This information is current as of July 29, 2017.

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*J Immunol* 1998; 161:2648-2654; 
http://www.jimmunol.org/content/161/5/2648

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IA-2 (Islet Cell Antigen 512) Is the Primary Target of Humoral Autoimmunity Against Type 1 Diabetes-Associated Tyrosine Phosphatase Autoantigens

Ezio Bonifacio, Vito Lampasona, and Polly J. Bingley

IA-2 (islet cell Ag 512) and IA-2β (phogrin/IAAR) are related autoantigens associated with type 1 diabetes. To determine the critical regions for autoantibody binding and which of these autoantigens is the primary target, mutant and chimeric constructs were used to characterize Ab epitope binding in sera from 217 new onset patients with type 1 diabetes and sequential samples from 141 islet cell Ab positive first degree relatives of patients. All 22 relatives and 121 of 129 patients with IA-2/IA-2β Abs had reactivity to IA-2-specific epitopes. These epitopes were in the juxtamembrane region (residues 601–682) and the protein tyrosine phosphatase (PTP)-like domain of IA-2. Chimeras showed that IA-2 residues 795–889 were important for IA-2-specific Ab binding in the PTP-like domain, and mutation of IA-2 residues 877 and 911, previously indicated as relevant for phosphatase activity, also reduced Ab binding. In contrast, Ab binding to IA-2-β2 was limited to its PTP-like domain, most IA-2β Abs recognized epitopes shared with IA-2, and only 20 patients and 2 relatives had Abs to IA-2-β specific epitopes. In 4 relatives, IA-2 and/or IA-2β Abs developed in follow-up samples. In each of these, Abs to IA-2-specific epitopes were the first detected. In three, spreading to epitopes shared between IA-2 and IA-2β in subsequent samples was seen. In the 17 relatives who developed type 1 diabetes, progression to disease was associated with reactivity to multiple IA-2/IA-2β epitopes. These data suggest that IA-2 is the primary phosphatase-like autoantigen associated with type 1 diabetes and that studying autoantibody epitope diversity may assist in disease prediction.

1995. Type 1 diabetes was diagnosed according to World Health Organization criteria. Patients had a median age of 12 yr (range, 1–40 yr). Serum samples were obtained from 141 first-degree relatives of patients with type 1 diabetes. All relatives had islet cell Abs (ICA) at >10 Juvenile Diabetes Foundation (JDF) units in at least two consecutive samples. These relatives were identified from all those tested in the Barts-Oxford family study (11). Initial samples and follow-up samples obtained every 1 to 2 yr were included in the study. A total of 441 samples was tested with a median follow-up of 7 yr (range, 0.02–11.25 yr). Of these, 17 developed type 1 diabetes after the collection of the initial blood sample (median time to diabetes, 4.8 yr; range, 0.02–11.25 yr). Serum was also obtained from 80 normal children and blood donors in the Milan area. Control subjects had normal blood glucose levels; median age was 12 yr (range, 1–40 yr); 37 of these were male. Diabetes, 4.8 yr; range, 0.02–11.25 yr). Serum was also obtained from 80 normal children and blood donors in the Milan area. Control subjects had normal blood glucose levels; median age was 12 yr (range, 1–40 yr); 37 of these were male.

**Cloning of IA-2 and IA-2β constructs**

Constructs used in this study are shown in Figure 1. The IA-2 605–979 construct was used to identify Abs binding to the juxtamembrane region of IA-2, and the IA-2 264–979 construct was used for Abs binding to the PTP-like domain of IA-2. These were prepared in the pSP64 poly(A) (IA-2 264–979) or pGEM-T (IA-2 264–979 and IA-2 264–605) cloning vectors (Promega, Madison, WI) under the control of the SP6 promoter as described previously (11).

**Human IA-2β and rat phogrin.** cDNA clones encoding the intracellular portion of human IA-2β (amino acids 662-1033) corresponding to the ICAAR cDNA sequence (GenBank accession no. Y08569) (16), the juxtamembrane region of rat phogrin (amino acids 662-741), and PTP-like domain (amino acids 741-1033) were subsequently obtained after PCR amplification (Perkin-Elmer, Norwalk, CT) of reverse transcribed (Amersham, Aylesbury, U.K.) total RNA extracted from human islets using the following specific primers in different combinations: IA-2β-IC-5′, 5′-GGTTACCATGGCT CAGCACAAGGCTGAAGAG-3′; EST T33353–3′, 5′-GGGGTGAT CCTAACAGGCGGCTGCTAC-3′; IA-2β-JM-3′, 5′-GGGTTACCC CAGGAAGTGCTTGGAGGA-3′; and IA-2β-PTP-5′, 5′-CAAGCC CAACATGGCACTTGC-3′.

The IA-2β-IC-5′ primer contains an artificial in-frame start codon followed by an alanine codon to allow efficient expression of the amplified product. A cDNA clone containing a single point mutation, which encodes a stop codon at amino acid 999, was also isolated during the clone screening procedure. The products were cloned into the pGEM-T vector. The IA-2β-662-1033 construct was used to identify IA-2β Abs. The IA-2β-662-741 construct was used for identification of Abs binding to the juxtamembrane region of IA-2β, and the IA-2β-264-1033 construct was used for Abs binding to the PTP-like domain of IA-2β. For bacterial expression, the cDNAs were subcloned into the pTrHis vector (Invitrogen, San Diego, CA).

Based upon the deposited sequence of the rat homologue of IA-2β (phogrin; GenBank accession no. Z50735), a cDNA encoding the intracellular portion (amino acids 629-1004) of the rat protein was PCR amplified from reverse-transcribed RIN 5AH cells total RNA, with the specific primers phogrin-IC-5′, 5′-ATGGCCGACACCAGTCACTAAAGTCAATGCGAA-3′ and phogrin-IC-3′, 5′-CTACACAGAAAGTACGAAATGATAT-3′. The phogrin-IC-5′ primer contained an artificial in-frame start codon to allow in vitro transcription/translation. The amplified cDNA was cloned into the pGEM-T vector.

### Chimeric IA-2/IA-2β PTP domains.

The following chimeric IA-2/IA-2β PTP domains were obtained by in-frame joining of selected portions of the IA-2 and IA-2β: IA-2 687–889/IA-2β 687–943; IA-2 687–943/IA-2β 687–909; IA-2 687–943/IA-2β 795–899; IA-2 687–943/IA-2β 849–909; IA-2 687–943/IA-2β 909–979; IA-2 687–943/IA-2β 925–1033. The 1A-2687–889/IA-2β 687–943 and IA-2 849–943/IA-2β 687–909 chimeras were obtained upon PCR amplification of respective IA-2 and IA-2β cDNAs introduced into pJEF215 restriction site, followed by restriction and ligation. Mutagenesis did not affect amino acid sequence. The chimeras IA-2 687–943/IA-2β 909–943 and IA-2 849–943/IA-2β 687–909 were obtained from the first two using the Seamless (Stratagene, La Jolla, CA) method with appropriate oligonucleotide primers.

### Antigenic IA-2β constructs

These were obtained and tested upon insertion of single nucleotide changes into the cytoplasmic portion of the IA-2 cDNA by the QuickChange (Stratagene) method using mutagenesis-specific oligonucleotides. The following amino acid residues were modified: aa 877 Ala ➔ Asp; aa 911 Asp ➔ Ala; aa 877 Ala ➔ Asp + aa 911 Asp ➔ Ala.

### In vitro translation and immunoassay

Purified plasmid DNA of the constructs was obtained by Qiapquick spin column purification (Diagen, Hilden, Germany) and in vitro transcribed and translated utilizing the TNT SP6-coupled rabbit reticulocyte lysate system (Promega) in the presence of [35S]methionine (Amersham), according to the manufacturer’s instructions. Unincorporated [35S]methionine was removed by gel chromatography on a NAP5 column (Pharmacia, Uppsala, Sweden). The correspondence of the molecular size for all translated proteins that predicted from the amino acid sequence was verified by SDS-PAGE and autoradiography of the products. The junctional protein and small product, probably derived from a downstream start codon was also obtained. For immunoassay, 2 μl of serum was added to 25 μl of TBST (50 mM Tris, 150 mM NaCl, pH 7.2. 1% Tween 20) containing 15,000 to 20,000 cpm of labeled recombinant proteins in wells of a 96-well deep well plate (Beckman, Fullerton, CA) and incubated overnight on ice. Immune complexes were recovered by adding 1 μg of protein A-Sepharose (Pharmacia) preswollen and resuspended in 50 μl of TBST, then incubated for 1 h at 4°C with shaking. Protein A-Sepharose beads were washed five times with 800 μl of TBST and centrifuged at 600 × g for 3 min to pellet beads between washes. After washing, Sepharose was transferred in 100 μl of TBST to a 96-well Optiplate (Packard, Groningen, The Netherlands), 150 μl of Microscint 40 (Packard) was added, and plates were shaken for 30 min and counted for 5 min in a TopCount (Packard) scintillation counter for measurement of the recovered cpm. In some experiments, protein A-Sepharose pellets were processed in 30 μl of denaturing loading buffer for SDS-PAGE and autoradiography. Results were expressed as arbitrary units relative to a standard curve prepared by measurement in each assay of a series of sera with high autoantibody levels serially diluted in normal serum. The upper first centile of 80 control sera was used as the threshold of autoantibody detection for each construct. This was 1.5 U for each of IA-2 687–794/IA-2β 687–943, IA-2 849–943/IA-2β 795–943, and 6 U for Abs against the juxtamembrane region of IA-2. Reactivity against the mutated IA-2β proteins was expressed relative to that observed with a mouse anti-IA-2 mAb (76F) that binds to an epitope contained within the juxtamembrane region of IA-2.

### Competition experiments

Identification of IA-2 and IA-2β PTP-like domain-specific and cross-reactive Abs was performed with competition by the alternate bacterially expressed PTP-like domain protein in the standard Ab radiobinding assay. The pTRC-His bacterial expression vector (Xpress expression and purification system, Invitrogen) containing IA-2β 687–794, and IA-2β 909–979 were transformed into the INVSuE Esherichia coli strain. After culturing to early log phase, protein expression was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside. Bacteria were harvested 4 h after induction and resuspended in 10 mM HEPES, 150 mM NaCl (pH 7.4) containing 10 mM benzamidine, 0.1% aprotinin, and 2% Triton X-100, followed by incubation for 2 h at 4°C with shaking. Insoluble material was removed by centrifugation at 15,000 × g for 15 min. Competition was conducted by preincubating the sera with the IA-2 or IA-2β PTP-like domain preparations containing ~5 μg of recombinant protein, or control E. coli extract, for 1 h at 4°C before adding, respectively, radiolabeled IA-2β or IA-2β PTP-like domain and conducting an immunoassay as described above. Sera were considered to contain cross-reactive PTP-like domain Abs if binding to radiolabeled IA-2β 687–794 was at least partially inhibited (>10%) by unlabeled IA-2β 687–943, and binding to radiolabeled IA-2β 687–794 was at least partially inhibited (>10%) by unlabeled IA-2β 687–979. Sera were considered to contain IA-2-specific PTP-like domain.
Abs if binding could only be detected against the IA-2\textsubscript{687-979} protein and not IA-2\textsubscript{741-1033} or if binding to IA-2\textsubscript{687-979} remained above 1.5 U after competition with unlabeled IA-2\textsubscript{741-1033}. Sera were considered to contain IA-2\textsubscript{β}-specific Abs if binding to IA-2\textsubscript{β}\textsubscript{741-1033} remained above 1.5 U after competition with unlabeled IA-2\textsubscript{687-979}.

**Statistical analysis**

A Kaplan-Meier survival analysis was used to determine the cumulative risk of the development of type 1 diabetes. Follow-up time was calculated from the date of the first sample found to contain IA-2 Abs to the date of diabetes onset or last contact. Diabetes-free survival was compared with the log rank test. The 5-yr risks are given as the cumulative risk with a 95% confidence interval (CI) calculated from the SE. The Statistical Package for Social Sciences (SPSS, Chicago, IL) was used for statistical analyses.

**Results**

**IA-2 and IA-2\textsubscript{β} Ab epitopes in type 1 diabetes and prediabetes**

IA-2 Abs were detected in 129 (59%) of 217 patients and in 22 (16%) of 141 relatives with ICA ≥ 10 Juvenile Diabetes Foundation (JDF) units; Abs to IA-2\textsubscript{β} were found in 90 (41%) patients and 14 (10%) of the relatives (Table I). None had Abs to IA-2\textsubscript{β} in the absence of IA-2 Abs. Among those with IA-2 Abs, reactivity was evenly distributed between epitopes found within the juxtamembrane region (84 (65%) of 129 patients and 17 (77%) of 22 relatives) and the PTP-like domain (112 (87%) patients and 17 (73%) relatives); 67 (52%) patients and 12 (55%) relatives with IA-2 Abs had reactivity to both regions. None of the sera binding IA-2\textsubscript{β} had Abs to construct IA-2\textsubscript{β}\textsubscript{662-744} representing the juxtamembrane IA-2\textsubscript{β} region, and all of the reactivity was confined within the PTP-like domain of IA-2\textsubscript{β} (Fig. 2). Sera from 2 patients showed binding to the human IA-2\textsubscript{β}, but not to the rat phogrin, indicating the presence of species-specific Abs. These two sera did not immunoprecipitate the 37-kDa islet tryptic polypeptide from rat insulinoma cells as was found in previous studies (11). Only a few sera bound to the short IA-2\textsubscript{β}\textsubscript{662-999}, indicating that the C-terminal amino acids are either important for maintaining correct Ag conformation or contain contact residues for autoantibody binding.

Ab binding to the PTP-like domains was IA-2 specific or cross-reactive between IA-2 and IA-2\textsubscript{β}, and occasionally IA-2\textsubscript{β}-specific (Fig. 3). Sera from 24 patients and 3 of the relatives had Abs that bound the IA-2 PTP-like domain and not the IA-2\textsubscript{β} PTP-like domain (Table II). As expected, binding to IA-2\textsubscript{β}\textsubscript{687-979} in these sera was, for the most part, unaffected by competition with IA-2\textsubscript{β}\textsubscript{741-1047} (e.g., serum 2 and 3, Fig. 3), although binding in occasional sera could be inhibited by excess IA-2\textsubscript{β} without showing direct binding to in vitro translated IA-2\textsubscript{β}; this suggested the presence of Abs with markedly reduced affinity to the IA-2\textsubscript{β} PTP-like domain as compared with the IA-2 PTP-like domain (data not shown). All sera with binding to both IA-2 and IA-2\textsubscript{β} PTP-like domains had Abs that recognized epitopes shared between the two molecules, since binding could be at least partially inhibited by either IA-2 or IA-2\textsubscript{β} PTP-like domains (e.g., sera 4 – 6, Fig. 3). In most, but not all, of these serum samples, binding to the IA-2 PTP-like domain was not completely inhibited by competition with IA-2\textsubscript{β}, and therefore these sera also contained IA-2-specific PTP-like domain Abs (e.g., sera 4 and 6, Fig. 3). Competition with increased amounts of IA-2\textsubscript{β} did not further reduce binding, indicating that incomplete inhibition was not due to insufficient competitor. For a minority of the serum samples, binding to IA-2\textsubscript{β} could not be completely inhibited by competition with excess IA-2, indicating the presence of Abs that recognized IA-2\textsubscript{β}-specific epitopes (e.g., sera 4 and 5, Fig. 3).

Combinations of Abs to the IA-2/IA-2\textsubscript{β} epitope specificities were heterogeneous. Most patients (96/129) and relatives (17/22) had Abs to more than one epitope. The majority of those with a single reactivity had IA-2 juxtamembrane-specific Abs. Of those with IA-2 or IA-2\textsubscript{β} Abs, 121 (94%) of 129 patients with type 1 diabetes and all 22 (100%) relatives had some reactivity to IA-2-specific epitopes contained within the juxtamembrane or PTP-like domains. Sera from only 20 patients and 2 relatives had Abs recognizing IA-2\textsubscript{β}-specific epitopes, and no sera had IA-2\textsubscript{β}-specific Abs only (Table II).

**Ab epitopes in seroconverters**

Of the 22 first-degree relatives with IA-2 or IA-2\textsubscript{β} Abs, 4 had undetectable Ab levels in their initial sample and developed IA-2/IA-2\textsubscript{β} Ab reactivity during follow-up (shown in Table III). In
each of the 4 relatives Abs were reactive with IA-2-specific epitopes, either in the juxtamembrane region or the PTP-like domain, in the first sample in which Abs were detected. In 3 of these subjects (A, B, and D in Table III), binding to IA-2 could not be inhibited with rIA-2β (data not shown). One had Abs to IA-2-specific PTP-like domain epitopes only (A), one to IA-2 juxtamembrane epitopes only (D), and one to both IA-2-specific PTP-like domain and juxtamembrane region epitopes (B). In relatives A and B, subsequent samples had additional Abs that bound to epitopes shared between the PTP-like domains of IA-2 and IA-2β. In relative C, Abs binding to IA-2-specific PTP-like domain epitopes were detected concurrently with weak Abs to either IA-2-specific PTP domain epitopes or epitopes shared between IA-2 and IA-2β. Both Ab nucleotides increased in titer in subsequent samples.

Table III. IA-2/IA-2β Ab reactivity in sequential samples from seroconverters

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age at Sample (yr)</th>
<th>IA-2/IA-2β Ab Reactivitya</th>
<th>Patients at Diabetes Onset (n = 217)</th>
<th>ICA+b Relatives (n = 141)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JM</td>
<td>IA-2PTP</td>
<td>X-react</td>
<td>βPTP</td>
<td>11 (5%)</td>
</tr>
<tr>
<td>JM</td>
<td>IA-2PTP</td>
<td>X-react</td>
<td>βPTP</td>
<td>32 (15%)</td>
</tr>
<tr>
<td>JM</td>
<td>IA-2PTP</td>
<td>X-react</td>
<td>βPTP</td>
<td>7 (3%)</td>
</tr>
<tr>
<td>JM</td>
<td>IA-2PTP</td>
<td>X-react</td>
<td>βPTP</td>
<td>14 (6%)</td>
</tr>
<tr>
<td>JM</td>
<td>IA-2PTP</td>
<td>X-react</td>
<td>βPTP</td>
<td>10 (5%)</td>
</tr>
<tr>
<td>JM</td>
<td>IA-2PTP</td>
<td>X-react</td>
<td>βPTP</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>JM</td>
<td>IA-2PTP</td>
<td>X-react</td>
<td>βPTP</td>
<td>20 (9%)</td>
</tr>
<tr>
<td>JM</td>
<td>IA-2PTP</td>
<td>X-react</td>
<td>βPTP</td>
<td>15 (7%)</td>
</tr>
<tr>
<td>JM</td>
<td>IA-2PTP</td>
<td>X-react</td>
<td>βPTP</td>
<td>10 (5%)</td>
</tr>
<tr>
<td>JM</td>
<td>IA-2PTP</td>
<td>X-react</td>
<td>βPTP</td>
<td>8 (4%)</td>
</tr>
<tr>
<td>JM</td>
<td>IA-2PTP</td>
<td>X-react</td>
<td>βPTP</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>JM</td>
<td>IA-2PTP</td>
<td>X-react</td>
<td>βPTP</td>
<td>88 (41%)</td>
</tr>
</tbody>
</table>

a JM, IA-2 juxtamembrane Abs; IA-2PTP, IA-2-specific PTP-like domain Abs; X-react, Abs binding both IA-2 and IA-2β PTP-like domains; βPTP, IA-2β-specific PTP-like domain Abs.
b Numbers in parentheses indicate how many have developed type 1 diabetes.
Ala$^{877}$ was replaced with Asp. The replacement of Asp$^{911}$ with Ala reduced binding by 55 to 87%, but the replacement of both Ala$^{877}$ and Asp$^{911}$ did not reduce binding beyond that seen with the substitution of Asp$^{911}$ alone. Ab binding to IA-2 juxtamembrane epitopes by patient sera was not affected by this change (Fig. 6).

IA-2/IA-2β Ab epitopes and progression to IDDM in relatives

Life table analysis in relatives with ICA showed that those also having Abs to IA-2 or IA-2β had a markedly increased progression to type 1 diabetes with a 5-yr risk of 40% (95% CI, 18–62) compared with 3.7% (95% CI, 0.2–7.2) in relatives without these Abs ($p < 0.0001$). Among those with IA-2/IA-2β Abs, progression to diabetes was not associated with any one of the Ab epitope specificities identified. Progression was, however, increased in those with multiple epitope reactivity. The 3-yr cumulative risk in those with >2 reactivities was 79% (95% CI, 44–100) vs 23% (95% CI, 1–45) in those with 1 or 2 reactivities ($p = 0.01$) (Fig. 7). Two relatives with a single IA-2/IA-2β epitope reactivity developed diabetes, and in both of them, Abs were against the IA-2 juxtamembrane region (Table II).

Discussion

Humoral autoimmunity to islet autoantigens is the single most consistently detected immune abnormality associated with type 1 diabetes. Over 90% of patients have islet autoantibodies at and before onset of clinical disease. These circulating autoantibodies are not considered directly pathogenic, but recent evidence showing an absence of insulitis and diabetes in B lymphocyte-deficient NOD mice suggest that B lymphocytes have a critical role in the disease process (17). Studying the early humoral responses is therefore

![FIGURE 5. Comparison of the PTP-like domain amino acid sequences of IA-2 and IA-2β. The region containing amino acids that are potentially critical for the binding of Abs to the IA-2-specific PTP-like domain epitopes, as determined from binding studies to the PTP-like domain chimeric proteins, is shaded.](http://www.jimmunol.org/)

![FIGURE 6. Effect of specific IA-2 amino acid mutations on autoantibody binding. Binding to unmutated IA-2ic (filled columns), IA-2ic 877 Ala > Asp (open columns), IA-2ic 911 Asp > Ala (grey columns), and IA-2ic 877 Ala > Asp plus aa 911 Asp > Ala (dotted columns) is shown for sera with IA-2 juxtamembrane-specific Abs only (serum 1), IA-2 cross-reactive Abs only (sera 2 and 3), IA-2-specific PTP-like domain Abs (sera 4–7), and IA-2β-specific Abs (serum 8). Serum 9 is from a normal subject.](http://www.jimmunol.org/)

![FIGURE 7. IA-2/IA-2β reactivity and progression to type 1 diabetes. Progression to disease in ICA-positive relatives of patients with respect to the total number of IA-2/IA-2β Ab specificities is expressed as cumulative diabetes-free survival in the Kaplan-Meier survival analysis. The numbers under the abscissa indicate the number of subjects remaining at each time point.](http://www.jimmunol.org/)
likely to be important for understanding the early immunization process in type 1 diabetes. The identification of two structurally related major humoral autoantigens associated with diabetes, IA-2 and IA-2\(β\), allows the study of these responses in detail with respect to epitope spreading and has enabled us to identify potentially critical regions and residues involved in early IA-2 autoimmunity. We have determined that both the juxtamembrane region and the PTP-like domain of IA-2 contain epitopes that are specific to IA-2 and that most patients have Abs that recognize these IA-2-specific epitopes. Moreover, analysis of sequential samples from relatives who seroconverted to IA-2/IA-2\(β\) humoral autoimmunity suggests that these IA-2-specific epitopes are those to which the early Abs against IA-2 are directed. IA-2\(β\) autoimmunity appears to arise later through spreading to epitopes in the IA-2 PTP-like domain that are shared with IA-2\(β\). These findings indicate that IA-2 is likely to be the primary target of humoral autoimmunity against diabetes associated PTP-like autoantigens.

Previous studies have suggested that autoimmunity to IA-2\(β\) rather than IA-2 may be more associated with the development of type 1 diabetes. This is based upon the suggestion that IA-2\(β\) may be more strongly expressed in \(β\) cells than is IA-2 and that Abs to IA-2\(β\) are less frequently detected in control subjects (2). Our findings do not support this view, since many patients had IA-2 Abs in the absence of IA-2\(β\) Abs, and importantly, several relatives developed the disease having only IA-2-specific epitope reactivity. We did find, however, that relatives with multiple IA-2/IA-2\(β\) epitope reactivity had a more frequent and rapid diabetes development. Thus, spreading of autoimmunity to other epitopes, including those found on IA-2\(β\), appears to be associated with the development of clinical disease. The more rapid progression in those with multiple IA-2/IA-2\(β\) reactivity may simply reflect their identification at a later stage of preclinical disease. However, studies in offspring of parents with type 1 diabetes suggest that multiple Ag reactivity often occurs very early in life (18). Autoantibodies to multiple autoantigen determinants from different autoantigens has also been found associated with an increased likelihood of progression to disease in several studies (19–21), and therefore, both a high level and a wide range of humoral islet autoimmunity appear to be important covariates of diabetes risk. Note is our previous demonstration that the detection of IA-2/IA-2\(β\) Abs, using extracts of rat insulinoma cell lines in relatives with ICA, was associated with a more rapid progression to diabetes than that of relatives with ICA and autoantibodies to glutamic acid decarboxylase and/or insulin only (19). Estimation of the risk associated with individual Ab specificities is problematic because of the variable presence of Abs to other autoantigens. Progression to disease in relatives, however, does occur regardless of which Ab specificities are detected, and we suggest that in relatives, the predictive value of autoantibodies appears to be less dependent upon the overall specificity of the autoantibodies than the broadness of the reactivity.

We have identified potential key residues involved in the initial humoral response to IA-2. IA-2-specific residues in both the juxtamembrane and PTP-like domains appear to be important in early Ab recognition of Ag. The IA-2 juxtamembrane region (amino acids 601–682), in particular, appears to harbor unique epitopes not found in IA-2\(β\), and many patients and relatives have Abs that recognize only this region of the IA-2 protein. Ab binding to IA-2/IA-2\(β\) PTP-like domain chimeras determined that the amino acids that are critical for Ab recognition of the IA-2-specific PTP-like domain epitopes are contained within residues 794–889 of the IA-2 protein. Within this region, there are a total of 19 residues that differ between IA-2 and IA-2\(β\), 9 of which represent only conserved changes. This finding needs to be confirmed using IA-2 with specific mutations and in studies examining the epitope specificities of IA-2/IA-2\(β\) that appear in the first years of life, but their identification is of potential importance for disease prevention. Identification of residues that stimulate autoimmunity but are not essential for function may enable the genetic engineering of functionally intact \(β\) cells with a lower capacity to induce or re-stimulate autoimmunity. Knowing these residues may also be helpful in designing vaccines. Immunization with either insulin or glutamic acid decarboxylase autoantigens in animal models of diabetes can induce T cell tolerance and delay diabetes onset (22–24). Thus far, there are no studies showing that IA-2/IA-2\(β\) or peptides of these Ags can induce T cell or B cell tolerance or prevent diabetes in these models. Moreover, humoral autoimmunity to IA-2 and IA-2\(β\) does not appear to be a feature of these animals (25). It still remains unclear whether immunization with any of the diabetes-associated Ags can induce tolerance in man, especially after autoimmunity has been initiated. Nevertheless, several proposed intervention strategies are based on the principle that Ag-induced modulation of the chronic inflammation leading to diabetes is possible either through tolerance induction or, more likely, specific regulation and/or Th2-like immune activation. Since IA-2 and IA-2\(β\) are expressed within islet \(β\) cells, it is expected that induction of Th2-like immunity against these proteins may also delay disease onset. Maintenance of residues that readily promote Ab responses is likely to be useful in the development of strategies for vaccination with Ag, during which an induction of a protective Th2-like immunity is desired.

Other IA-2 residues appear to be important for structure and function as well as Ab binding (13, 15). Residue 911 has been shown to restore partial classic PTPase activity of IA-2 if changed to an alanine; this activity was further enhanced by the additional change of residue 877 to an aspartic acid. The finding that these changes also affect binding of autoantibodies to the PTP-like domain suggests that they may be important for overall PTP-like domain structure. Residue 877 is interesting because it is an aspartic acid in native IA-2\(β\), and it would therefore be expected that the cross-reactive PTP-like domain Abs would preferentially bind to IA-2. Evidence for the presence of Abs that recognize both IA-2 and IA-2\(β\) PTP-like domains with different affinities is given by the ability of IA-2\(β_{θ=1-1047}\) to compete for the binding of IA-2_{θ=741-1047} in some sera. Finally, sera from two patients in our study had Abs that bound to human but not rat IA-2\(β\). Both of these patients had cross-reactive PTP-like domain Abs but not IA-2\(β\)-specific Abs. Therefore, residues that are not conserved between the rat and human IA-2\(β\) PTP-like domains must be involved in the Ab recognition of some of the epitopes that are shared between IA-2 and IA-2\(β\). Since only a minority of sera with cross-reactive PTP-like domain Abs did not bind to rat IA-2\(β\), it is likely that there are several different epitopes that are shared between IA-2 and IA-2\(β\).

In conclusion, this study shows that autoantibody binding to the PTP-like autoantigens associated with type 1 diabetes is most frequently against IA-2 rather than IA-2\(β\); that almost all individuals with IA-2/IA-2\(β\) Abs have Abs that bind specifically to IA-2 and not IA-2\(β\); and that these IA-2-specific Abs are most frequently those that can be identified in the early Ab response to IA-2/IA-2\(β\). These findings suggest that IA-2 is the primary PTP-like autoantigen in type 1 diabetes. Potentially critical residues for the recognition of the IA-2-specific epitopes have been identified, and their identification may be useful for the design of analogues that can be used for preventing destructive autoimmunity. The finding of an increased disease risk associated with increased diversity of the Ab reactivity to IA-2/IA-2\(β\) epitopes suggests that studying this diversity may assist in disease prediction and, moreover, may
be a valuable inclusion as a surrogate marker when evaluating the efficacy of intervention therapies to prevent type 1 diabetes.

Acknowledgments

We thank William Moore for his assistance in sample preparation.

References


