Comparison of Insulin Autoantibodies in Diabetes-Related and Healthy Populations by Precise Displacement ELISA

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The second international workshop on insulin autoantibodies (IAAs) demonstrated improved concordance among laboratories with both radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) when measurements were based on signals displaceable by preincubation with excess insulin. This feature has been incorporated into our original ELISA for IAAs, and the assay was used to compare IAAs in diabetes-related and healthy populations. The serum from a healthy islet cell antibody (ICA)$^+$ and IAA$^+$ subject was used to construct standard curves with and without preincubation with 1 U/ml human insulin. Reporting results in arbitrary displacement ($\Delta \pm$IAA) units derived from the standard curve improved precision and increased the specificity of the assay. Frequency analysis of the results from 200 control adults did not show a normal distribution, and cumulative frequency analysis demonstrated two populations: 10 of 200 (5%) control adults, 8 of 241 (3.3%) healthy schoolchildren, and 18 of 229 (7.9%) non-insulin-dependent diabetes mellitus (NIDDM) patients had IAAs >12 $\Delta \pm$IAA units. On the same basis, we tested samples from 89 individuals in the prospective Bart's Windsor Family Study for insulin-dependent diabetes mellitus (IDDM), 31 of whom had ICAs >5 Juvenile Diabetes Foundation (JDF) units. Of those with ICAs >5 JDF units, 12 developed IDDM and 1 developed NIDDM in 10 yr of study. IAAs >12 $\Delta \pm$IAA units were found in 6 of 12 (50%) who became diabetic and in 4 of 18 (22%) who remain healthy. A significant association of IgG IAAs with ICAs >5 JDF units is confirmed in this IDDM-susceptible cohort ($P < .0005$) but was not observed in the other groups studied. The predictive value of a positive test with IgG IAAs alone is poor (20%), but the combined positive predictive value of both markers conjointly for IDDM is 60%, and the predictive value for health is 98.7%. We suggest that screening for IAAs by ELISA should be restricted to those with ICAs >5 JDF units. *Diabetes* 38:1275–81, 1989

Insulin 1 μM = 0.139 U/ml

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Received for publication 28 November 1988 and accepted in revised form 6 June 1989.

In pursuit of the goal of prevention of insulin-dependent diabetes mellitus (IDDM), attention has recently been focused on circulating autoantibodies as possible predictors for the future onset of IDDM. Together with knowledge of metabolic and human leukocyte antigen (HLA) status, information on autoantibodies might enable early identification of high-risk individuals with confidence. Although international workshops have fully recognized islet cell antibodies (ICAs) as markers of ongoing β-cell destruction (1) and much progress has been achieved toward the standardization of their measurement in the past 2 yr (2–5), the role of spontaneous insulin autoantibodies (IAAs) and their usefulness in disease prediction has yet to be fully substantiated.

There are conflicting reports concerning the association of IAAs with ICAs (for review, see ref. 6). Although some discrepancies may arise from differences in the selection of patient groups with regard to disease susceptibility and/or ethnic origin, others are undoubtedly methodological.

IAA methods based solely on direct binding of antibody to antigen can lead to a wide variation of signal, regardless of whether liquid-phase radioimmunoassay (RIA) or solid-phase enzyme-linked immunosorbent assay (ELISA) is used (7). With either technique, improved concordance between laboratories was achieved when measurements were based on signals that were quenchable by preincubation with excess human insulin (8). In addition, international workshops for the standardization of anti-nuclear antibodies (ANAs) (9)

and ICAs (5) have shown that the use of standard curves considerably improves assay precision (10).

Accordingly, we incorporated both these features into our original ELISA for IgG IAAs and reassessed our initial findings in the prospective Bart's Windsor Family Study (BWFS) for IDDM (11,12). To evaluate the displacement method, we investigated 229 non-insulin-dependent diabetic (NIDDM) patients, 200 control adults, and 241 schoolchildren for the presence of IAAs, ICAs, and other autoantibodies. This study provides comparative values for ICAs and IAAs in terms of the sensitivity, specificity, and predictive values of these markers for diabetes onset.

RESEARCH DESIGN AND METHODS

The criteria for inclusion of families into the BWFS were that the IDDM proband was diagnosed before 21 yr of age and that there was at least one nondiabetic sibling under that age. One hundred ninety-eight families were recruited, most between September 1978 and April 1979. All family members were tissue typed for HLA-A, -B, -C, and -DR and screened for ICAs at 3- to 6-mo intervals (13). Retrospective titration of all ICA^+ serums and conversion to Juvenile Diabetes Foundation (JDF) units have shown that 36 nondiabetic first-degree relatives, from a total cohort of 719, had ICAs >5 JDF units during the 10-yr study. By July 1989, 16 first-degree relatives (10 of 11 siblings and 4 of 5 parents) with ICAs >5 JDF units had developed IDDM, and 1 father had developed NIDDM. However, 21 subjects (16 siblings and 5 parents) with ICAs >5 JDF units remained healthy, together with 366 parents and 316 siblings who have ICAs ≤5 JDF units.

In this study, all available sequential serums from 31 of 36 first-degree relatives with ICAs >5 JDF units were investigated for IAAs. We were unable to include 1 child and 1 father who had progressed to IDDM (2 of 14) and 2 mothers and 1 child (3 of 21) who remain healthy. In addition, all sequential samples from 58 ICA^- family members were screened for IAAs. In total, >1500 serums were tested by the direct-binding ELISA for IgG IAAs (11,12). The peak response samples of all IAA^+ individuals have been retested by the displacement ELISA described below.

Serum samples were obtained from 229 NIDDM subjects, composed of 160 outpatients (mean ± SD age 57.4 ± 6.2 yr, range 42–65 yr) from the Royal Hallamshire Hospital (Sheffield, UK) and 69 patients (mean age 67 ± 10 yr, range 50–87 yr) from Leeds General Infirmary (Leeds, UK). These patients were treated only with oral hypoglycemic agents or diet, and in all cases, lack of exposure to administered insulin was ascertained by rigorous questioning and reference to medical records.

Serums from 200 adult blood bank donors (mean age 35 ± 11.4 yr, range 19–65 yr) and 241 healthy schoolchildren (mean age 11.6 ± 2.1 yr, range 8–16 yr) were used as control samples. The latter were taken during a longitudinal study of growth and endocrine changes in puberty that was approved by the Ethical Committee of the Great Ormond Street Hospital for Sick Children (London).

All subjects were believed to be of Caucasian origin. In addition to screening for IAAs, all samples were tested for ICAs (expressed in JDF units), gastric parietal cell autoantibodies (PCAs), thyroglobulin autoantibodies (TGAs), thyroid microsomal autoantibodies (TMAs), and ANAs. IAA^+-ICA^- samples were also tested for rheumatoid factor. Samples were stored frozen at −20°C for up to 10 yr.

Measurement of IgG IAAs. Recrystallized monocomponent human insulin was kindly donated by Novo (Copenhagen). Human proinsulin and human C-peptide were made available by Lilly (Indianapolis, IN).

A class-specific micro-ELISA (11) was used with the following modifications. Nunc Immunoplate I microplates (Gibco, Paisley, UK) were used. The optimal antiserum dilution of the affinity-purified goat anti-human IgG (γ-chain–specific) horseradish peroxidase conjugate (Tago, Burlingame, CA) for the assay was 1/8000. Improved stability and reproducibility of the dye system has been achieved with stock solutions of 3,3′,5,5′-tetramethylbenzidine in 10 mg/ml dimethyl sulfoxide and diluting immediately before use in 0.05 M citric acid–sodium citrate buffer (1 ml/dl), pH 6.0, with the addition of 75 μl of 6% (wt/vol) H_2O_2. Plates were read at an optical density (O.D.) of 450 nm with the dual-wavelength facility of an automatic ELISA plate photometer (EAR 400 FW, SLT Lab Instruments, Salzburg, Austria) at O.D. 690 nm. Raw O.D. readings for insulin-coated wells were corrected for nonspecific binding by subtraction of values (mean of triplicates for each sample) obtained from wells treated with the coating buffer and blocking agent alone (11).

Serums (10 μl) were preincubated for 1 h at room temperature in 1 ml buffer (0.015 M phosphate-buffered saline containing 5% Tween 20 and 10% normal goat serum, pH 7.4) with and without 1 U human insulin. Aliquots of serum from an ICA^+ (15 JDF units) nondiabetic first-degree relative who is IgG IAA^+ (IgM IAA^-) were stored frozen at −20°C. This standard serum was designated to contain 100 IAA units by direct binding and 100 Δ±IAA units by the displacement assay at 1/100 dilution (working dilution of assay). In each assay, 8 dilutions of the standard were used, covering the 0- to 40-unit range. Standard curves were constructed plotting the difference in O.D. obtained with and without insulin preincubation (ΔO.D.) at each dilution against the relevant Δ±IAA units. Within the studied range, the serum was well matched in both direct-binding and displacement characteristics to the standard serum supplied in the second international workshop for the standardization of IAA measurement (8). Serum Δ±IAA unit values were calculated from the appropriate linear equations derived from standards in the 0- to 40-Δ±IAA unit range. O.D. readings >40 standard units were interpolated by extrapolation of the linear regression line.

Examples of the responses obtained for the standard serum in the dilution range 1/2000–1/250 (5–40 Δ±IAA units) are shown in Fig. 1. When human insulin was replaced by an equimolar amount of human proinsulin in preincubation, the observed displacement was identical, leading to a standard Δ±IAA unit curve indistinguishable from that obtained by preincubation with human insulin. When an equimolar amount of human C-peptide was used, no displacement occurred at any of the serum dilutions tested, indicating that specificities for the antigen-binding sites reside in the A- and/or B-chain of the insulin/proinsulin molecule but not in the C-peptide moiety.

In 15 consecutive assays, a linear regression was ob-

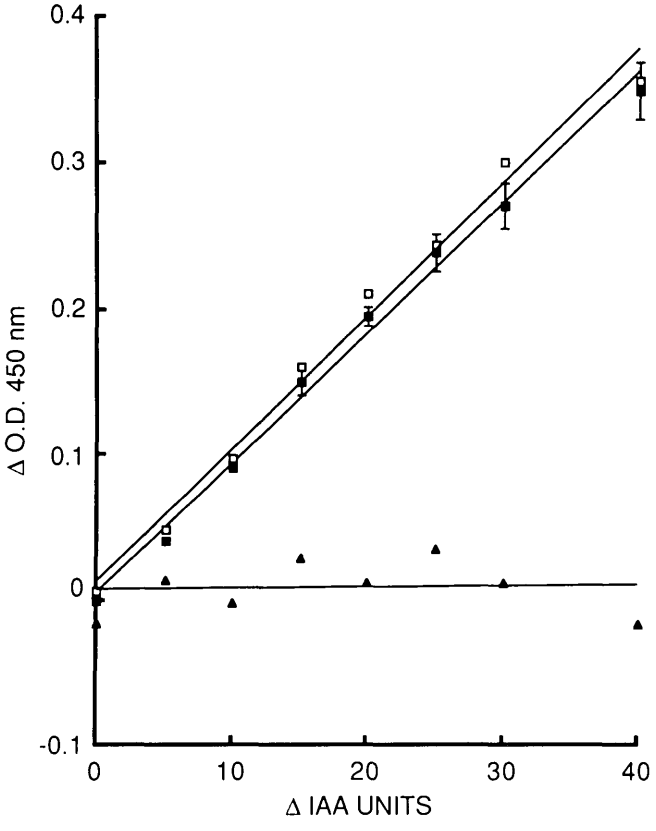

FIG. 1. Examples of standard curves used in interpolation of insulin autoantibodies (IAAs) in arbitrary standard units (see RESEARCH DESIGN AND METHODS). □, Line (y = 0.0092x + 0.0124, r = .99) relates direct-binding optical density (O.D.) values to designated IAA units; ■, line (y' = 0.0090x' + 0.0051, r = 1.0) describes corresponding values after subtraction of O.D. values obtained by preincubation of samples with 1 U/ml human insulin. Mean ± SD of triplicate observations are shown for points relating to displaceable Δ±IAA units. When preincubation occurred under same conditions with equimolar amount of human C-peptide, no displacement occurred (▲).

served over the 5- to 40-unit range for both the direct-binding and the displacement standard curves (mean r^2 = .98, range .96–1.0). The mean value (±SD) of the slope (m′) of the displacement curves given by $y' = m'x' + c'$ was 0.0095 ± 0.00083, indicating an interassay coefficient of variation (C.V.) of 8.7% (n = 15) for the curve slopes. The intra-assay C.V. of triplicate ΔO.D. measurements of the 10 and 40 Δ±IAA unit standards ranged from 2.6 to 13.1%.

Conversion of O.D. values to arbitrary units from the standard curves improved interassay precision. The C.V. of ΔO.D. values obtained for control serum A, tested in 15 consecutive assays, was 17.1% (mean ΔO.D. ± SD 0.082 ± 0.014) and was reduced to 9.5% for the corresponding Δ±IAA units after interpolation from the standard curves (mean Δ±IAA units ± SD 8.74 ± 0.826). Likewise, control serum B, tested in 9 consecutive assays, had a C.V. of 16.8% (mean ΔO.D. 0.201 ± 0.034) reduced to 9.3% (mean Δ±IAA units 20.18 ± 1.87).

ICA assay. Undiluted serums were screened with indirect immunofluorescence on sections of blood group 0 pancreas following the standard protocol used in the first international workshop for the standardization of ICA determination (2). End-point titration was carried out retrospectively on all positive samples, and ICA titers were converted to JDF units with standard curves constructed from our own standard serum, which was calibrated from the JDF standard (4). With our substrate, the JDF standard serum (designated to contain 80 JDF units) gave end-point titers of 32 when tested blind during two separate serum exchanges organized by the ICA workshops. Fluoresceinated rabbit antihuman IgG (Dakopatts, Glostrup, Denmark) was used.

Other autoantibodies. TGA and TMA were measured with Serodia kits (Fujirebio, Tokyo). PCA and ANA were detected by indirect immunofluorescence with undiluted serum on human stomach and pancreas, and the latter was confirmed at 1/10 serum dilution on rat liver and kidney sections. Rheumatoid factor was measured by latex agglutination.

Statistics. Standard curve equations and correlation coefficients were computed with Cricket Graph programs on an Apple Macintosh computer. For evaluation of the statistical differences between groups, the Mann-Whitney U test, χ^2-test, or Fisher's exact test was used where appropriate. $P < .05$ was regarded as the level of statistical significance.

RESULTS

The IAA frequency distribution for 200 control adults was expressed in direct-binding units and displacement Δ±IAA units (Fig. 2). A normal distribution was not observed by

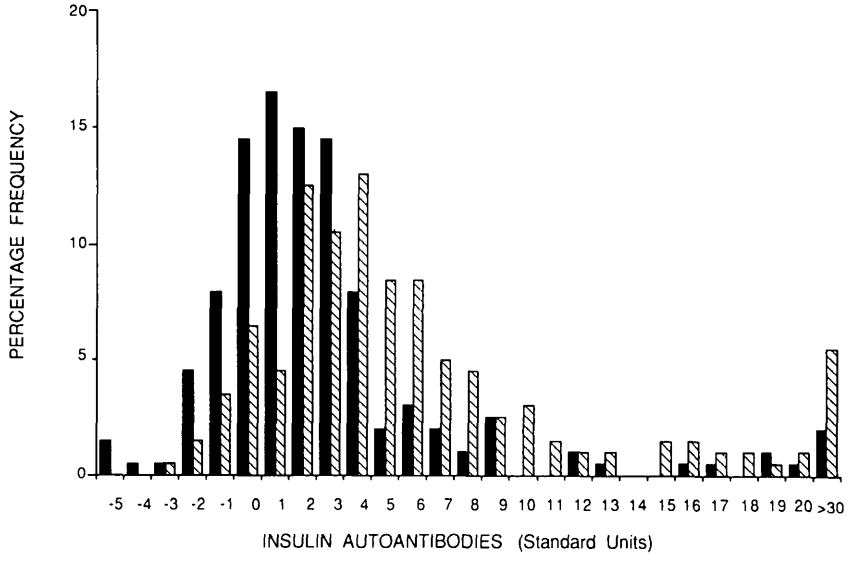

FIG. 2. Frequency distribution of insulin autoantibodies (IAAs) in 200 adult control subjects. *Hatched bars*, values expressed in direct-binding units; *solid bars*, distribution when displaceable Δ±IAA units are used.

either method, but use of the displacement assay narrowed and reduced the main frequency peak from 3 IAA units to 1.5 Δ±IAA units and considerably reduced the number of serums in the 20- to 40-Δ±IAA unit range.

Analysis of the cumulative percentage frequency for displacement IAAs in the adult control group reveals a dual population when plotted on a probability scale (Fig. 3). From this plot, most of the group define an apparently IAA^- population, with a median value of +1.5 and an upper 95% confidence limit at 6.5 Δ±IAA units. However, a second population is clearly defined and consequently such a low cutoff point would result in low specificity.

IAAs in diabetes-related versus control groups. We ascertained where an appropriate upper cutoff level could be set to use the assay to distinguish between disease and health by a comparison of displacement IAAs in all groups studied (Fig. 4). First, the range of measurements in the control adults and children spans that of the disease-related groups. Similar results were obtained previously in the prospective BWFS subjects with a different adult control population and the direct-binding ELISA (11). These findings suggest that there is no cutoff level that will afford a high disease specificity for IAA, and therefore, it is of little use as a marker of prediabetes in the general population. Second, most IAA^+ observations in all groups lie between 12 and 40 Δ±IAA units, thus illustrating the necessity to demonstrate the precision within this range (Fig. 4).

With the nonparametric method of Herrera (14), the 90th percentile of the adult control group lies at 7 Δ±IAA units, with an upper 95% confidence limit at 12 Δ±IAA units. (Corresponding figures for the direct-binding data are 14 and 18 units, respectively.) Apart from the adult control group, few serums were within the 7- to 12-Δ±IAA unit range (Fig. 4), indicating that 12 Δ±IAA units is a satisfactory and statistically sound cutoff level for IAA positivity in this assay.

With the cutoff set at 12 Δ±IAA units, the prevalence of IAAs in the adult control group became 10 of 200 (5%) compared to 6.5% by direct binding with 18 units as the cutoff. The relative frequencies for IAAs in the groups with these cutoff limits are shown in Table 1. In the BWFS subjects, the IAA frequency for those with ICAs >5 JDF units was unaffected whether direct-binding or displacement units were used. In addition, these results did not differ essentially from those reported for first samples at entry into the study (11). For relatives with ICAs ≤5 JDF units, use of Δ±IAA units significantly reduced ($P < .03$) the IAA frequency to 3.5%, a value not significantly different from that of either the child or adult control groups. Approximately 8% of the NIDDM patients were IAA^+ when assessed by either method (NS vs. adult control group).

Although 10 of 12 IAA^+ BWFS relatives were ICA^+, ICAs were infrequently associated with IAAs in the other groups ($P < .0001$): 0 of 8 control children, 1 of 10 control adults, and 1 of 18 NIDDM patients had ICAs >5 JDF units. A low prevalence of other autoantibodies was observed in those who were IAA^+. PCA, TGA, TMA, ANA, and rheumatoid factor were absent in 12 BWFS relatives, 1 control child had TMA, 1 control adult TMA and PCA, and of the 18 NIDDM patients, 3 had ANA, 1 had PCA, 1 had TMA, and 1 had rheumatoid factor. These frequencies were not different for IAA^- individuals.

Comparative predictive value of IAAs and ICAs for IDDM. In first-degree relatives with ICAs >5 JDF units, IAAs were observed in 6 of 10 with ICAs >40 JDF units and in 4 of 21 with ICAs >5–40 JDF units ($P < .03$). Both groups had significantly higher IAA frequency than those with ICAs <5 JDF units ($P < .002$ and $P < .05$, respectively). IAAs were found in 6 of 12 (50%) who progressed to IDDM and in 4 of 18 (22.2%) who remain healthy (Table 1). Mann-Whitney *U* analysis confirmed that peak Δ±IAA units are significantly higher ($P < .008$) in those who developed IDDM than in those who remain healthy.

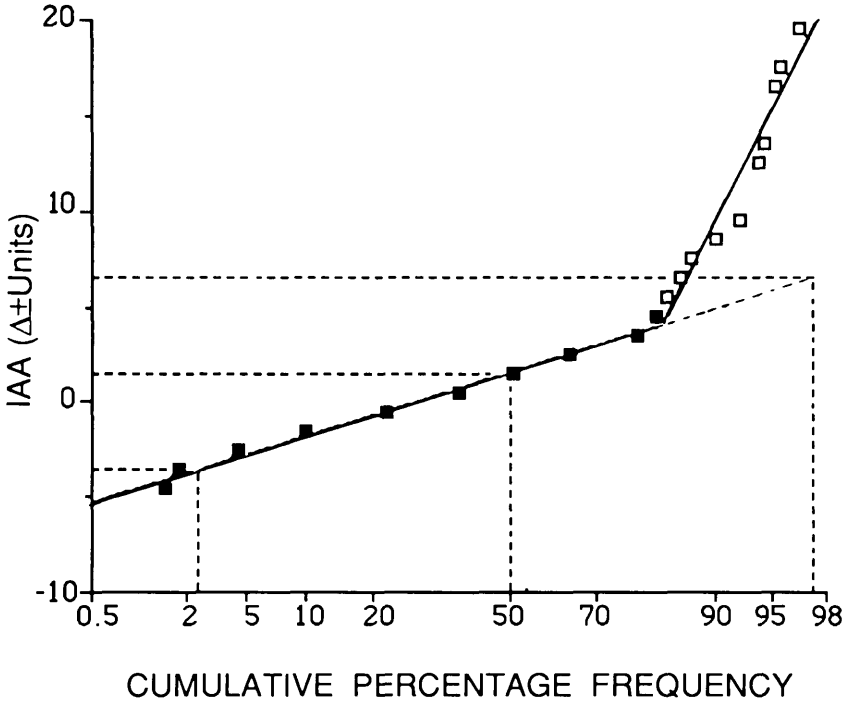

FIG. 3. Cumulative percentage frequency of insulin autoantibodies (IAAs) describes dual population (■, □) in 200 adult control subjects when results by displacement enzyme-linked immunosorbent assay are plotted on probability scale. Only 88% of this group have IAA values that lie within 95% confidence limits (*dotted lines*) of median value for whole group (1.5 Δ±IAA units).

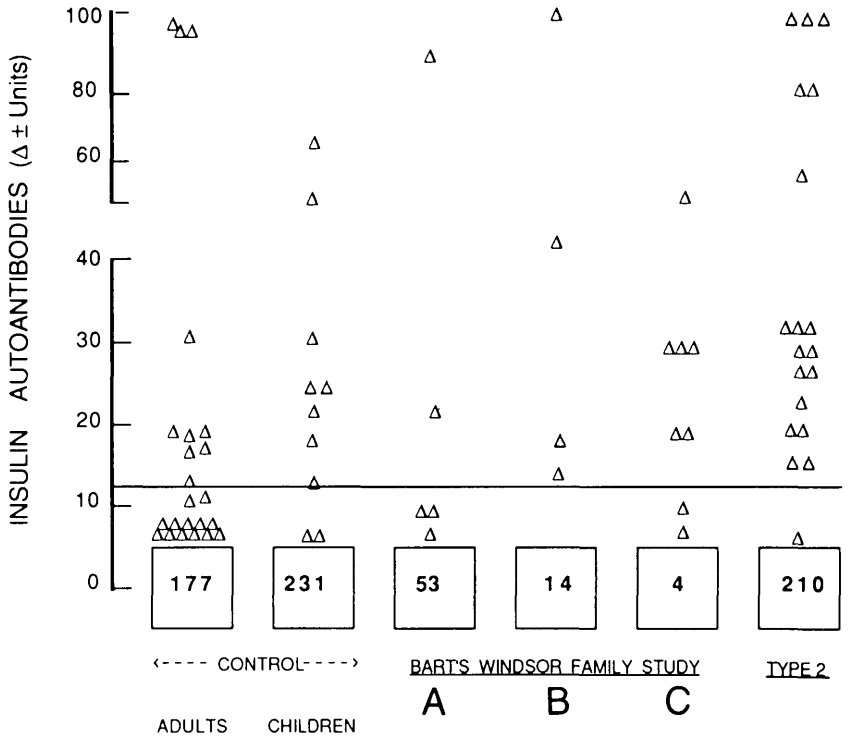

FIG. 4. Distribution of insulin autoantibodies (IAAs) by displacement enzyme-linked immunosorbent assay (Δ ± IAA units) in control adults and children, first-degree relatives of prospective Bart's Windsor Family Study (*A*: nondiabetic, islet cell antibodies [ICAs] ≤5 Juvenile Diabetes Foundation [JDF] units; *B*: nondiabetic, ICAs >5 units; *C*: insulin-dependent diabetic, ICAs >5 JDF units), and non-insulin-dependent (type 2) diabetic subjects without exogenous insulin therapy.

When the predictive values of the two autoantibodies are compared (Table 2), it is clear that IAAs alone are poor predictors of future IDDM development. The sensitivity and positive predictive value P+ for IAAs are only half that for ICAs >5 JDF units, although comparable values for the specificity and negative predictive value P− are seen. When both markers occur conjointly, P+ is raised from 40 (ICAs) to 60%, with increased specificity (99.3%) but reduced sensitivity (42.9%).

DISCUSSION

The recent detection of IAAs in prediabetes has been achieved by optimizing existing RIAs for antibodies to injected insulin (15) and the introduction of ELISA (16,17). This sensitive displacement ELISA confirms earlier reports of a significant association of IAAs with ICAs in an IDDM-susceptible cohort (11,12,18,19), fails to show any association of IAAs with ICAs in NIDDM patients, and clearly demonstrates the existence of IAAs in both adult and child control groups. Incorporation of standard curves and displacement has improved the precision and increased the specificity of the assay.

It has yet to be established whether common or different epitopes are recognized by RIA and ELISA, and although the latter readily discriminates between class IgG and IgM IAAs, this is not true for RIA. Both methodologies have reported IAAs in ~40% of untreated IDDM subjects (6), a frequency ~50% that observed for ICAs in newly diagnosed IDDM (1). Despite this apparent concordance, serum exchanges in the past two IAA international workshops have revealed systematic variation between the two methodologies.

It has also emerged from IAA workshops that serums suitable for the construction of standard curves in solid-phase assays may not be applicable for liquid-phase assays. In fact, the standard serum used in this study was scored pos-

TABLE 1
Relative frequency of insulin autoantibodies (IAAs) by direct-binding and displacement of enzyme-linked immunosorbent assay (ELISA)

		ELISA	
	n	Direct binding (IAA >18 units)	Displacement (IAA >12 Δ±units)
Control children	241	12 (5)	8 (3.3)
Bart's Windsor Family Study			
ICAs ≤5 JDF units (nondiabetic)	58	7 (12.1)	2 (3.5)*
ICAs >5 JDF units (nondiabetic)	18	3 (16.6)	4 (22.2)
ICAs >5 JDF units (developed IDDM)	12	6 (50)	6 (50)
NIDDM	229	20 (8.7)	18 (7.9)

Values in parentheses are percentages. ICAs, islet cell antibodies; JDF, Juvenile Diabetes Foundation; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus. Units (see RESEARCH DESIGN AND METHODS) were measured in the same sample simultaneously. NIDDM subjects had no exogenous insulin therapy.
*$P < .03$ vs. direct binding.

TABLE 2
Comparison of predictive values for insulin-dependent diabetes mellitus (IDDM) with insulin autoantibodies (IAAs) and islet cell antibodies (ICAs) in Bart's Windsor Family Study cohort

	IAA alone		ICA alone		Both markers	
	+	−	+	−	+	−
Developed IDDM	6	8	12	2	6	8
Healthy	24*	577*	18	583*	4	597
Assay characteristics						
Sensitivity	6 of 14 (42.9)		12 of 14 (85.7)		6 of 14 (42.9)	
Specificity	577 of 601 (96)		583 of 601 (97)		597 of 601 (99.3)	
P+	6 of 30 (20)		12 of 30 (40)		6 of 10 (60)	
P−	577 of 585 (98.6)		583 of 585 (99.7)		597 of 605 (98.7)	

Values in parentheses are percentages. P+, predictive value of positive test for IDDM; P−, predictive value of negative test for health. Positive values based on >12 Δ±units for IAAs and >5 Juvenile Diabetes Foundation units for ICAs. Negativity for both markers includes positivity for either alone.
*Values were calculated in relation to proportion of total cohort studied for IAAs.

itive in 100% of ELISAs but negative in 100% of RIAs in the first IAA workshop. However, we have shown that this serum not only binds to human insulin in the solid phase in a dose-dependent manner at 1/2000–1/250 dilution but also that the binding can be effectively displaced by human insulin and human proinsulin and not by human C-peptide, which indicates that specificities for the antigen-binding sites reside in the A- and/or B-chain of the insulin/proinsulin molecule but not in the C-peptide moiety. We know that IAAs in the standard serum are detected by an antibody to the $F(ab)_2$ portion of human IgG, do not contain IgM IAAs, and bind similarly to human, porcine, and bovine insulins and human proinsulin but not to human C-peptide in the solid phase. In addition, solid-phase binding can be quenched dose dependently by preincubation with human, porcine, or bovine insulin or human proinsulin in the 0.01- to 1-U/ml range (data not shown).

Although our displacement ELISA has been shown to be concordant with a recently described displacement RIA for antibodies to exogenous insulin (20), in an initial comparison, the latter method was unable to detect any of the ELISA IAA^+ serums from either the BWFS or control groups. ELISA can readily detect low-affinity antibodies in serums, but the ratio of antigen to antibody in liquid-phase assays may be more attuned to the detection of higher-affinity antibodies. Herein lies a plausible explanation for the reported normal distribution of a control population in the displacement RIA (20), whereas a normal distribution was not observed for the control group in this study.

In our ELISA and others, antibody binding is unaffected by endogenous levels of insulin in normal or diabetic serums (16,17). In preliminary experiments to establish human insulin levels required to displace solid-phase binding, signals from insulin-treated diabetic and ICA^+ individuals ($n > 100$) were quenched 75–95% with 1 U/ml human insulin. Conversely, some ICA^- serums with 5–20 IAA units required 10 to 100-fold more human insulin to achieve >50% quenching. Because nonspecific binding is accounted for in our assay, we conclude that preincubation at 1 U/ml human insulin provides an excellent monitor to distinguish higher- from lower-affinity antibodies.

The contradictory results reported here by two assays that have both been well defined in terms of precision, sensitivity, and specificity confirm earlier suspicions that they are in fact recognizing different binding properties of the antibodies under widely differing experimental conditions. Sodoyez-Goffaux et al. (21) have recently shown that iodine substitution on the A14-terminal of insulin may significantly affect the affinity of some insulin antibodies for the ligand, a factor that could also explain some of the observed discrepancies between ELISA and RIA.

The data from this and other ELISAs indicate a higher detection rate in ICA^+ susceptible individuals than that for RIA (18,22–25), with one exception (19). Whether this is due to lower affinity in the prodromal period remains to be clarified, but supportive evidence comes from our finding of >12 Δ±IAA units in control adult and child groups (5 and 3.3%, respectively) in comparison with the failure of displacement RIAs to detect this antibody in control groups (19,20).

The combined positive predictive value of both markers for IDDM, when a cutoff of 5 JDF units is used for ICAs, is only 60%, with a predictive value for health of 98.7% in their absence (Table 2). Vardi et al. (19), with a modified displacement RIA and an ICA assay with a high detection threshold, found 40.3% of 62 ICA^+ first-degree relatives had elevated IAAs and postulated that 90% of newly diagnosed children are positive with one or the other marker. Subdivision of the group showed a higher IAA prevalence (55.6%) in those <30 yr of age than in those >30 yr of age (19.2%). With our lower ICA cutoff of 5 JDF units, 9 of 10 siblings and 3 of 4 parents who developed IDDM were ICA^+ (a sensitivity for ICA alone of 86%); 5 of 9 (55.5%) and 1 of 3 (33.3%) of these had IAAs, but neither of the 2 ICA^- relatives (1 sibling and 1 parent) who developed IDDM were IAA^+. Because of the small number of siblings, it is difficult to ascertain from our study whether IAAs are more frequently found in younger siblings. Although we observed a similar IAA frequency in ICA^+ siblings who developed IDDM, as in the group discussed above, analysis of our sibling data alone did not increase the combined predictive power of the two markers.

In our study, the presence of IAAs without ICAs did not incur an increased risk for IDDM. Therefore, we suggest that IAA screening by ELISA should be restricted to those of known ICA status. It appears important to continue the search for new markers that might narrow the "window" dur-

ing the long latency period and thus improve our ability to predict diabetes onset. Selective immune intervention at that time could ultimately lead to prevention of the disease.

ACKNOWLEDGMENTS

We are grateful to all families who cooperated enthusiastically in the Bart's Windsor Family Study and the physicians who were responsible for their welfare and the meticulous collection of samples during the past 10 yr, in particular Drs. A. Gorsuch, K. Spencer, A. Tarn, and P. Bingley. In addition, we thank and acknowledge the collaboration of Dr. A. Kurtz for comparative radioimmunoassay measurements, Drs. J. Bodansky and K. Spencer for samples and data from the non-insulin-dependent diabetic groups, Prof. M.A. Preece for allowing access to the control child serums, and Dr. G. Schwarz for enabling us to obtain a reference bank of control adult serums. We are also indebted to A. Saunders and M. Shattock for skilled technical assistance with islet cell antibody and other autoantibody screening and M. Pine for help in preparing the manuscript.

This work was partially supported by the British Diabetic Association, the Medical Research Council (UK), and Novo Research Institute (Copenhagen).

During this study, E.B. was on study leave from the Department of Clinical Immunology, Queen Elizabeth II Medical Centre, Perth, Western Australia.

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