Protein Tyrosine Phosphatase–Like Proteins: Link With IDDM

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IDDM is an autoimmune disease that is clinically evident when <10% of the residual β-cell mass has been destroyed (1). Either humoral or cellular abnormalities are present before the appearance of the clinical onset of the disease process (2). In humans, islet lymphocytic infiltrate is characterized mainly by $CD8^+$ cells that can be found in the islets of newly diagnosed IDDM patients (3,4). Interestingly, $CD8^+$ T-cell islet infiltration also develops after a pancreatic transplant from an HLA-identical twin donor (5). Lymphocyte migration from the bloodstream into the islet target tissue is a complex process involving a cascade of events (6,7). It has been postulated that a selective expression of cell adhesion molecules may lead to migration of lymphocytes into the pancreatic islets and ultimately to localized chronic inflammation that results in destruction of the islet of Langerhans (8,9). The presence of antibodies to islet autoantigens can be detected in the sera of first-degree relatives of IDDM patients years before the development of the clinical onset of IDDM, and they represent markers for prediction of clinical IDDM (10,11).

The development of IDDM in humans is linked to the presence of susceptibility genes. Two chromosomal regions are associated with and linked to IDDM: the HLA region on chromosome 6p21 (12,13) and the insulin gene region on chromosome 11p15 (14). At present, many new putative IDDM susceptibility loci have been proposed as a result of genome-wide searches (15,16).

Several epidemiological observations support the etiologic role of environmental factors in IDDM (17).

Over the past decade a large effort has been made to improve our knowledge of islet cell autoantigens. With the screening of human islet cDNA expression libraries using human sera from individuals with autoimmune abnormalities related to IDDM, it has been possible to identify at the molecular level a growing number of islet autoantigens.

To date, the most studied autoantigens characterized at the molecular level include insulin (18), GAD (19), carboxypeptidase H (20), the glycolipid GM2-1 (21), islet cell autoantigen 69 (ICA69) (22), imogen 38 (23), and the protein tyrosine phosphatase (PTP)–like protein ICA512(IA-2) (24–26) and its homologue, phogrin (27) (Table 1). Autoantibodies to islet autoantigens have yet to be shown to play a major role in the pathogenesis of the disease process and possibly represent epiphenomena of a β-cell attack. Nevertheless, they provide valuable markers to predict IDDM in first-degree relatives and ultimately in the general population. ICA512(IA-2) autoantibodies, in combination with additional immunological markers such as GAD_{65} and insulin autoantibodies, are to date considered the most reliable markers for the diagnosis and prediction of IDDM (28).

IDENTIFICATION OF ICA512(IA-2) AND ITS HOMOLOGUE, PHOGRIN —

ICA512 was initially cloned by screening a human islet cDNA expression library using a pool of sera from IDDM patients (24,25). The same molecule, termed IA-2, was independently cloned using a subtraction library (subtracting glucagonoma from insulinoma cDNAs) (26). Subsequent analysis of ICA512 clones showed that the published discrepancies of the deduced cDNA sequence between IA-2 and ICA512 were due to technical reasons. Therefore, as far as the coding region, ICA512 and IA-2 appeared to be identical in sequence.

The deduced ICA512(IA-2) cDNA sequence predicts a 979–amino acid protein with a single transmembrane region encompassing residues 577–601 and with significant homology to receptor-type PTP (RT-PTPase).

An insulin granule membrane PTP homologue, termed phogrin, was recently cloned (27) by screening a rat insulinoma cDNA library with polyclonal antisera to highly enriched insulinoma secretory granule membranes. The deduced polypeptide sequence of 1,004 amino acids encoded a protein of 111,876 Da of a moderately acidic character (predicted pI 5.66), with a single-functioned NH_2-linked glycosylation site. A consensus sequence for the catalytic site of a PTP appears at amino acid 931–942 close to the COOH-terminus of the molecule. EMBL (European Molecular Biology Laboratory) and GenBank database searches revealed homology with a large number of different PTPs over a stretch of ~260 residues commencing 100 amino acids after the transmembrane domain and extending to the COOH-terminus. Among the 250 positively scoring PTP homologues, phogrin showed the highest homology with ICA512(IA-2). The alignment of phogrin and ICA512(IA-2) over their entire lengths revealed 42% identity and 57.1% similarity, with additional homologies in a short Cys-rich region near the NH_2-terminus (Gly_{36}-Lys_{112}; 41.5% identity; 64.9% similarity) and in the region that corresponds approximately to the 60/64-kDa mature protein (Gln_{415}-Gln_{1003}; 64% identity; 78.9% similarity). The identity within the PTP domain between ICA512(IA-2) is ~80% (Fig. 1).

In vitro translation of mRNA transcribed from the full-length cDNA, gener-

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ICA, islet cell antigen; PBMNC, peripheral blood mononuclear cell; PTP, protein tyrosine phosphatase; ROC, receiver operating characteristic; RT-PTPase, receptor-type protein tyrosine phosphatase.

Table 1—*Most-studied islet cell autoantigens related to IDDM*

	Localization	Autoantibodies	T-cell responses
Insulin*	Secretory granules of pancreatic β-cells	Insulin autoantibodies are found in ~100% of young children (<5 years of age) before the onset of IDDM. Correlation with younger age and faster progression of IDDM in first-degree relatives of IDDM patients. Prophylactic subcutaneous injection of insulin may prevent IDDM.	T-cells from humans and NOD mice react with insulin β-chain.
GAD_{65}* and GAD_{67}	Synaptic-like microvesicles of islets and neurons (GAD_{65}). GAD is also present in neurons, testis, ovary, adrenal, pituitary, thyroid.	Autoantibodies to GAD_{65} are present in 70–80% of prediabetic subjects or new-onset IDDM patients, in patients with stiff-man syndrome, and those with autoimmune thyroid diseases. A subset of anti–64-kDa autoantibodies recognize GAD. Radioimmunoassay of in vitro transcribed/translated GAD_{65} useful for large-scale screening.	PBMC responses to GAD_{65} in newly diagnosed diabetic patients and in NOD mice.
PTP-like proteins: ICA512(IA-2)* and phogrin	Neurosecretory granules (pancreatic β-cells, central nervous system, pituitary, adrenal)	Autoantibodies to ICA512(IA-2) are present in 62% of prediabetic subjects or newly diagnosed IDDM patients. Relationship between 37,000 and 40,000 Da tryptic fragments and PTP-like proteins. Radioimmunoassay of in vitro transcribed/ translated ICA512(IA-2) useful for large-scale screening.	PBMC responses to ICA512(IA-2) in newly diagnosed diabetic patients.
Islet cell autoantigen 69 kDa (ICA69)	Human islet cells (ICA69 transcripts found in brain, heart, kidney, liver)	Autoantibodies to ICA69 can be detected in 43% of prediabetic subjects by Western blotting.	T-cell responsiveness to ICA69 present in newly diagnosed diabetic patients. Association with the presence of HLA-DR3 and T-cell responsiveness. Regions of similarity with bovine serum albumin.
Carboxypeptidase H	Neurosecretory granules (pancreatic β-cells, adrenal, pituitary)	Autoantibodies to carboxypeptidase H found in 10% of prediabetic subjects.	Present
Ganglioside GM2-1	Pancreatic islet cells	Autoantibodies to GM2-1 detected in ~80% of prediabetic subjects and NOD mice.	?
Imogen 38 (38 kDa)	Mitochondria; widely distributed with variable levels of expression	Presence of circulating antibodies to 38-kDa proteins. Possible presence of antibodies to imogen 38.	T-cells from newly diagnosed IDDM patients respond to imogen 38.
Glima 38	Membrane glycoprotein; expressed in cells of neuroendocrine origin	Autoantibodies to Glima 38 can be detected in 19% of newly diagnosed diabetic patients. Most of these patients are negative for GAD_{65} and/or ICA512(IA-2) autoantibodies.	?
Peripherin	Neuronal cells	Autoantibody response against peripherin in NOD mice.	T-cell response against peripherin in NOD mice
Heat-shock protein 60 (Hsp60)	Ubiquitously inducible	Antibodies to Hsp60 in prediabetic NOD mice.	Hsp60-reactive T-cells can accelerate disease in prediabetic NOD mice.

*High sensitivity and positive predictive value without loss of specificity if radioimmunoassays for detecting autoantibodies against insulin, GAD_{65}, and ICA512(IA-2) are combined (27). For specific references see Pietropaolo and Eisenbarth (2).

ated a protein of 128 kDa, which is compatible with the deduced protein sequence given its acidic character.

EXPRESSION OF ICA512(IA-2) IN NEUROENDOCRINE CELLS —

Double immunofluorescence studies have been performed using anti-glucagon and anti-somatostatin and specific rabbit antisera generated against either the cytoplasmic (amino acid residues 89–59) or the luminal (amino acid residues 92–18) domain of the human ICA512(IA-2), which indicate that the molecule is detectable in β-, α-, and δ-cells (29). Other neuroendocrine tissues expressing ICA512(IA-2) included pituitary and adrenal medulla. The highest levels of ICA512(IA-2) are found in the infundibular tract and posterior pituitary.

In addition, ICA512(IA-2) immunoreactivity is detectable in autonomic nerve fibers and ganglia and has been reported to be particularly intense at the level of synaptic terminals (29). In brain, ICA512(IA-2) is predominantly expressed in the amygdala and hypothalamus. Of note, the amygdala and hypothalamus are considered the neuroendocrine regions of the brain that contain the highest concentration of dense-core secretory granules as compared with other regions of the central nervous system. In other regions of the cerebrum, such as cerebral cortex, cerebellar cortex, hippocampus, striatum, and thalamus, which are regions with low levels of secretory granules, the expression of ICA512(IA-2) appears to be lower.

The fact that ICA512(IA-2) and the array of islet autoantigens associated with IDDM (Table 1), with the exception of insulin, are found in multiple cell types is intriguing. The reason(s) why a β-cell–specific autoimmune disease such as IDDM is associated with the presence of autoimmunity to autoantigens that do not show a tissue-specific distribution is still a subject of discussion. The cytotoxic immune attack, which selectively targets the pancreatic β-cells, may not be due to the tissue distribution of a certain antigen, but rather may depend on the sensitivity of the β-cell to cytotoxic T-cells. A β-cell may simply be more prone to die than an α-cell under the effect of cytotoxic T-cells. The unique sensitivity of β-cells in vitro has been proposed to explain the non–major histocompatibility complex–restricted destruction of pancreatic β-cells (30). However, in vivo exposure of histocompatible islets to cytokines during an allogeneic immune reaction does not lead to their destruction (31).

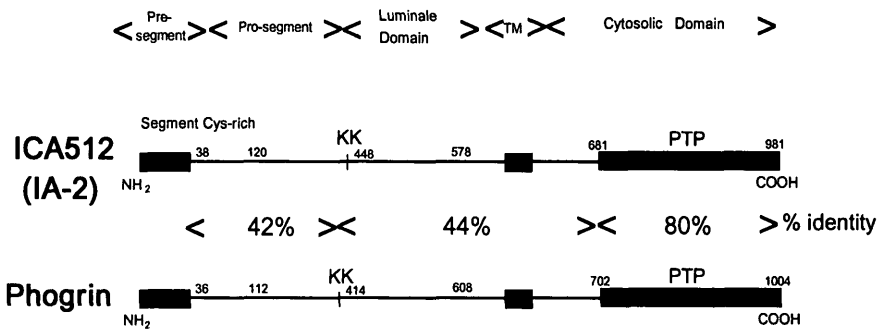

Figure 1—*Schematic representation of the regions of similarity between ICA512(IA-2) and phogrin. TM, Transmembrane domain.*

SUBCELLULAR LOCALIZATION OF ICA512(IA-2) AND PHOGRIN —

The subcellular localization of ICA512(IA-2) has been investigated by immunoelectron microscopy (29). A prominent immunogold labeling of neurosecretory granules was evident using the anti-ICA512 89-59 and 92-18 antibodies. It appears that synaptic vesicles do not contain ICA512(IA-2). Posttranslational processing studies of ICA512(IA-2) show that ICA512(IA-2) recycles to the Golgi complex region and is sorted into newly formed secretory granules (29).

Posttranslational processing studies of phogrin (27), the ICA512(IA-2) homologue, indicate that it is cotranslationally inserted into the endoplasmic reticulum as a type I transmembrane protein and passed rapidly to the Golgi and the trans-Golgi network (TGN) before undergoing proteolytic cleavage in the luminal domain to produce 60- and 64-kDa mature forms, which migrate in a similar fashion on SDS-PAGE to the major forms of the protein found in pancreatic islets and insulinoma tissue by Western blotting. Subcellular fractionation of insulinoma tissue showed that the 60- and 64-kDa products of the breakdown of phogrin had a very similar distribution of insulin and carboxypeptidase H, and it was concluded that phogrin was predominantly localized to the secretory granules (27).

RELATIONSHIP BETWEEN THE 37,000- AND 40,000-M_r TRYPTIC FRAGMENTS AND PTP-LIKE PROTEINS —

It has been reported that antibodies to the 37,000- and 40,000-M_r proteolytic fragments were found in up to 80% of recent onset IDDM patients (32), and similar frequencies were reported in twins who developed clinical disease (67–75%). Several reports (33–36) suggest that trypsinization of the recombinant intracellular domain of IA-2 generated fragments identical in size to the 40-kDa insulinoma trypsinized fragment. Sera from recent-onset IDDM patients reacting to the 40,000 M_r insulinoma fragment gave strong reactivity with the ICA512(IA-2). In particular, a strong association ($r = 0.85$, $P < 0.001$) was detected between the two assays that measured antibodies to the 40,000-M_r insulinoma fragment and the IA-2 antigen respectively (33). It is likely that the 37,000-M_r fragment derives from a different protein, one of which is related to ICA512(IA-2) (33–36). Additional studies performed with anti-phogrin antibodies suggest that the 37,000 tryptic fragment recognized by sera from diabetic patients corresponds to the phogrin molecule (27,34).

AUTOANTIBODIES AND T-CELL IMMUNE RESPONSES TO PTP-LIKE PROTEINS —

The radioimmunoassay for detecting antibodies to ICA512(IA-2) is probably the most specific used for studies on prediction of IDDM (28,37), having specificity of >99%, and a low prevalence in low-risk first-degree relatives of IDDM patients (28).

We have constructed a receiver operating characteristic (ROC) curve (38,39) for the ICA512(IA-2) assay by plotting the true-positive rate (sensitivity) among 409 newly diagnosed IDDM patients (including new-onset IDDM patients from the Children's Hospital of Pittsburgh Registry) against the false-positive rate (100 − specificity) among 280 healthy control subjects (144 male, 136 female; median age 26, range 2.7–61

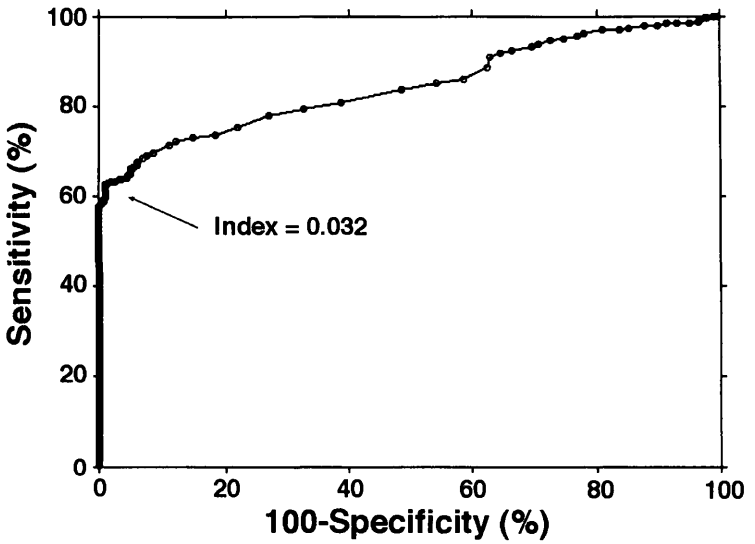

Figure 2—*ROC curves for ICA512(IA-2) autoantibodies measured by radioimmunoassay. The ROC curve for the ICA512(IA-2) shown was generated analyzing 409 newly diagnosed diabetic patients and control subjects. In general, in an ROC curve, the highest and the farthest point to the left is the preferred cutoff point for distinguishing normal from abnormal test results. The ROC curves support the use of the 99th percentile as cutoff point for the ICA512(IA-2) radioimmunoassay.*

years). The new-onset IDDM patients were 198 male subjects, 211 female subjects; median age 10.3 years, range 0.3–44.6 years. An ideal test is one that reaches the upper left corner of the ROC plot (100% sensitivity and 100% specificity). A worthless test follows the diagonal from the lower left to the upper right corners: each incremental gain in sensitivity is matched by an equal loss in specificity. The ROC curve for the radioimmunoassay that detects antibodies to ICA512(IA-2) indicates that high cutoff points can be selected that preserve high sensitivity without a significant loss of specificity (Fig. 2). Usually, the best cutoff point is where the ROC curve "turns the corner," in this case when the index for ICA512(IA-2) antibodies is 0.032. This cutoff point corresponds to the 99th percentile among the control population analyzed. The prevalence of ICA512(IA-2) antibodies is 62% in IDDM patients at the clinical onset of the disease (Fig. 2). The prevalence of IA512(IA-2) autoantibodies was reported as 48% in prediabetic subjects (followed to the clinical onset of IDDM) and only 1.4% (10 of 694) in low-risk first-degree relatives (islet cell antibody–negative first-degree relatives of IDDM patients) (28). In comparison to radioimmunoassays such as those to detect islet cell autoantibodies, or radioimmunoassays to detect GAD_{65} or insulin autoantibodies, the radioimmunoassay for measuring autoantibodies to ICA512(IA-2) seems to be a very specific candidate test for predicting type I diabetes. ICA512(IA-2) autoantibodies can be detected in either newly diagnosed or prediabetic patients younger than 20 years of age and in those older than 20 years of age (40).

Recent observations by Durinovic-Belló et al. (41) have shown that peripheral blood mononuclear cells (PBMCs) from newly diagnosed IDDM patients proliferate in response to ICA512(IA-2) and that such proliferation was exceptionally high compared with that of other recombinant islet autoantigens like GAD and insulin.

CORRELATION OF AUTOANTIBODIES AGAINST ICA512(IA-2) WITH ANTI-PHOGRIN AUTOANTIBODIES

— Levels of ICA512(IA-2) autoantibodies correlate with those of anti-phogrin autoantibodies (r = 0.82, $P < 0.0001$) (Fig. 3) (37). In particular, in 58 patients positive for anti-phogrin autoantibodies, 57 (98%) were also positive for ICA512(IA-2) autoantibodies. One of 57 (1.7%) newly diagnosed diabetic patients was anti-phogrin autoantibody positive but ICA512(IA-2) autoantibody negative. Five of 57 (9%) newly diagnosed diabetic patients and 4 of 44 (9%) prediabetic relatives were ICA512(IA-2) autoantibody positive but anti-phogrin autoantibody negative. Levels of anti-phogrin autoantibodies correlated minimally with insulin autoantibodies (r = 0.20, P = 0.05), whereas there was no correlation between anti-phogrin autoantibodies and GAD_{65} autoantibodies (r = 0.16, P = 0.12). It was concluded from these studies that the major epitope is between ICA512(IA-2) and phogrin and localized to the conserved COOH-terminal PTP domain. It is noted that this portion of the molecule is oriented toward the cytoplasm of the β-cell and thus it is considered an unlikely target for complement-mediated autoimmune attack on the β-cell.

AUTOANTIBODIES TO ISLET AUTOANTIGENS IN COMBINATION IMPROVE PREDICTION OF IDDM

— Islet cell antibodies represented an important step

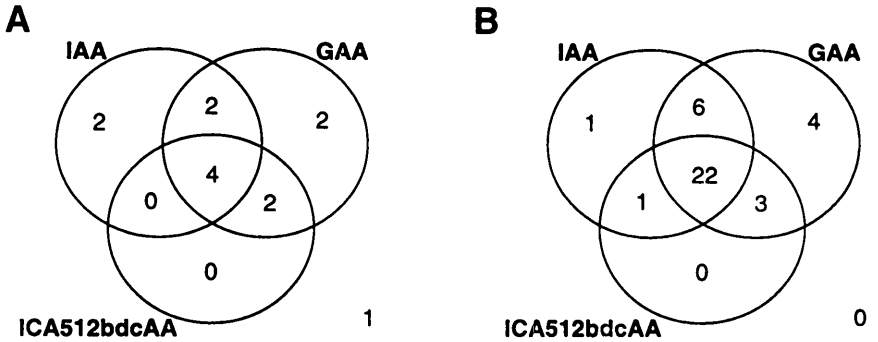

Figure 3—*Frequency of autoantibodies against insulin, GAD_{65}, and ICA512(IA-2) detected by radioimmunoassay in 50 first-degree relatives who developed diabetes during follow-up, according to islet cell antibody status: negative* (A), n = *13; positive* (B), n = *37. From Verge et al.*, Diabetes *45:926–933, 1996.*

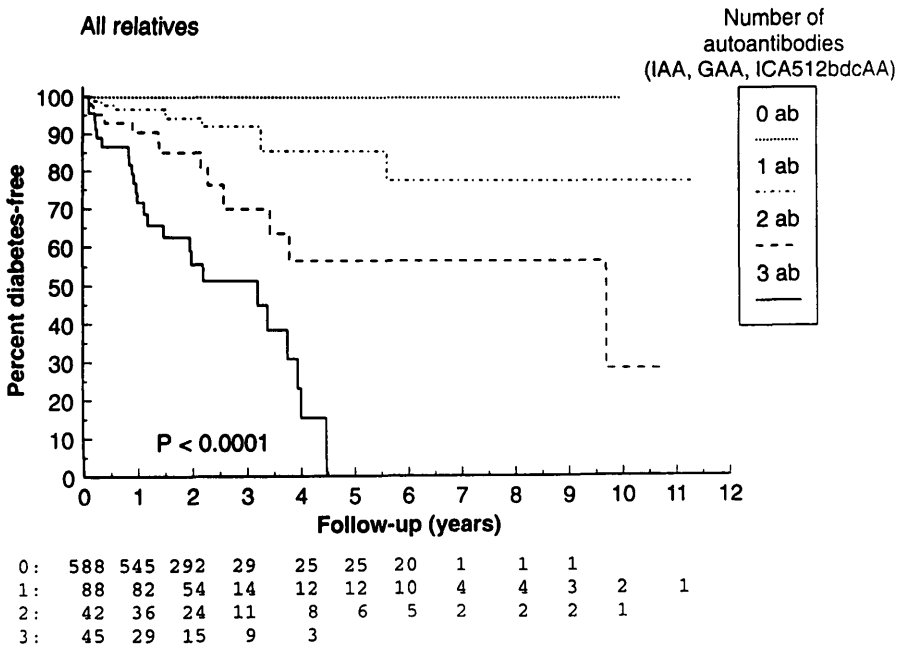

Figure 4—*The diabetes-free survival of first-degree relatives according to the number of autoantibodies (ab) present at baseline, considering insulin autoantibodies (IAA), GAD_{65} autoantibodies (GAA), and ICA512(IA-2) autoantibodies (ICA512bdcAA) (from Verge et al.,* Diabetes *45:926–933, 1996).*

forward in understanding that IDDM was an autoimmune disease (10). Nevertheless, although islet cell antibodies are predictive of IDDM, the assay for detecting them has proven difficult to standardize. A concrete possibility for having an alternative to the islet cell antibody assay is represented by a combination of radioimmunoassays for detecting autoantibodies against molecularly characterized autoantigens.

Figure 3 shows the frequency of autoantibodies to ICA512(IA-2), in addition to insulin and GAD_{65}. All three autoantibodies were detected by radioimmunoassay in 50 prediabetic relatives, according to islet cell antibody status. Only one prediabetic relative was negative for all three immunological markers, and this individual was also negative for islet cell antibodies. There was no significant difference in frequency of insulin, GAD_{65}, and ICA512(IA-2) autoantibodies, comparing islet cell antibody–positive and –negative prediabetic relatives.

We found that two or more autoantibodies to the islet autoantigens insulin, GAD_{65}, and ICA512(IA-2) are highly predictive of diabetes risk in first-degree relatives of IDDM patients regardless of age (28). Prediction can be improved by the measurement of first-phase insulin response. It is of importance that the presence of two or more autoantibodies to the above-mentioned islet autoantigens has 68% positive predictive value, 80% sensitivity, and 100% specificity. In first-degree relatives of IDDM patients, there is increase in risk relative to the number of autoantibodies (detected by radioimmunoassays) against insulin, GAD_{65}, and ICA512(IA-2), comparing zero, one, two, and three of these autoantibodies ($P < 0.0001$, log-rank test; Fig. 4).

CONCLUDING REMARKS

— PTP-like proteins are molecules localized in the dense-core secretory granules of a number of neuroendocrine tissues, including the pancreatic β-cell. In IDDM, pancreatic β-cell secretory granules, which contain insulin and carboxypeptidase H, and other organelles, such as synaptic-like microvesicles, which contain GAD, are considered prominent targets involved in the induction of a specific autoimmune response. It is conceivable that a complex of single islet autoantigens capable of driving an autoimmune response is indeed an aggregate of molecules forming subcellular particles (2). In IDDM, autoimmunity could be induced by several components of these particles.

The identification of the molecular structure of islet autoantigens in IDDM leads to the obvious question of which antigens are central to the pathogenesis of IDDM. We do not have an answer to this question yet, but the optimization of radioimmunoassays to specifically detect autoantibodies to molecularly characterized autoantigens represents the foundation on which prediction of IDDM can be improved. Radioimmunoassays for detecting antibodies against ICA512(IA-2) and its homologue, phogrin, are quantitative and have the important practical advantage of being semi-automated. Compared with GAD_{65} autoantibodies, ICA512(IA-2) autoantibodies have high specificity and low prevalence in low-risk relatives of IDDM patients. The ICA512(IA-2) autoantibody radioimmunoassay has a sensitivity of 62%, as indicated by analyzing a large series of serum samples from new-onset IDDM patients and control subjects. The ROC curve for this radioimmunoassay indicates that very high cutoff points can be selected for distinguishing normal from abnormal test results without significant loss of specificity.

It is more than likely that a fourth and perhaps a fifth radioimmunoassay for additional islet autoantigens, such as ICA69 (22), GM2-1 (21), the 52-kDa molecule (42), and imogen (23) (Table 1), will be required to further improve the positive predictive value of these markers in combination. At present, combined analyses of specific islet humoral markers represent a prerequisite for the development of simple predictive screening for IDDM in first-degree relatives of IDDM patients and in the general population and will help in designing intervention trials for prevention of the disease in highly susceptible individuals who will develop IDDM.

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