Crystal Structure of the Major Diabetes Autoantigen Insulinoma-Associated Protein 2 Reveals Distinctive Immune Epitopes

Seung Jun Kim, Dae Gwin Jeong, Sook Kyung Jeong, Tae-Seong Yoon, and Seong Eon Ryu

Insulinoma-associated protein-2 (IA-2) is a major autoantigen in type 1 diabetes that occurs through autoimmune-mediated β-cell destruction. We present here the crystal structure of the protein tyrosine phosphatase (PTP)-like domain of human IA-2. The structure reveals a canonical PTP domain with the closed WPD loop over the active site pocket, explaining the lack of enzyme activity in the native protein. The structural interpretation of previous mutagenesis studies indicates that the B-cell epitopes are concentrated on two distinctive regions on peripheral loops of the central β-sheet surrounding T-cell epitopes within the sheet. The detailed structural information on immune epitopes provides a framework for the future development of immune intervention strategies against diabetes. Diabetes 56:41–48, 2007

Type 1 diabetes is an autoimmune disease involving β-cell destruction by immune responses against self-proteins (1). Together with GAD65 and insulin, the islet antigen insulinoma-associated protein-2 (IA-2) is a major autoantigen in type 1 diabetes (2). Autoantibodies against IA-2 are detected in sera of 60–80% of recent-onset type 1 diabetic patients (3,4). They are usually found several years before onset of clinical symptoms, and thus have been used as markers of diabetes diagnosis. The presence of autoantibodies to IA-2 and GAD65 in serum indicates 50% probability of type 1 diabetes development within 5 years (5).

IA-2 is a type I membrane protein comprising an NH₂-terminal glycosylated extracellular region, a membrane-spanning region, and a COOH-terminal cytoplasmic region (6). The immune epitopes of IA-2 are exclusively located in the cytoplasmic region (7–9). Within the cytoplasmic region of IA-2, the PTP-like domain is the major region for autoantibody recognition, and the juxtamembrane region has a few immune recognition epitopes for the autoantibodies found early in the disease. Dominant T-cell epitopes of IA-2, which also reside in the protein tyrosine phosphatase (PTP)-like domain, have significant similarity with a region of VP7, the major immunogenic protein of human rotavirus, suggesting a molecular mimicry mechanism for IA-2-mediated autoimmunity (10). However, the pathogenic mechanism of autoimmune responses by IA-2 has not been clearly revealed yet.

Targeted disruption of IA-2 in mice results in a significant elevation of glucose and depressed insulin release, indicating that IA-2 is involved in glucose-stimulated insulin secretion (11–13). Besides the regulatory role of IA-2 in secretory vesicle mobilization and recruitment in exocytosis, it was found that IA-2 heterodimerizes with other receptor-type PTPs, such as receptor PTP (RPTP)-α and -ε, influencing the signaling pathway in the regulation of exocytosis of hormones and peptides (14). The IA-2 PTP-like domain lacks enzyme activity and has substitutions in the conserved active site residues (15). Back mutations of the substituted residues in IA-2 partially restored PTP enzyme activity (16,17). However, the evolutionary conservation of the substitutions indicates that the lack of enzyme activity is likely required for the protein’s physiological function (18).

Extensive studies on the immune epitopes of IA-2 have found that mutations influencing autoantibody recognition are scattered around the primary sequence of the PTP-like domain (19). However, epitope mapping of conformational antibodies and their potential relationships with T-cell responses were hampered by the lack of three-dimensional structure of IA-2. To understand the conformational immune epitopes and biological activity of IA-2, we determined the crystal structure of the IA-2 PTP-like domain. The structure reveals the mechanism of inactivity in the PTP-like domain as well as detailed information on spatial relationships between immune-epitope residues, providing important implications on autoimmune responses mediated by IA-2.

From the Systemic Proteomics Research Center, Korea Research Institute of Bioscience and Biotechnology, Yuseong-gu, Daejeon, Korea.

Address correspondence and reprint requests to Seong Eon Ryu, PhD, or Seung Jun Kim, PhD, Systemic Proteomics Research Center, Korea Research Institute of Bioscience and Biotechnology, 52 Eui-eun-dong, Yuseong-gu, Daejeon 305-333, Korea. E-mail: ryuse@kribb.re.kr or ksj@kribb.re.kr.

Received for publication 20 February 2006 and accepted in revised form 5 October 2006.

IA-2, insulinoma-associated protein 2; IA-2p, PTP-like domain of IA-2; LAR, leukocyte antigen-related; MHC, major histocompatibility complex; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PTP, protein tyrosine phosphatase; RPTP, receptor PTP.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

RESEARCH DESIGN AND METHODS

The gene encoding the PTP-like domain of IA-2 (IA-2p; residues 709–979) was subcloned into pET28a and expressed in the Escherichia coli BL21(DE3) strain by 0.1 mmol/l isopropyl β-D-thiogalactopyranoside induction. Cells were harvested and resolved in a lysis buffer containing 50 mmol/l Tris-HCl, pH 7.5, 200 mmol/l NaCl, and 1 mmol/l phenylmethylsulfonyl fluoride. After cell lysis by sonication, the His-tagged IA-2p protein was purified by nickel-affinity chromatography. The His tag was removed by thrombin protease digestion. The IA-2p protein was further purified by Q-Sepharose ion exchange and gel filtration chromatographies. The purified protein was dialyzed against a buffer...
**TABLE 1**

Data collection and refinement statistics

|                         | Values |
|-------------------------|--------|
| **Data collection**     |        |
| Space group             | C222_1 |
| Cell parameter          | a = 76.6 Å, b = 80.5 Å, c = 239.9 Å |
| Highest resolution (Å)  | 2.1    |
| Unique reflections (total) | 44,502 (270,952) |
| Completeness (%)*       | 99.9 (99.9) |
| Rmerge (%)†             | 6.4 (33.2) |
| I/σ                    | 8.4 (2.3) |
| **Refinement**          |        |
| Number of reflections   | 43,742 |
| Number of atoms         |        |
| Protein/nonprotein      | 4,370/349 |
| Rcryst/Rfree            | 19.7/22.8 |
| Bond distances (Å)      | 0.009  |
| Bond angles (°)         | 1.48   |
| Improperς (°)           | 0.99   |
| Dihedrals (°)           | 23.5   |

*The values in parentheses (completeness and Rmerge) are for the highest resolution bin; \( R_{\text{merge}} = \Sigma_i \frac{|I_i - \langle I \rangle_i|}{|I_i|} \), where \( I_i \) is the intensity for the \( i^{\text{th}} \) measurement of an equivalent reflection with the indices h, k, and l. Rms, root mean square.

Crystallization and data collection. Crystallization was performed at 18°C by the hanging-drop vapor diffusion method, and initial trials were carried out using commercial screening kits (Hampton Research). The best crystals were grown by mixing 1.8 μl of protein solution and an equal volume of reservoir solution containing 0.1 mol/l Bis-Tris (pH 6.5), 13% (vol/vol) monomethoxy polyethylene glycol (MW 5 kDa), 19% (vol/vol) glycerol, and 5 mmol/l dithiothreitol. The crystals were flash-cooled into nitrogen gas stream at −180°C. The crystal diffracted to 2.1 Å and belonged to the space group C222_1, with unit cell parameters of a = 76.6 Å, b = 80.5 Å, and c = 239.9 Å. The collected diffraction data were processed and scaled with the programs MOSFLM (20) and SCALA (21), respectively. The statistics for data collection and refinement are summarized in Table 1.

Structure solution and refinement. The structure of IA-2p was determined by the molecular replacement method, using the leukocyte antigen-related (LAR) PTP D1 domain (PDB code: 1LAR) as a search model (22). The program EPMR (23) placed two monomers in the asymmetric unit of the crystal. The Ramachandran plot drawn by the program Procheck (26) shows that 88.2% of residues fall within the generously allowed region and another two residues (Asp181, Ala227, and Gln262) are found at the end of strand β3, extending the central β-sheet laterally. The overall architecture of IA-2p is similar to other classical PTP structures, such as PTP1B and PTPζ (27,28). However, there are several important structural differences in the various regions, including the region corresponding to the PTP active site (see below). The crystal contains two IA-2p molecules (A and B) per asymmetric unit making noncrystallographic two-fold interactions with each other (A:B interaction). There is another intermolecular interaction made by the crystallographically observed dimers are unlikely to exist in solution.

**Enzyme activity.** In marked contrast to other PTPs, IA-2p lacks enzyme activity toward conventional PTP substrates (15). The lack of activity has been attributed to substitutions of highly conserved residues that are essential to catalysis (17). Sequence alignment of IA-2p with other PTPs shows that four residues essential for PTP enzyme activity are not conserved in IA-2p (Fig. 2). The four residues in IA-2p are His740, Ala877, Arg911, and Arg954 corresponding to Tyr46, Asp181, Ala227, and Gln262, respectively, in the conventional enzyme PTP1B. In PTP1B, Tyr46 is involved in hydrophobic interactions with phosphotyrosine substrate; Asp181 in the WPD loop functions as a general acid in the enzyme reaction; Ala227 is located in the loop containing the catalytic cysteine (the catalytic loop); and Gln262 coordinates scissile oxygen during catalysis and activates the attacking water.

In the IA-2p structure, Asp911 points inside the catalytic loop, making a salt bridge interaction with Arg954 (Fig. 1). In addition, two short β-strands (β4 and β5) are found at the end of strand β3, extending the central β-sheet laterally. The overall architecture of IA-2p is similar to other classical PTP structures, such as PTP1B and PTPζ (27,28). However, there are several important structural differences in the various regions, including the region corresponding to the PTP active site (see below). The crystal contains two IA-2p molecules (A and B) per asymmetric unit making noncrystallographic two-fold interactions with each other (A:B interaction). There is another intermolecular interaction made by the crystallographically observed dimers are unlikely to exist in solution.
FIG. 2. Sequence alignment of IA-2p and related PTPs. Based on the experimental structure, sequences of four PTPs (IA-2p, PTP1B, LAR, and TCPTP [T-cell PTP]) are aligned using the program CLUSTALW (45). Secondary structural elements of IA-2p are indicated above the aligned sequences. Residues with 100% identity are colored yellow, and those with a conservation value >5.0 defined in the program ALSCRIPT (46) are colored magenta. The residues important for PTP activity are indicated as inverted triangles. Among those, the nonconserved residues in IA-2p are enclosed in black boxes.

FIG. 3. The PTP catalytic site. A: Structures of IA-2p (cyan) and PTP1B (PDB code: 2HNP) (gray) are superimposed in the PTP catalytic site. Side chains of IA-2 are drawn as a ball-and-stick model. The hydrogen bonding interaction between Arg954 and Asp911 is indicated by the dotted line. B: The electrostatic potential surface. Positive and negative potentials are colored blue and red, respectively. Electrostatic surface was calculated with the program GRASP (47). The conserved catalytic Cys909 and the WPAE loop residues (Ala877 and Glu878) are labeled.
Autoantibody epitopes and solvent accessibility

| Antibody* | Critical amino acids | FSA >0.5 | 0.25 < FSA < 0.5 |
|-----------|----------------------|----------|------------------|
| 76/12     | 794–845, 815, 818, 829, 830, 834 | (D826), (E827), (H843), D815, K818 | A829, S830, (V813), (W845) |
| 96/3†     | i) 858, 836, 799; ii) 836, 838, 855 | P876, E878 | A877, T880 |
| 96/5      | 876, 877, 878, 880 | N862 | V859 |
| 2_sera    | 859, 862, 867 | — | — |
| 7_sera    | 876, 877, 878, 880 | — | — |
| 6_sera    | 876, 877, 878, 880, 862, 822 | P876, E878, N862 | A877, T880, R822 |
| M13       | 777–808 | (S788), (H789) | (M777), (P778), (A779), (T804) |

The fractional solvent accessibility of each amino acid residue in the IA-2p structure was calculated using the program QUANTA as the ratio of the side chain fractional solvent accessibility for residue X to the fractional solvent accessibility obtained after reducing the structure to a Gly-X-Gly tripeptide. The epitope residues mapped by regional deletion were designated by parentheses to distinguish from those mapped by point mutations. *Antibody names from Kolm-Litty et al. (76/12 and 96/5) (19), Dromey et al. (96/3) (35), Bearzatto et al. (2_sera, 7_sera and 6_sera) (34), and Ananieva-Jordanova et al. (M13) (48); †for the antibody 96/3, the residues implicated by both the i) homology-modeled structure and ii) the crystal structure were analyzed by fractional solvent accessibility calculation. FSA, fractional solvent accessibility.

This salt bridge interaction greatly diminishes the exposed area of the catalytic loop and thereby blocks access of substrates into the catalytic loop. When the IA-2p structure is superimposed with the ligand-bound PTPs (29,30), the position of the salt bridge overlaps that of substrates. The side chain of His740 points away from the catalytic loop, losing the pTyr stabilizing effect of the corresponding residue in PTP1B.

In classical PTPs, the WPD loop near the catalytic loop plays an important role in substrate binding and catalysis (31). In the ligand-free states, the WPD loop is open for the substrate access and, on ligand binding, the loop is closed to stabilize the bound ligand. The WPD loop in the closed state presents the aspartic acid (D) of the loop in an appropriate position for the role of general acid. In IA-2p, the aspartic acid is changed to Ala877-Glu878, resulting in a WPAE loop. Because of the insertion of alanine (A) before an acidic residue (E), the acidic side chain of Glu878 is not properly positioned to perform the role of general acid (Figs. 2 and 3A). In addition, the WPAE loop of IA-2p exhibits a closed conformation even in the absence of a substrate in the active site. The closed WPAE loop conformation is likely to prohibit access of peptide or large molecule substrates. Although the closed WPAE loop narrows the pocket entrance by half of the size, the remaining pocket has a size of ~6–10 Å that is able to accommodate small molecule substrates or inhibitors (Fig. 3B and see below). Back mutations of some of these residues to the PTP consensus amino acids partially restore the ability to dephosphorylate PTP substrates (16). However, the restored activity was not very high compared with that of the usual PTP enzymes, consistent with the lack of activity in IA-2p due to the combination of multiple structural factors found in the IA-2p structure.

Like IA-2p, the D2 domain of RPTPs, including RPTPa and LAR, usually lacks catalytic activity. However, two point mutations of the D2 domain residues to corresponding PTP1B residues (RPTPa:V555Y and RPTPa:E690D; and LAR:L1644Y and LAR:E1779D) result in similar catalytic activity of the D1 domain (22,32). In comparison, catalytic activity of IA-2p did not reach that of other PTPs, even with extensive back mutations (16). It would be also of interest to consider PTEN phosphatase (phosphatase and tensin homolog deleted on chromosome 10), whose physiological substrate is not phosphoprotein but phosphatidylinositol. In PTEN, an enlarged active pocket and some positively charged residues accommodate the large substrate PI(3,4,5)P3 (33). Despite PTEN's unique active site pocket shape, the catalytically important residues are well conserved (33).

**B-cell epitopes.** Epitope mapping of the patient sera was carried out using site-directed mutagenesis and chimeric constructs of IA-2 with the less immunogenic IA-2β (34). The study identified three groups of sera (2_sera, 7_sera and 6_sera) in which different IA-2 residues were critical for antibody binding (Table 2). In another study, human monoclonal antibodies were generated from peripheral B-cells isolated from type 1 diabetic patients, and subsequent mutagenesis studies identified IA-2 residues critical for binding of each monoclonal antibody (19) (Table 2). These studies found various residues whose mutations affect autoantibody binding toward IA-2. However, epitope information in the primary sequence cannot discriminate mutations disrupting direct antibody interaction from those destabilizing protein structure. Because the side chain solvent accessibility of each residue can be a good measure of direct interaction between antibodies and IA-2, we calculated the side chain solvent accessibility for each residue found to affect autoantibody binding (Table 2).

Recently, it was found that the monoclonal antibody 96/3 competes with autoantibodies in most patient sera. Phage display experiments found that aromatic, asparagine, and glutamic acid residues were likely involved in the binding of IA-2 (35). Based on homology modeling of IA-2 and phage display experiments, the authors proposed that Asn858, Glu836, and Trp799 might be a binding site for 96/3 antibody. However, in the crystal structure of IA-2p, Asn858 and Trp799 are located on the opposite side of Glu836, indicating that the three residues cannot form a binding surface (Fig. 4). Instead, we found a surface comprising clustered aromatic, asparagine, and glutamic acid residues (Tyr855, Asn838, and Glu836) on the other side of the molecule. (Fig. 4). There is no other prominent surface of clustered aromatic, asparagine, and glutamic acid residues in the IA-2p structure. The E836K mutation completely abolished antibody binding, whereas the N858A and W799A mutations exhibited 40% inhibition, supporting the principal role of Glu836 in 96/3 binding (35). However, the effects N858A and W799A mutations, whose side chain solvent accessibility are 0.07 and 0.15, respectively, may be attributable to structural perturbation.

Structural display of residues implicated in the autoantibody interactions reveals two major epitope regions in IA-2p: one in the WAPE loop region and the other in the lower part of the central β-sheet region (Fig. 5). The WAPE loop residues (Pro876, Ala877, Glu878, and Thr880) were...
found to recognize a group of sera, including 7_sera and 2_sera, and the monoclonal antibody 96/5. The epitope of antibody 76/12 (Asn815 and Lys818) is also near the WPAE loop, constituting the first cluster of epitope residues. The lower part of the central H-sheet contains many highly exposed residues that are possibly involved in the autoantibody interactions. The residues implicated as the epitope of 2_sera (Asn862 and Val859) and antibody 96/3 (Tyr855, Asn838, and Glu836) are located in the same region, constituting the second cluster of the B-cell epitope. Among the three residues implicated in antibody 96/3 binding, the critical role of Glu836 was verified by previous point mutagenesis studies (35). The residues implicated in 2_sera binding (Asn862 and Val859) were also verified by point mutations (34). Clustering of residues implicated in two independent studies indicates that the surface comprised of Asn862, Val859, and Glu836 is likely an antigenic hot spot, and this hot spot may be extended to include Tyr855 and Asn838 (Fig. 5).

Interaction of B- and T-cell epitopes. The antibody-antigen complex can remain intact during antigen processing and presentation for T-cell receptors, and the antigen-bound antibodies affect T-cell responses by protecting the antigen residues from proteolysis or by triggering conformational changes (36, 37). In the major histocompatibility complex (MHC) class II-restricted presentation of tetanus toxin antigen, antigen processing and MHC class II loading of the footprinted region was hindered, whereas presentation of other regions was boosted (36). Human autoantibodies for the autoantigen thyroid peroxidase influenced the activity of self-reactive T-cells (37). In type 1 diabetes, the critical role of B-cells and their antigen presentation function was revealed, indicating an interplay of B- and T-cell responses (38). Because the immunodominant IA-2p or GAD are located in the cytoplasmic space, B-cells are likely to recognize low amounts of the islet autoantigens, which are released into circulation through cell rupture during the initial islet cell damage (39).

FIG. 4. Location of 96/3 antibody epitope residues. A and B: Potential epitope residues on the molecular surface drawn from the IA-2p structure. The epitope residues are presented as a ball-and-stick model. The point of view in B is the rotation of 90° along the vertical axis of A. C: Electron density. The 2Fo-Fc electron density of the epitope residues are presented at a level of 1.2 sigma.
Various reports describing T-cell epitopes of IA-2 have found that regions of residues 787–817 (T1 region) and 841–869 (T2 region) are the most prevalent T-cell epitope regions (40,41). In the IA-2p structure, the T1 region starts from the middle of helix α4 and continues through strands β3–β5 (Fig. 5). The T2 region encompasses strands β7–β9 in the central β-sheet. The two T-cell epitope regions are located closely to each other and form strong interactions. Residues Trp795, Val798, and I806 of T1 form hydrophobic interactions with residues Phe854, Leu856, and Leu867 of T2, resulting in a strong hydrophobic core in the middle of the protein. Strand β3 of T1 is involved in hydrogen bonding interactions with the neighboring strand β9 of T2, completing the central β-sheet. The two B-cell epitope clusters flank the T-cell epitope regions with partial overlaps between them (Fig. 5). For example, Tyr855, Val859 and Asn862 reside in the edge of T2, whereas Asp815 is located in an exposed loop of T1. The closely related location of the B- and T-cell epitopes is reminiscent of the finding in tetanus toxin (36), suggesting the autoantibody binding to IA-2 may influence antigen processing for the T-cell response. When the autoantibodies bind to the ends of the central β-sheet region, the antibodies are likely to block the sites from processing by proteolytic enzymes. In the meantime, antibody binding may stabilize the T1 and T2 cores, helping an appropriate processing of the region for presentation on the MHC class II molecules.

In the IA-2 structure, there are several B-cell hot spot residues residing within the potential T-cell epitope regions (Fig. 5), indicating that certain residues of B-cell epitopes may participate in T-cell–mediated immune response. Previously, it was proposed that T-cells would recognize very different antigenic determinants from B-cells because T-cells bind to linear peptides, whereas B-cell epitopes of IA-2 are likely conformational (9). In our IA-2 structure, the B-cell hot spots contain specific structural features such as turns and β-strands, indicating the B-cell epitopes of IA-2 are highly conformational. The epitope residues are also highly exposed to solvent and accessible for autoantibody binding. However, when the IA-2 protein is proteolytically cleaved into linear peptides for presentation on MHC molecules, the tertiary structure of IA-2 is disrupted, and the B-cell epitope residues are able to form very different antigenic structures for recognition by T-cell receptors.

**DISCUSSION**

The current structure provides detailed information on the location and solvent exposure of residues implicated in the autoimmune responses of IA-2. The structural information would be valuable for design and analysis of further site-directed mutagenesis studies aiming at comprehensive understanding of IA-2–mediated autoimmunity. The understanding could eventually lead to the development of novel diagnostic and therapeutic strategies against type 1 diabetes. First, the atomic level definition of antigenic hot spots in the structure could be used to design conformation-constrained peptide antigens. Previously, it was shown that conformation-constrained peptides could mimic native structure, and these could be used as synthetic vaccines or modulators of receptor-ligand interactions (42,43). The conformation-constrained peptides mimicking antigenic hot spots of IA-2 may also be used for developing peptide enzyme-linked immunosorbent assay protocols for detection of IA-2 autoantibodies in blood, which are cheaper and more efficient than conventional protein-based diagnostic methods (44). Second, the finding that B- and T-cell epitopes of IA-2 overlap may be exploited for the design of immunomodulation strategies. By using the IA-2 structure and epitope mapping information, one would be able to engineer the protein or to design conformation-constrained peptide antigens to raise novel antibodies that bind the T-cell epitope regions. The novel antibodies may interfere with the usual proteolytic processing of IA-2 in the antigen-presenting cells, resulting in alleviation of T-cell–mediated pathogenic autoimmunity. Third, the surface structure of the antigenic hot spots could be used to design novel small molecules that bind to surface pockets and inhibit auto-
antibody binding. The most representative surface pocket implicated in the hot spots is the one corresponding to the PTP-active site (the cysteine pocket) near the WPAE loop, a major antigenic hot spot (Fig. 3F). The small molecule binding to the cysteine pocket may trigger a conformation change in the WPAE loop, resulting in disruption of autoantibody binding.

ACKNOWLEDGMENTS

This work was supported by a Korea Research Institute of Bioscience and Biotechnology research initiatives grant.

We thank Drs. H.S. Lee (Pohang Light Source BL4A) and N. Sakabe (Photon Factory BL18B) for help with data collection.

REFERENCES

1. Palmer JP, Asplin CM, Clemons P: Insulin autoantibodies in insulin-dependent diabetes before insulin treatment. Science 222:1337–1339, 1983
2. Atkinson MA, Eisenbarth GS: Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 355:221–229, 2000
3. Leslie RDG, Atkinson MA, Notkins AL: Autoantigens IA2 and GAD in type 1 (insulin dependent) diabetes. Diabetologia 42:3–14, 1999
4. Nishino M, Ikegami H, Kawaguchi Y, Fujisawa T, Kawahata Y, Shintani M, Ono M, Horiki M, Kawasaki E, Oghara T: Polymorphism in gene for islet autoantigen IA-2, and type 1 diabetes in Japanese subjects. Hum Immunol 62:518–522, 2001
5. Notkins AL, Lernmark A: Autoimmune type 1 diabetes: resolved and unresolved issues. J Clin Invest 108:1247–1252, 2001
6. Solimena M, Dirks R Jr, Herrel JM, Pleasance-Williams S, Shapiro JA, Caron LG, Janeway CA Jr, Shlomchik MJ: Investigation of the role of B-cells in insulin-dependent diabetes mellitus. Curr Opin Immunol 16:759–767, 2004
7. Drake PG, Peters GH, Andersen HS, Hendriks W, Møller NPH: A novel strategy for the development of selective active-site inhibitors of the protein tyrosine phosphate-like protein IA-2 eluted from HLA-DR4. J Clin Invest 104:1449–1457, 1999

S.J. KIM AND ASSOCIATES

DIABETES, VOL. 56, JANUARY 2007
41. Hawkes CJ, Schloot NC, Marks J, Willemen SJ, Drijfhout JW, Mayer EK, Christie MR, Roep BO: T-cell lines reactive to an immunodominant epitope of the tyrosine phosphatase-like autoantigen IA-2 in type 1 diabetes. *Diabetes* 49:356–366, 2000

42. Moreno R, Jiang L, Moehle K, Zurbriggen R, Gluck R, Robinson JA, Pluschke G: Exploiting conformationally constrained peptidomimetics and an efficient human-compatible delivery system in synthetic vaccine design. *ChemBiochem* 2:838–843, 2001

43. Berezov A, Chen J, Liu Q, Zhang HT, Greene MI, Murali R: Disabling receptor ensembles with rationally designed interface peptidomimetics. *J Biol Chem* 277:28330–283339, 2002

44. Plagemann PGW: Peptide ELISA for measuring antibodies to N-protein of porcine reproductive and respiratory syndrome virus. *J Virol Methods* 134:99–118, 2006

45. Thompson JD, Higgins DG, Gibson TJ: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acid Res* 22:4673–4680, 1994

46. Barton GJ: ALSCRIPT: a tool to format multiple sequence alignments. *Protein Eng* 6:37–40, 1993

47. Nicholls A, Sharp KA, Honig B: Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins* 11:281–296, 1991

48. Ananieva-Jordanova R, Evans M, Nakamatsu T, Premawardhana LD, Sanders J, Powell M, Chen S, McGrath V, Belton C, Arnold C, Baker S, Betterle C, Zanchetta R, Smith BR, Furmaniak J: Isolation and characterization of a human monoclonal autoantibody to the islet cell autoantigen IA-2. *J Autoimmun* 24:337–345, 2005