ICA512 Autoantibody Radioassay

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As part of a general program of screening islet expression libraries we have identified a clone from a λgt11 human islet expression library that reacts with human diabetic sera and, upon sequencing, was determined to be the neuroendocrine islet autoantigen ICA512 (islet cell antigen 512). In the current communication, we describe the development of a radioassay for autoantibodies to ICA-512 (ICA512AA) using in vitro transcribed and translated protein for production of labeled antigen. Our initial results indicate that this radioassay is significantly more sensitive than the enzyme-linked immunosorbant assay, which uses a COOH-terminal fragment of ICA512. The ICA512AA radioassay uses a 96-well format with membrane separation of antibody bound from free antigen and should be readily adaptable to automated large-scale screening. Only 7 μl of serum is required for triplicate determinations. In order to determine the specificity and sensitivity of this assay and estimate its positive predictive value, we have studied 42 new-onset diabetic patients, 33 first-degree relatives of diabetic patients followed to diabetes, 694 islet cell antibody–negative (ICA−) relatives, and 205 normal control subjects. Thirty-eight percent of new-onset patients and 48% of relatives followed to diabetes express autoantibodies to ICA512 exceeding the 99th percentile of the normal control subjects. In contrast, only 1.4% of ICA− first-degree relatives were positive for ICA512 autoantibodies. By using three radioassays for islet autoantibodies against insulin, glutamic acid decarboxylase 65 (GAD_{65}), and ICA512, 100% of the prediabetic sera we have studied have been shown to express antibodies to at least one antigen, and the majority (88%, 27 of 31) express two or more. ICA512 autoantibodies provide a specific marker for type I diabetes and, in combination with antibodies to GAD_{65} and insulin, should facilitate the prediction of type I diabetes. *Diabetes* 44:1340–1344, 1995

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AA, autoantibody; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; GAA, GAD_{65} autoantibody; GAD, glutamic acid decarboxylase; IAA, insulin autoantibody; ICA, islet cell antibody/antigen; IDDM, insulin-dependent diabetes mellitus; PCR, polymerase chain reaction.

During the past several years, an increasing number of islet autoantigens have been identified (1–3), including insulin (4), glutamic acid decarboxylase 65 (GAD_{65}) and GAD_{67} (5), a 37-kDa molecule (6), islet cell antigen 69 (ICA69) (7), carboxypeptidase H (8), and a GM2–1 ganglioside (9). To date, of assays with high specificity and sensitivity, only assays for autoantibodies to insulin (IAAs) and GAD_{65} (GAAs) have been developed to the stage where thousands of serum samples have been evaluated. Workshops for both IAA and GAA assays have fostered international standardization of these assays (10). Other molecules have not yet been fully characterized (37 kDa) and some assays are not in a radioassay format (ICA69, GM2–1 autoantibodies). For predicting type I diabetes with assays for antibodies to defined islet autoantigens, there is a need for at least one more quantitative assay (in addition to GAA and IAA assays). This is particularly important if algorithms, which depend on the detection of two or more autoantibodies in at-risk populations, are to be developed. In this report, we describe the development of a radioassay for autoantibodies reacting with ICA512, which can substantially contribute to a panel of biochemical markers.

RESEARCH DESIGN AND METHODS

Subjects. The subjects of the current study consisted of 42 patients with new onset of type I diabetes (tested within 7 days of diagnosis), 33 prediabetic relatives of patients with type I diabetes (from the Joslin Diabetes Center and the Barbara Davis Center, who were followed to the onset of overt diabetes), 694 nondiabetic islet cell antibody–negative (ICA−) relatives (with sera obtained at the time of their initial screening), and 205 healthy control subjects with no family history of type I diabetes. The median age of the new-onset patients was 11.0 years (range 2.2–33 years), and the median age of the control subjects was 16.2 years (range 0.4–67.5 years). The median age of prediabetic relatives at the time of obtaining the serum sample for determination of autoantibodies was 11.2 years with a range of 2.5–66.4 years. The median age at diagnosis of diabetes in the prediabetic group was 11.6 years with a range of 3.9–69 years. For these control subjects, the mean follow-up time (from sample collection to diabetes onset) was 1.78 years (SD = 1.44) with a range of 0.03–5.6 years. The median age of the ICA− relatives at testing was 13 years with a range of 0.1–76.8 years. Studies were carried out with the oversight of our Institutional Review Board and with informed consent for obtaining research sera.

Diagnosis of diabetes. Type I diabetes was diagnosed by standard adult National Diabetes Data Group criteria. With the close follow-up of first-degree relatives, diabetes in the majority of relatives was diagnosed by either the finding of fasting hyperglycemia at the time of routine follow-up or, upon oral glucose tolerance testing, the finding of 1- and 2-h glucose levels >200 mg/dl.

Molecular screening, sequencing, and cloning of ICA512. A human islet λgt11 expression library was screened with a pool of five sera according to techniques previously published (7,8). These serum samples were collected from three ICA-positive (ICA^+) first-degree relatives

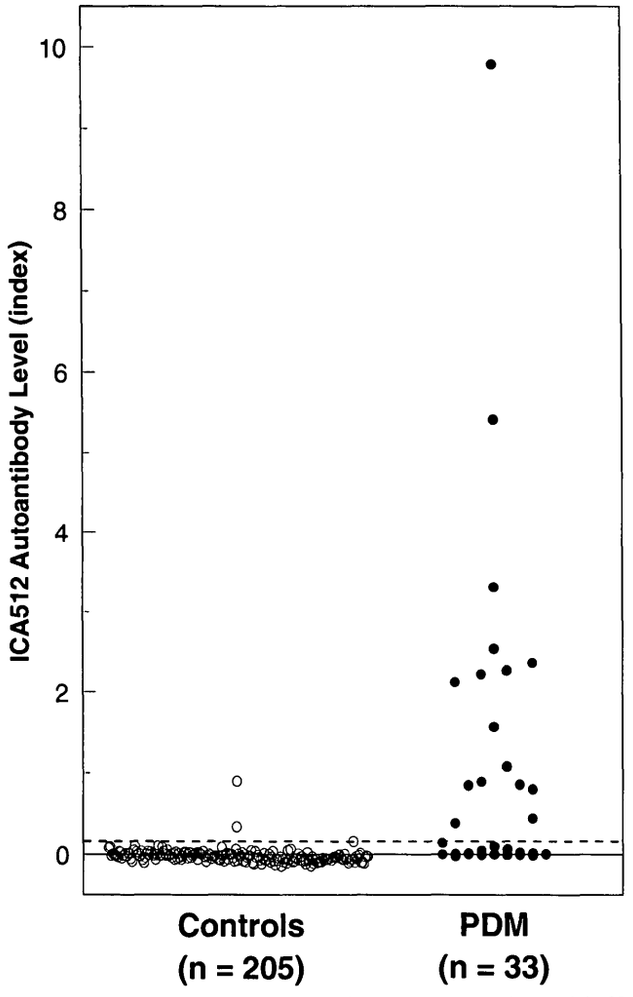

FIG. 1. The levels of ICA512 antibodies (determined by radioassay) in 33 prediabetic relatives (PDM) and 205 healthy control subjects. - - - -, 99th percentile of the control subjects (index of 0.16). The difference between the two groups is highly statistically significant ($P < 0.001$, Wilcoxon's test).

of type I diabetes patients who subsequently developed diabetes and from two ICA$^+$ relatives who did not progress to type I diabetes. Approximately 1.5×10^5 plaque forming units were screened until a positive clone (HB-1) was found. Two out of five individual sera reacted with HB-1.

The insert from the positive clone was amplified by polymerase chain reaction (PCR) using λgt11-specific primers, separated on 1% agarose gel, and subsequently purified using a QIA-EX kit (Qiagen, San Diego, CA). This DNA was directly sequenced using PCR-based sequencing (New England Biolabs, Beverly, MA) and was identified as the islet antigen ICA512 previously described by Rabin et al. (11). In order to amplify the coding region of the λgt11 molecule, the following primers were designed on the basis of the published sequence: 5′ TAT GGA TCC GTT GGA GCT GAT ATC 3′ and 5′ TTA TCT AGA GGT CAC GGA CAT GCT3′. The PCR-amplified product of the HB-1 λgt11 clone was 1.6 kb consistent with the full-length ICA512 coding region. This insert was subcloned in a TA-cloning plasmid pCRII (InVitrogen) and used for in vitro translation.

Autoantibody assays

Insulin. IAAs were measured with our standard IAA radioassay (12), using competition with unlabeled insulin and 600 μl of sera per determination (150-μl duplicates with and without unlabeled insulin), which in Immunology of Diabetes Workshop proficiency testing, generally gives 100% sensitivity and specificity. The cutoff for this assay, based on the 99th percentile of levels in the 205 control subjects, is 42 nU/ml. The interassay coefficient of variation (CV) is 10.3% (n = 7) (12).

GAD_{65}. GAAs were determined with the assay described by Lernmark et al. (13). The assay uses in vitro transcribed and translated GAD_{65} and immunoprecipitation. The results are expressed as an index (index = sample cpm − negative control cpm/positive control cpm − negative control cpm). The 99th percentile of levels in the 205 control subjects for this assay was an index of 0.032. The interassay CV was 6.5% (n = 10) and the intra-assay CV was 5.0% (n = 6).

ICA512 ELISA. The ICA512 enzyme-linked immunosorbent assay (ELISA) was performed as described by Rabin et al. (11) using the COOH-terminal 1/2 of ICA512 as antigen. The ELISA interassay CV was 29% (n = 20).

ICA512 radioassay. The ICA512 radioassay used a format similar to the one used for GAA determination (13). The ICA512 cDNA cloned in the TA-cloning plasmid pCRII was in vitro transcribed/translated with a TNT rabbit reticulocyte lysate kit (Promega), and the translated protein was labeled with [^{35}S]methionine (Amersham). ^{35}S-labeled ICA512 (20,000 cpm) was used for incubation with patients' serum at a 1:25 dilution overnight at 4°C in buffer (20 mmol/l Tris-HCl (pH 7.4), 150 mmol/l NaCl, 0.1% aprotinin, 10 mmol/l benzamidine, 0.1% bovine serum albumin, and 0.15% Tween-20). Of this incubate, 50 μl was added to 50

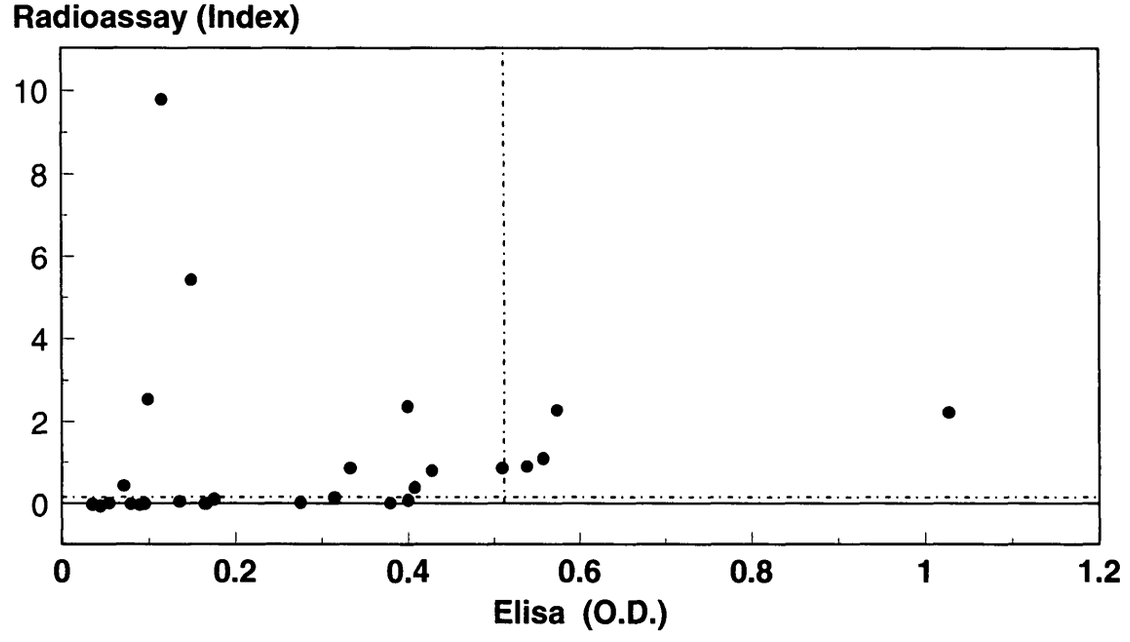

FIG. 2. Autoantibody levels of prediabetic relatives measured by radioassay (y-axis, index values) and by ELISA (x-axis, optical density units). - - - -, 99th percentile of normal control subjects for the two assays.

μl of protein A–sepharose (50%, Pharmacia, Uppsala, Sweden) in a MultiScreen-DV 96-well filtration plate (Millipore), with shaking for 45 min at 4°C, and washed with cold washing buffer for three cycles (each cycle with three washes and 5 min shaking at 4°C between cycles). Using the MultiScreen punch system, the sepharose and the membrane at the bottom of the well was punched out into scintillation vials and radioactivity (cpm) was counted in a beta counter. Results were expressed as an index, calculated with the same formula used in the GAD_{65} radioassay (13) (see GAA radioassay). The cutoff for this assay, based on the 99th percentile of the 205 control subjects, is an index of 0.16. The ICA512 radioassay has an interassay CV of 9.6% (n = 12). The intra-assay CV was 6.0% (n = 7).

ICA. The ICA assay was performed using indirect immunofluorescence (for samples from the Barbara Davis Center) or protein A immunoperoxidase staining (for samples from the Joslin Diabetes Center) on unfixed frozen pancreatic sections. In the 1993 8th Immunology of Diabetes ICA Proficiency Test (organized by Noel Maclaren at the University of Florida), the Barbara Davis Center assay achieved a rating of 100% for all workshop parameters.

Statistical analysis. SAS software (SAS Institute, Cary, NC) was used for Wilcoxon's rank-sum test.

RESULTS

The λgt11 PCR-amplified product of clone HB-1 was 2.8 kb in length. Using direct PCR-based sequencing, the clone was identified as ICA512. The coding region of the molecule was PCR amplified from the original HB-1 insert using appropriate primers based on the ICA512 GenBank sequence and cloned into a TA-cloning plasma pCRII vector (InVitrogen). [^{35}S]methionine-labeled ICA512 was produced with transcription and translation of ICA512 plasmid DNA and was used in our radioassay without further purification.

Forty-eight percent (16 of 33) of prediabetic subjects (Fig. 1) and 38% of new-onset patients (16 of 42) had ICA512AA levels exceeding the 99th percentile of the control group versus 1.4% (10 of 694) of ICA− nondiabetic relatives of patients with type I diabetes. We did not observe any significant difference in ICA512AA positivity when comparing relatives progressing to diabetes before (13 of 25) and after (3 of 8) the age of 20 years. Both normal control subjects and ICA− first-degree relatives were evaluated to estimate assay specificity in populations at low risk for progression to diabetes. The prevalence of ICA512AAs is not significantly different between these two low-risk groups (Table 2).

Twenty-seven prediabetic sera were evaluated with both the ICA512 ELISA and radioassay (Fig. 2). The radioassay detected as positive three times as many prediabetic relatives as the ELISA assay.

Discrepancies between the ELISA and the radioassay do not appear to reflect simply quantitative differences; some of the strongest sera with the radioassay were completely negative with the ELISA assay. It is possible that these individuals expressed antibodies to epitopes that were not present in the truncated ICA512 molecule used in the ELISA assay.

Table 1 summarizes data for ICA512, GAD_{65}, and IAAs of the prediabetic subjects, as well as the age of onset of diabetes and the presence or absence of cytoplasmic ICA. Relatives of diabetic patients are listed in Table 1 in order of their ICA512AA values. All prediabetic relatives expressed at least one autoantibody determined by radioassay, 88% (27 of 31) expressed two or more of these autoantibodies, and 33% (9 of 27) expressed all three autoantibodies. This compares with 95% (40 of 42) of new-onset patients positive for at least one autoantibody determined by radioassay, 64% (27 of 42) positive for two or more, and 21% (9 of 42) positive for all three. Positive ICA levels are expressed in Juvenile Diabetes Foundation units or, in earlier measurements, on a qualitative scale from weakly positive to strongly positive. Six of the prediabetic subjects were negative for cytoplasmic ICAs on the serum sample evaluated for autoantibodies measured by radioassay. Of note, with the number of control subjects evaluated to date (n = 205) and cutoffs representing 99th percentiles, none of the control subjects were positive for more than a single autoantibody. Table 2 indicates sensitivity, specificity, and the estimated positive and negative predictive values for the three radioassays used in this study among first-degree relatives and in the general population.

TABLE 1
Relatives of patients with type I diabetes followed to the development of diabetes

ID No.	Age at onset	ICA512AA (index)	GAA (index)	IAA (nU/mll)	Anti-bodies	ICA
6573	37	−0.065	**1.23**	29	1	320
420	12.7	−0.035	**1.58**	**139**	2	80
3185	35.4	−0.032	**1.73**	13	1	320
4244	10.1	−0.023	**0.31**	**313**	2	3*
7354	9.5	−0.016	**0.35**	**133**	2	0
2192	9.1	−0.016	**0.05**	−8	1	320
3440	69	−0.013	**1.26**	**79**	2	5*
2054	11.5	−0.012	**1.05**	**179**	2	4*
5032	12.5	−0.005	**0.05**	**184**	2	20
3291	4	−0.002	0.004	**372**	1	3*
7353	5.8	0.010	**0.154**	**562**	2	40
10791	5.1	0.011	**1.43**	**183**	2	640
11429	10.3	0.017	**0.76**	**441**	2	0
4989	59	0.043	NA	NA	NA	2*
10507	5.6	0.057	−0.483	**147**	2	0
3716	35	0.093	NA	8	NA	0
571	17.2	0.135	**1.23**	**50**	2	0
3626	16.8	**0.375**	**0.48**	29	2	0
2201	28.6	**0.429**	**1**	5	2	160
1587	16.2	**0.793**	**0.35**	**88**	3	2*
18684	4.4	**0.841**	**1.672**	**2823**	3	320
8911	3.9	**0.850**	**1.598**	**1953**	3	640
205707	7.1	**0.888**	−0.0234	**419**	2	40
8105	10.9	**1.073**	0.0291	**159**	2	160
5097	13.5	**1.565**	0.028	**288**	2	80
4674	8.9	**2.119**	0.0316	**147**	2	320
2279	11.6	**2.215**	**0.41**	**54**	3	3*
533	11.4	**2.263**	**1.07**	**268**	3	160
3687	14.2	**2.358**	**0.6364**	**116**	3	320
2360	29.4	**2.534**	**1.2382**	**142**	3	6*
1450	8.8	**3.308**	**1.00**	**221**	3	4*
4147	14.4	**5.409**	**0.284**	**162**	3	80
2316	60.6	**9.782**	**1.04**	37	2	6*

Levels of autoantibodies of 33 prediabetic relatives with their age at onset and numbers of autoantibodies determined by radioassay (positive values in bold type and NA = sample not available). The ICA values are expressed in Juvenile Diabetes Foundation (JDF) units when available. However, for measurements before the introduction of the JDF standard (*), the values are expressed as a subjective rating (with 0 as negative and positive rated 1–6).

DISCUSSION

The molecule ICA512 is a 548–amino acid neuroendocrine protein with significant homology to the T-cell CD45 tyrosine phosphatase. Its sequence is compatible with a single transmembrane spanning region and a cytoplasmic domain. ICA512 messenger RNA is expressed in the pancreas and brain (11). The neuroendocrine expression of ICA512 is similar to that reported for a series of islet autoantigens,

TABLE 2
Estimates of sensitivity, specificity, and positive and negative predictive values in relatives and the general population

				Number of autoantibodies (ICA512AA, GAA, IAA)	
	ICA512AA (%)	GAA (%)	IAA (%)	≥1 AB (%)	≥2 AB (%)
Sensitivity (new-onset patients)	38.1	78.6	64.3	95.2	64.3
Sensitivity (prediabetic relatives)	48.5	83.9	78.1	100†	87.7
Specificity (control subjects)	99.0	99.0	99.0	97.1	100*
Specificity (ICA^- relatives)	98.6	91.1	94.2	87.3	96.5
Positive predictive value (general population)	16.4	28.8	24.9	14.1	100*
Positive predictive value (relatives)	74.5	44.9	54.1	40.7	68.8
Negative predictive value (general population)	99.7	99.9	99.8	99.98	99.8
Negative predictive value (relatives)	95.7	98.5	98.0	100†	98.9

For the general population, sensitivity was determined as the proportion autoantibody positive among 42 new-onset patients, specificity as the proportion autoantibody negative among 205 control subjects, and positive and negative predictive values were estimated from these figures using Bayes' theorem with a disease prevalence of 0.5%. For relatives, sensitivity was determined as the proportion autoantibody positive among 33 prediabetic relatives (subsequently developing diabetes), specificity was estimated as the proportion autoantibody negative among 694 low-risk (ICA^-) relatives, and positive and negative predictive values were estimated from these figures using Bayes' theorem with a disease prevalence of 8%. Specificity is likely to be underestimated in that some ICA^- relatives do progress to diabetes. *None of the 205 control subjects was positive for ≥2 autoantibodies, but if 1 of 205 was positive (a proportion that cannot be excluded with this data) then the estimate of positive predictive value falls to 40%. †In a similar manner, if one relative progressed to diabetes without expressing any of the three autoantibodies, negative predictive value decreases to 99.7%.

including GAD (14) and carboxypeptidase H (8). Recently, Lan et al. (15) reported the isolation of a neuroendocrine molecule termed IA-2, whose DNA sequence is nearly identical to ICA512, over 3.3 kb and with 310 additional bases at the 5′ end. Further studies are needed to elucidate the relationship between the two molecules.

The physiological function of ICA512 is unknown. Our independent isolation of ICA512 from an islet expression library makes it the first repeat molecule to our knowledge retrieved from an islet expression library with patients' sera. It is currently unknown how many islet molecules will be found to be the target of autoantibodies and thus how many additional molecules will be detected with the screening of such libraries. One advantage of identification of autoantigens by screening expression libraries is the rapidity with which it is now possible to obtain sequence information, determine the tissue distribution of messenger RNA expression, and most importantly, develop an autoantibody radioassay with in vitro transcription and translation of the cloned DNA.

The current report is the initial description of a radioassay detecting autoantibodies reacting with recombinant ICA512. With assay cutoff at the 99th percentile of normal control subjects, autoantibodies for ICA512 were present in 48% of relatives who progressed to diabetes and in 38% of new-onset patients but in only 1.4% of ICA− nondiabetic relatives. Therefore, autoimmunity to ICA512 is strongly associated with type I diabetes, with sensitivity of 38% in new-onset patients (and 48% in relatives with β-cell autoimmunity subsequently progressing to overt diabetes), and specificity of 99%. Applying Bayes' theorem and using figures of 8 and 0.5% for disease prevalence in relatives and the general population respectively, we calculate positive predictive values for this test of 74.5% in relatives and 16.4% in the general population (Table 2). The assay is in a format similar to that used for the detection of GAD_{65}AAs, and it is likely that given the similar formats and serum volumes, it will be possible to determine both autoantibodies in the same assay with differential labeling (e.g., [^{35}S]methionine, [^{3}H]leucine). This should facilitate the screening of the thousands of samples necessary to fully evaluate the prognostic significance of ICA512AAs in conjunction with other autoantibodies. Our preliminary studies of 33 prediabetic individuals suggest that the detection of these three autoantibodies with the assays used will give a sensitivity higher than reported for cytoplasmic ICA testing.

In a recent report, Bingley et al. (3) analyzed insulin, GAD, and anti-37-kDa autoantibodies in type I diabetic relatives who subsequently did or did not progress to type I diabetes. The present report is consistent with the finding that a panel of antigens is more useful than any individual autoantigen in the prediction of type I diabetes. Though Bingley et al. (3) include ICA in their algorithm for predicting type I diabetes, it is possible that a combination of defined antigens will supplant this test. In terms of the current sensitivity from 95 to 100% for any single antibody and 88% for two autoantibodies with radioassay determination of GAA, IAA, and ICA512AA, there may be little need to determine cytoplasmic ICA. To fully validate the above concepts, large numbers of relatives who have and have not progressed to diabetes, as well as the general population, will need to be studied. The convenience of the three autoantibody assays described in this report makes it likely that such studies can be readily accomplished.

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REFERENCES

1. Eisenbarth GS, Ziegler A, Colman P: Pathogenesis of insulin-dependent (type I) diabetes mellitus. In *Joslin's Diabetes Mellitus*. Weir GC, Kahn CR, Eds. Philadelphia, Lea & Febiger, 1994, p. 216–239
2. Atkinson MA, Maclaren NK: Islet cell autoantigens in insulin-dependent diabetes. *J Clin Invest* 92:1608–1616, 1993
3. Bingley PJ, Christie MR, Bonifacio E, Bonfanti R, Shattock M, Fonte M,

Bottazzo G, Gale EAM: Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. *Diabetes* 43:1304–1310, 1994
4. Srikanta S, Ricker AT, McCulloch DK, Soeldner JS, Eisenbarth GS, Palmer JP: Autoimmunity to insulin, β-cell dysfunction and development of insulin-dependent diabetes mellitus. *Diabetes* 35:139–142, 1986
5. Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ: Autoimmunity to two forms of glutamate decarboxylase in insulin dependent diabetes mellitus. *J Clin Invest* 89:283–292, 1992
6. Christie MR, Richard YM, Tun RYM, Lo SSL, Cassidy D, Brown TJ, Hollands J, Shattock M, Bottazzo GF, Leslie DG: Antibodies to GAD and tryptic fragments of islet 64K antigen as distinct markers for development of IDDM: studies with identical twins. *Diabetes* 41:782–787, 1992
7. Pietropaolo M, Castano L, Babu S, Buelow R, Martin S, Martin A, Powers A, Prochazka M, Naggert J, Leiter EH, Eisenbarth GS: Islet cell autoantigen 69 kDa (ICA69): molecular cloning and characterization of a novel diabetes associated autoantigen. *J Clin Invest* 92:359–371, 1993
8. Castano L, Russo E, Zhou L, Lipes MA, Eisenbarth GS: Identification and cloning of a granule autoantigen (carboxypeptidase H) associated with type I diabetes. *J Clin Endocrinol Metab* 73:1197–1201, 1991
9. Dotta F, Previti M, Lenti L, Dionisi S, Casetta B, D'Erme M, Eisenbarth G, DiMario U: GM2–1 pancreatic islet ganglioside: identification and characterization of a novel islet-specific molecule. *Diabetologia.* In press
10. Wilkin T, Palmer J, Kurtz A, Bonifacio E, Diaz J: The second international workshop on the standardization of insulin autoantibody (IAA) measurement. *Diabetologia* 31:449–450, 1988
11. 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: 3183–3187, 1994
12. Vardi P, Dib SA, Tuttleman M, Connelly JE, Grinbergs M, Rabizadeh A, Riley WJ, Maclaren NK, Eisenbarth GS, Soeldner JS: Competitive insulin autoantibody RIA: prospective evaluation of subjects at high risk for development of type I diabetes. *Diabetes* 36:1286–1291, 1987
13. Grubin CE, Daniels T, Toivola B, Landin-Olsson M, Hagopian WA, Li L, Karlsen AE, Boel E, Michelsen B, Lernmark A: A novel radiogland binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. *Diabetologia* 37:344–350, 1994
14. Petersen JS, Russel S, Marshall MO, Kofod H, Buschard K, Cambon N, Karlsen A, Boel E, Hagopian WA, Hejinaes KR, Moody A, Dyrberg T, Lernmark A, Madsen OD, Michelsen BK: Differential expression of glutamic acid decarboxylase in rat and human islets. *Diabetes* 42:484–495, 1993
15. 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