-2 antibody frequencies in these type 1 diabetic patients include

young age at diagnosis and the presence of human leukocyte antigen DR4 (18).

We determined antibody reactivity in type 1 diabetes to *1*) the whole molecule IA-2 (amino acids 1–979) and *2*) the intracellular portion of IA-2, designated IA-2ic (amino acids 603–979). We have observed a striking association between positivity for antibodies to IA-2 and the intracellular portion of that molecule, strongly supporting the proposal that the carboxy-terminal of the intracellular fragment contains the immunodominant epitope (10,12,21). Epitopes recognized by IA-2 antibodies are likely to be different from those recognized by GAD_{65} antibodies, since a number of subjects had antibodies to only one of the antigens. In line with this argument, other studies of a probable fragment of IA-2, designated ICA512, also found antibody positivity in some type 1 diabetic patients to the protein but not to GAD_{65} (22). We cannot determine the sequence, if any, of autoantibody production in the prediabetic period from this study; those prediabetic twins with these antibodies already had them when ascertained. Antibodies detected in the initial sample were remarkably stable thereafter, being detected throughout the prediabetic period in most twins. Variation in positivity occurred infrequently. For example, in 47 IA-2 antibody–positive subjects (twins and singletons), only two became IA-2 antibody–negative on follow-up. Antibodies occurred with similar frequency in newly diagnosed type 1 diabetic patients, type 1 diabetic patients at 1 year, and type 1 diabetic identical twins.

Significantly, more monozygotic than dizygotic twin pairs were concordant for antibody combinations. Thus, the detection of antibody combinations may indicate a genetically determined expansion of the altered humoral immune response. The striking discordance for the presence of ICAs, GAD_{65}, IA-2, and IA-2ic antibodies between identical twins suggests that antibody production was not entirely genetically determined. The detection of a similar frequency of these antibodies in monozygotic and dizygotic twins (whether diabetic or nondiabetic) is consistent with a common environmental effect. The detection of autoimmune changes in twins who remain nondiabetic and at low disease risk (nondiabetic dizygotic twins have ~10% disease risk) would also be consistent with the proposal that the frequency of type 1 diabetes–associated autoimmunity in selected subjects can be higher than the frequency of overt diabetes (1,4,15,22,23).

The observation that GAD_{65} and IA-2 are major type 1 diabetes–associated antigens has practical implications. In particular, it could enhance our capacity to predict type 1 diabetes. Previous studies have demonstrated the value of ICAs as a predictor of type 1 diabetes (4,6,15). However, ICAs are a technically difficult to assay, requiring human pancreatic tissue, and as such, not readily applicable to large scale analysis (25). A positive ICA reaction may reflect the presence of antibodies

TABLE 2
Comparison of humoral immune changes at a single time point in 18 monozygotic and 18 dizygotic twin pairs obtained from the total group of 31 monozygotic and 22 dizygotic twin pairs and matched for both age and disease duration in the index twin

	Total	IA-2	IA-2ic	GAD_{65}	ICAs >4 JDF U
Monozygotic type 1 diabetic twins	18	13	16	15	12
Monozygotic nondiabetic twins	18	5	5	4	5
Dizygotic type 1 diabetic twins	18	9	9	9	15
Dizygotic nondiabetic twins	18	2	2	2	4

TABLE 3
Humoral immune changes in 11 prediabetic monozygotic twins during follow-up before the onset of type 1 diabetes

Twin number	Sex	Total number of tests	IA-2$^+$	IA-2ic$^+$	GAD$_{65}$	ICAs >4 JDF U
1	F	2	2	2	2	2
2	F	12	12	12	3	12
3	F	7	7	7	7	7
4	M	8	8	5	8	8
5	F	2	2	2	2	2
6	M	5	5	5	1	4
7	M	3	3	3	3	2
8	F	1	1	1	0	1
9	M	5	0	0	5	5
10	M	10	10	10	1	5
11	M	1	1	1	1	1

to one or more islet cell antigens. Thus, it might be possible to use recombinant islet proteins identified as type 1 diabetes–associated antigens in place of ICAs for population screening. The tendency for subjects with ICAs in this and other studies to be positive for IA-2 or GAD antibodies is consistent with the proposal that ICAs are composed, to a degree, of antibodies to these two antigens. Our present study, in line with the only other study to assess antibodies to fragments of IA-2 and GAD, confirms the potential for prediction of type 1 diabetes, using antibody combinations to specific islet antigens (26–28). As we and others have found that some subjects have ICAs but neither GAD$_{65}$ nor IA-2 antibodies, it seems that ICAs, despite their technical limitations, should still be used in primary screening for optimal sensitivity of detection.

ICAs may be represented by antibodies other than GAD$_{65}$ and IA-2, such as IA-2β/phogrin or Glima 38, so that in time primary screening might be performed using only antibodies to a range of recombinant antigens (9,29,30). In our study, the numbers of ICA-negative twins or newly diagnosed type 1 diabetic patients were too small to be certain whether GAD or IA-2 antibodies are associated with disease risk, independent of ICAs. The identification of antibodies to IA-2 in prediabetic twins who were GAD$_{65}$ antibody–negative and vice versa suggests that testing for both GAD$_{65}$ and IA-2 antibodies could increase the sensitivity of prediction in comparison with either alone. We found that only ~50% of newly diagnosed type 1 diabetic patients and prediabetic twins had a combination of IA-2 and GAD$_{65}$ antibodies, implying that this particular combination might improve the specificity of disease prediction but at the cost of sensitivity.

It is possible to induce immunological tolerance to specific proteins, using a variety of strategies including the alteration of peptide ligands that bind to the T-cell receptor. These approaches have been effective in preventing the progression to disease in animal models of immune-mediated diseases, including the injection of GAD$_{65}$ into NOD mice, an animal model of type 1 diabetes (31). The identification of additional major type 1 diabetes–associated antigens, a protein tyrosine phosphatase–like molecule, and its related molecule IA-2β, extends our repertoire of potential predictive and intervention strategies.

ACKNOWLEDGMENTS

REFERENCES

1. Leslie RDG, Gale EAM: Aetiology of insulin dependent diabetes. In *Diabetes: Clinical Science in Practise.* Leslie RDG, Robbins DC, Eds. Cambridge, Cambridge Univ. Press, 1995, p. 134–156
2. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Paquette TL: Insulin antibodies in insulin-dependent diabetes before insulin treatment. *Science* 222:1337–1339, 1983
3. Baekkeskov S, Aanstoot HJ, Christgau S, Christgau S, Retz A, Solimena M, Cascalho M, Folli F, Richter-Ohlsen H, De Camilli P: Identification of the 64K autoantigen an insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 347:151–156, 1990
4. Christie MR, Tun RYM, Lo SSS, Cassidy D, Brown TJ, Hollands J, Shattock M, Bottazzo GF, Leslie RDG: Antibodies to GAD and tryptic fragments of islet 64K antigen as distinct markers for development of IDDM. *Diabetes* 41:782–787, 1992
5. Rabin DU, Pleasic SM, Palmer-Crocker R, Shapiro JA: Cloning and expression of IDDM-specific human autoantigens. *Diabetes* 41:183–186, 1992
6. Bingley PJ, Christie MR, Bonfanti R, Bonifacio E, Shattock M, McNally JM, Gale EAM, Bottazzo GF: Combined analysis of autoantibodies enhances prediction of IDDM in islet cell antibody (ICA) positive relatives. *Diabetes* 43:1304–1310, 1994
7. Rabin DU, Pleasic SM, Shapiro JA, Yoo-Warren H, Oles J, Hicks JM, Goldstein DE, Rae PMM: Islet cell antigen 512 is a diabetes-specific islet autoantigen related to protein tyrosine phosphatases. *J Immunol* 152:3183–3188, 1994
8. Lan MS, Wasserfell C, MacLaren NK, Notkins AL: IA-2, a transmembrane protein of the protein tyrosine phosphatase, is a major autoantigen in insulin-dependent diabetes mellitus. *Proc Natl Acad Sci USA* 93:6367–6370, 1996
9. Jia Lu, Qing Li, Hong Xie, Zhi-Jian Chen, Borovitskaya AE, Maclaren NK, Notkins AL, Lan MS: Identification of a second transmembrane protein tyrosine phosphatase, IA-2β, as an autoantigen in insulin-dependent diabetes mellitus: precursor of the 37-kDa tryptic fragment. *Proc Natl Acad Sci USA* 93:2307–2311, 1996
10. Payton MA, Hawkes CJ, Christie MR: Relationship of the 37,000 and 40,000 Mr tryptic fragments of islet antigens in insulin-dependent diabetes to the protein tyrosine phosphatase-like molecule IA-2 (ICA512). *J Clin Invest* 96:1506–1511, 1995
11. Lan MS, Lu J, Goto Y, Notkins AL: Molecular cloning and identification of a receptor-type protein tyrosine phosphatase, IA-2, from human insulinoma. *DNA Cell Biol* 13:505–514, 1994
12. Lu J, Notkins AL, Lan MS: Isolation, sequence and expression of a novel mouse brain cDNA, mIA-2, and its relatedness to members of the protein tyrosine phosphatase family. *Biochem Biophys Res Commun* 204:930–936, 1994
13. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories with glucose intolerance. *Diabetes* 28:1039–1057, 1979
14. Olmos P, A'Hern R, Heaton DA, Millward BA, Risley D, Pyke DA, Leslie RDG: The significance of the concordance rate for type 1 (insulin-dependent) diabetes in identical twins. *Diabetologia* 31:747–750, 1988
15. Tun RYM, Peakman M, Alviggi L, Hussain MJ, Lo SSS, Shattock M, Pyke DA,

Bottazzo GF, Vergani D, Leslie RDG: The importance of persistent cellular and humoral immune changes in the prediabetic period: a prospective identical twin study. *BMJ* 308:1063–1068, 1994

16. Schmidli RS, Colman PG, Bonifacio E, and Participating Laboratories: Disease sensitivity and specificity of 52 assays for glutamic acid decarboxylase antibodies. *Diabetes* 44:636–640, 1995

17. Tuomilehto J, Zimmet P, Mackay IR, Koskela P, Vidgren G, Toivanen L, Tuomilehto-Wolf E, Kohtamaki K, Stengard J, Rowley MJ: Antibodies to glutamic acid decarboxylase as predictors of insulin-dependent diabetes mellitus before clinical onset of disease. *Lancet* 343:1383–1385, 1994

18. Genovese S, Bonfanti R, Bazzigaluppi E, Lampasona V, Benazzi E, Bosi E, Bonifacio E: Association of IA-2 autoantibodies with HLA DR4 phenotypes in IDDM. *Diabetologia* 39:1223–1226, 1996

19. Morales J, Puig-Domingo M, Mauricio D, Piquer S, Vinets I, Lampasona V, Bonifacio E, de Leiva A: IA2 and GAD 65 antibodies complement islet cell antibodies in IDDM prediction (Abstract). *Diabetologia* 39 (Suppl. 1):327, 1996

20. Gorus KF, Goubert P, Semakula C, Vandevalle CL, Schepper JD, Scheen A, Christie MR, Pipeleers DG, The Belgian Diabetes Registry: IA-2 autoantibodies compliments GAD_{65} autoantibodies in new onset IDDM patients and help predict impending diabetes in their siblings. *Diabetologia* 40:95–99, 1997

21. Lampasona V, Bearzatto M, Genovese S, Bosi E, Ferrari M, Bonifacio E: Autoantibodies in insulin-dependent diabetes recognise distinct cytoplasmic domains of the protein tyrosine phosphatase-like IA-2 autoantigen. *J Immunol* 157:2707–2711, 1996

22. Gianani R, Rabin DU, Verge CF, Yu L, Babu SR, Pietropaolo M, Eisenbarth GS: ICA512 autoantibody radioassay. *Diabetes* 44:1340–1344, 1995

23. Leslie RDG, Elliott RB: Early environmental events as a cause of IDDM: evidence and implications. *Diabetes* 43:843–850, 1994

24. Verge CF, Gianani R, Yu L, Pietropaolo M, Smith T, Jackson RS, Soeldner JS, Eisenbarth GS: Late progression to diabetes and evidence for chronic cell

autoimmunity in identical twins of patients with type 1 diabetes. *Diabetes* 44:1176–1179, 1995

25. Bingley P, Bonifacio E, Shattock M, Gillmor HA, Sawtell PA, Dunger DB, Scott RDM, Bottazzo GF, Gale EAM: Can islet cell antibodies predict IDDM in the general population? *Diabetes Care* 16:45–50, 1993

26. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, Chase HP, Eisenbarth GS: Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes* 45:926–933, 1996

27. Seissler J, Morgenthaler NG, Achenbach P, Lampeter EF, Glawe D, Payton M, Christie M, Scherbaum WA, the DENIS Study Group: Combined screening for autoantibodies to IA-2 and antibodies to glutamic acid decarboxylase in first degree relatives of patients with IDDM. *Diabetologia* 39:1351–1356, 1996

28. Bonifacio E, Genovese S, Braghi S, Bazzigaluppi E, Lampasona V, Bingley PJ, Rogge L, Pastore MR, Bognetti E, Bottazzo GF, Gale EAM, Bosi E: Islet autoantibody markers in IDDM: risk assessment strategies yielding high sensitivity. *Diabetologia* 38:816–822, 1995

29. Hawkes CJ, Wasmeier C, Christie MR, Hutton CJ: Identification of the 37-kDa antigen in IDDM as a tyrosine phosphatase–like protein (phogrin) related to IA-2. *Diabetes* 45:1187–1192, 1996

30. Aanstoot H-K, Kang S-M, Kim J, Lindsay L, Roll U, Knip M, Atkinson M, Mose-Larson P, Fey S, Ludvigsson J, Landin M, Bruining J, Maclaren N, Akerblom HK, Baekkeskov S: Identification and characterisation of glima 38, a glycosylated islet cell membrane antigen, which together with GAD_{65} and IA2 marks the early phases of autoimmune response in type 1 diabetes. *J Clin Invest* 97:2772–2783, 1996

31. Kaufman DL, Care-Salzier M, Tian J, Forsthuber T, Ting GSP, Robinson P, Atkinson MA, Sercarz EE, Tobin AJ, Lehmann PV: Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature* 366:69–72, 1993