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Beyond HLA-A*0201: New HLA-Transgenic Nonobese Diabetic Mouse Models of Type 1 Diabetes Identify the Insulin C-Peptide as a Rich Source of CD8+ T Cell Epitopes

Zoltan Antal, Jason C. Baker, Carla Smith, Irene Jarchum, Jeffrey Babad, Gayatri Mukherjee, Yang Yang, John Sidney, Alessandro Sette, and Teresa P. DiLorenzo

Type 1 diabetes is an organ-specific autoimmune disease characterized by T cell responses to β cell Ags, including insulin. Investigations employing the NOD mouse model of the disease have revealed an essential role for β cell-specific CD8+ T cells in the pathogenic process. As CD8+ T cells specific for β cell Ags are also present in patients, these reactivities have the potential to serve as therapeutic targets or markers for autoimmune activity. NOD mice transgenic for human class I MHC molecules have previously been employed to identify T cell epitopes having important relevance to the human disease. However, most studies have focused exclusively on HLA-A*0201. To broaden the reach of epitope-based monitoring and therapeutic strategies, we have looked beyond this allele and developed NOD mice expressing human β2-microglobulin and HLA-A*1101 or HLA-B*0702, which are representative members of the A3 and B7 HLA supertypes, respectively. We have used islet-infiltrating T cells spontaneously arising in these strains to identify β cell peptides recognized in the context of the transgenic HLA molecules. This work has identified the insulin C-peptide as an abundant source of CD8+ T cell epitopes. Responses to these epitopes should be of considerable utility for immune monitoring, as they cannot reflect an immune reaction to exogenously administered insulin, which lacks the C-peptide. Because the peptides bound by one supertype member were found to bind certain other members also, the epitopes identified in this study have the potential to result in therapeutic and monitoring tools applicable to large numbers of patients and at-risk individuals. The Journal of Immunology, 2012, 188: 5766–5775.

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Abbreviations used in this article: IGRP, islet-specific glucose-6-phosphatase catalytic subunit-related protein; Ins, insulin; β2m, β2-microglobulin.

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glucose-6-phosphatase catalytic subunit-related protein (IGRP) are recognized by CD8+ T cells in most individuals. For example, in one study, 85% of recent-onset HLA-A*0201-positive patients showed T cell responses to the HLA-A*0201-binding peptide Ins L15–24, and one quarter showed responses to IGRP 265–273 (27).

Although multiple β-cell peptides have been identified as the antigenic targets of CD8+ T cells in type 1 diabetes patients, nearly all of these are recognized in the context of HLA-A*0201 (12). Although this is a common class I MHC molecule expressed in nearly 50% of certain ethnic groups (31), elucidation of the insulin and IGRP peptides recognized in the context of other human class I molecules would allow broader coverage of the patient population in terms of those that could benefit from the development of peptide-based predictive, diagnostic, and therapeutic strategies (32). In the past, we have used HLA-A*0201-transgenic NOD mouse models to identify epitopes of insulin and IGRP that are recognized by islet-infiltrating CD8+ T cells (28, 30, 33), and this strategy successfully identified epitopes (e.g., IGRP 228–236, IGRP 265–273) in B3a14 and B1R18 that were also found to be recognized in HLA-A*0201-positive type 1 diabetes patients (25–27, 34–38). In our efforts to look beyond HLA-A*0201, in this study we have generated NOD mouse strains for at least 10 generations and fixed to homozygosity for markers of NOD mice transgenic for H-2Db (KH95) and murine CD8α, and human CD8α and human CD8β (43, 44). Both strains were backcrossed to NOD mice for at least 10 generations and fixed to homozygosity for markers of NOD background, resulting in the NOD.hCD8 strain.

Materials and Methods

Derivation of NOD.mβ2mnull.hβ2m mice transgenic for HLA-A*1101 or HLA-B*0702

C57BL/6 mice expressing a chimeric HLA-A*1101 H chain (α1 and α2 domains of HLA-A*1101, and α3, transmembrane, and cytoplasmic domains from H-2Kb) or a fully human HLA-B*0702 H chain were obtained from C. David (Mayo Clinic) and have been described previously (43, 44). Both strains were backcrossed to NOD mice for at least 10 generations and fixed to homozygosity for markers of NOD origin delineating known Idd loci. Murine β2m was removed and human β2m introduced by intercrossing with the NOD.mβ2mnull.hβ2m strain (45), yielding the two new strains designated NOD.mβ2mnull.hβ2m HLA-A11 and NOD.mβ2mnull.hβ2m HLA-B7.

Derivation of NOD.nCD8 mice

C57BL/6 mice expressing human CD8α and human CD8β under the control of the murine p56 Lck proximal promoter (line αβ57) were obtained from The Jackson Laboratory (46). They were backcrossed to NOD mice for at least 10 generations and fixed to homozygosity for markers of NOD origin delineating known Idd loci, resulting in the NOD.nCD8 strain.

Flow cytometric analysis of splenocytes

Single-cell spleen suspensions were obtained by gentle grinding between frosted slides and passing through a 40-μm cell strainer. Splenocytes from NOD.mβ2mnull.hβ2m HLA-A11, NOD.mβ2mnull.hβ2m HLA-B7, and murine β2m-expressing NOD.HLA-A11 mice were analyzed by multicolor flow cytometry after staining with labeled Abs to class I HLA H chains (B9,12.1; Beckman Coulter), human β2m (TD99), H-2Kb (SF1-1.1), and H-2Dβ (KH95). Splenocytes from NOD.nCD8 and NOD mice were analyzed after staining with labeled Abs specific for murine CD4 (RM4-5), murine CD8α (53-6-7), and human CD8α (RPA-T8). All Abs for flow cytometry were obtained from BD Biosciences except where indicated.

Assessment of diabetes development

Mice were monitored weekly for glucosuria with Diasstix reagent strips for urinalysis (Bayer). Mice were considered diabetic when two positive measurements were obtained. The time of onset of disease was recorded as the date of the first of the two positive readings.

Cyclophosphamide treatment

Female mice (12–21 wk age) were treated with two injections of 200 mg/kg cyclophosphamide (Sigma-Aldrich) i.p. 2 wk apart (47, 48). Diabetes development was monitored weekly after the first injection.

Peptides

Peptide libraries containing all of the 8-mer, 9-mer, 10-mer, and 11-mer peptides that can be derived from murine preproinsulin 1, preproinsulin 2, or IGRP were synthesized by Mimotopes using their proprietary Truncated PepSet technology. Each library mixture contained four peptides with a common C terminus and having a length of 8, 9, 10, or 11 residues. The four peptides in each mixture were present in approximately equimolar amounts. Concentrated peptide stocks (2.75 mM) were prepared in 50% acetonitrile/H2O, and 40 μM (i.e., ~10 μM for each peptide in the mixture) working stocks were obtained by serial dilution in PBS (pH 6.5). Individual peptides, having a purity of ≥90%, were obtained from Mimotopes. Concentrated stocks (10 mM) were prepared in DMSO, and 10 μM working stocks were obtained by dilution in PBS.

Islet-infiltrating T cells

Pancreatic islets were isolated from female mice by collagenase perfusion of the common bile duct and islet-infiltrating T cells were cultured as described (49). Briefly, islets were handpicked and cultured for 7 d in 24-well cell culture plates (~50 islets/well) in RPMI 1640 medium supplemented with 10% FBS (HyClone), 1 mM sodium pyruvate, nonessential amino acids, 28 μM Zn 2-ME, and 50 U/ml recombinant human IL-2 (PeproTech).

IFN-γ ELISPOT

IFN-γ ELISPOT was performed as described (49) using 2 × 106 islet-infiltrating T cells as responders and 2 × 104 syngeneic splenocytes pulsed with 1 μM peptide as APCs. Spots were developed after incubation at 37°C for 40 h and were counted using an automated ELISPOT reader system (Autoimmun Diagnostika). Where indicated, CD8+ T cells were first purified by negative selection from cultured islet infiltrates using magnetic separation (Miltenyi Biotec).

Tetramer staining of islet-infiltrating T cells

PE-labeled HLA-A*1101 tetramers were prepared following standard procedures (50) using a chimeric H chain (α1 and α2 domains of HLA-A*1101, and α3 domain of H-2Kb) and human β2m. Islet-infiltrating T cells were first stained with the tetramers and then with a labeled Ab to murine CD8α (53-6-7) and analyzed by flow cytometry.

MHC purification and peptide-binding assays

Purification of MHC molecules by affinity chromatography has been detailed elsewhere (51). Briefly, EBV-transformed homoygous cell lines or single MHC allele-transfected 721.221 or C1R lines were used as sources of HLA class I MHC molecules. HLA molecules were purified from cell pellet lysates by repeated passage over protein A-Sepharose beads conjugated with the W6/32 (anti-HLA A, B, C) Ab. In some cases, HLA-A molecules were separated from HLA-B and -C molecules by prepassage over a B1.23.2 (anti-HLA B, C, and some A) column. Protein purity, concentration, and the effectiveness of depletion steps were monitored by SDS-PAGE and bicinchoninic acid protein assay.

Assays to quantitatively measure peptide binding to class I MHC molecules were based on the inhibition of binding of a high-affinity radiolabeled peptide to purified MHC molecules, and were performed essentially as detailed elsewhere (51, 52). Briefly, 0.1–1 nM radiolabeled
peptide was coincubated at room temperature with 1 μM to 1 mM purified MHC in the presence of a mixture of protease inhibitors and 1 mM β2m. Following a 2-d incubation, MHC-bound radioactivity was determined by capturing MHC/peptide complexes on W6/32 (anti-class I) Ab-coated Lumitrac 600 plates (Greiner Bio-One), and measuring bound cpm using the TopCount microscintillation counter (Packard Instrument). In the case of competitive assays, the concentration of peptide yielding 50% inhibition of the binding of the radiolabeled peptide was calculated. Under the conditions used, where [label] < [MHC] and IC50 > [MHC], the measured IC50 values are reasonable approximations of the true Kd values (53, 54).

Each competitor peptide was tested at six different concentrations covering a 100,000-fold dose range, and in three or more independent experiments. As a positive control, the unlabeled version of the radiolabeled probe was confirmed the cell-surface expression of the human HLA-A11 or HLA-B7 molecule in addition to the endogenous murine class I MHC molecules H-2Kd and H-2Db was also observed (Fig. 1A, 1B).

Results

NOD.mβ2mnull.hβ2m mice transgenic for HLA-A11 or HLA-B7 are diabetes-susceptible to varying degrees

Splenocytes from NOD.mβ2mnull.hβ2m mice transgenic for HLA-A11 or HLA-B7 were analyzed by flow cytometry, which confirmed the cell-surface expression of the human β2m and HLA molecules (Fig. 1A, 1B). As expected, expression of the endogenous NOD class I MHC molecules H-2Kd and H-2Db was also observed (Fig. 1A, 1B).

Both the NOD.mβ2mnull.hβ2m.HLA-A11 and NOD.mβ2mnull.hβ2m.HLA-B7 mice were diabetes-susceptible, albeit to varying degrees (Fig. 2A, 2B) and far less so than standard NOD mice, which had a diabetes incidence of 90% (Fig. 2C). As had been reported previously (45), even non-HLA–transgenic NOD.mβ2mnull.hβ2m mice showed reduced disease compared with standard NOD mice (Fig. 2A–C). This phenomenon is thought to be due to the adoption of a nondiabetogenic conformation by murine class I MHC molecules containing human β2m (45). NOD mice expressing the endogenous murine β2m (instead of human β2m) along with the transgenic HLA-A11 H chain (designated NOD.HLA-A11 mice) still exhibited HLA-A11 expression (Fig. 1C), but they showed a diabetes incidence curve indistinguishable from that of NOD mice (Fig. 2C), showing that HLA-A11 expression on its own does not protect from disease. We did not examine NOD.HLA-B7 mice, because prior work showed that HLA-B7 is poorly expressed in the absence of human β2m (55).

To further characterize the NOD.mβ2mnull.hβ2m.HLA-A11 and NOD.mβ2mnull.hβ2m.HLA-B7 mice, we investigated whether cyclophosphamide treatment could accelerate disease as it does in standard NOD mice (48). For these experiments, female mice (12–21 wk age) were treated with two injections of 200 mg/kg cyclophosphamide i.p. 2 wk apart. Diabetes incidence was monitored weekly after the first injection. As depicted in Fig. 2D, cyclophosphamide treatment induced disease in most of the treated animals. As cyclophosphamide acceleration of diabetes is associated with a reduction in regulatory T cells (47), these findings suggest that diabetogenic effectors are generated in the NOD.mβ2mnull.hβ2m.HLA-A11 and NOD.mβ2mnull.hβ2m.HLA-B7 mice, but that they are generally kept in check by regulatory mechanisms.

Identification of HLA-A11–restricted epitopes of IGRP and insulin

We previously showed that IGRP is an important antigenic target of pathogenic CD8+ T cells in both standard (56) and HLA-A2–transgenic NOD mice (30). To determine whether NOD.mβ2mnull.hβ2m.HLA-A11 mice similarly harbor HLA-A11–restricted T cells specific for this β cell Ag, we used cultured islet-infiltrating T cells to screen, by IFN-γ ELISPOT assay, a peptide library containing all possible 8- to 11-mer sequences that can be derived from murine IGRP. The library was offset by one residue, and the peptide mixtures in the library contained four sequences with a common C terminus, but having a length of 8, 9, 10, or 11 residues. As shown in Fig. 3A, a strong T cell response was observed to peptide mixture 273. We next synthesized and tested the individual 8-, 9-, 10-, and 11-mer peptides comprising mixture 273 and found that the 9-mer peptide IGRP272–280, having the sequence AINSEMF, was the minimal epitope recognized by T cells (Fig. 4A). This sequence is consistent with the peptide-binding motif reported for HLA-A11, that is, L, M, I, V, S, A, T, G, or N at position 2, and K or R at the C terminus (57). In-
FIGURE 2. NOD.mβ2mnull.hβ2m mice transgenic for HLA-A11 or HLA-B7 are diabetes-susceptible to varying degrees. (A–C) Female HLA-transgenic mice of the indicated strains and their corresponding non-transgenic littermates were monitored weekly for diabetes development. (D) Female mice of the indicated strains (12–21 wk of age) were treated with two injections of 200 mg/kg cyclophosphamide i.p. 2 wk apart. Diabetes incidence was monitored weekly after the first injection. The horizontal axis indicates weeks following the first injection.

Interestingly, the peptide overlaps the HLA-A2–binding epitope IGRP265–273. Note that recognition of the 10- and 11-mer peptides from mixture 273 (Fig. 4A) most likely reflects proteolytic generation of the 9-mer peptide during the assay period. However, it cannot be ruled out that these longer peptides represent epitopes distinct from the 9-mer.

Peptide libraries representing the two insulin proteins expressed in mice, that is, Ins1 and Ins2, were screened similarly using islet-infiltrating T cells pooled from two independent sets of NOD.mβ2mnull.hβ2m.HLA-A11 mice (Fig. 3B, 3C). Several peptide mixtures elicited positive responses, some of which were observed with only one of the T cell pools (e.g., Ins281–82), and some of which were in common to both pools (e.g., Ins179). We identified Ins1 C21–30 (TLALEVARQK) as the minimal epitope present in mixture Ins179, and Ins2 C23–32 (TLALEVAQQK) was found to be the minimal epitope present in mixture Ins281 (Fig. 3D).
4A). Although these two peptides differ at only a single position, their status as independent epitopes is suggested by the finding that some T cell pools responded only to mixture Ins179 and not to Ins281 (Fig. 3B, 4B).

When screening peptide libraries designed as were ours, a doublet of reactivity is sometimes seen. In this case, the true epitope is generally present in the first positive peptide mixture, but reactivity is sometimes observed to the next mixture due to proteolytic generation of the epitope from a longer peptide during the assay period. This likely explains the reactivity observed to Ins2 mixtures 81 and 82, as we did not see reactivity to one but not the other in any of our experiments (Fig. 3C and data not shown).

However, careful examination of our peptide library screening results revealed that the doublet of reactivity observed for Ins1 mixtures 79 and 80 (Fig. 3B) likely represents two independent epitopes, as the T cells tested in Fig. 3C responded to mixture 79 but not mixture 80. To confirm that mixture 80 contained an epitope independent from Ins1 C21–30 (the peptide responsible for reactivity to mixture 79), we identified another T cell pool that showed reactivity to both mixtures, and we screened it against the individual peptides comprising these mixtures. If reactivity to mixture 80 was due to proteolytic cleavage of a peptide generating Ins1 C21–30, we would have expected the 11-mer of this mixture to give the strongest response. However, we found instead that the
MHC binding capacity of mouse peptides recognized by islet-infiltrating HLA-restricted T cells in HLA-A*1101-transgenic NOD mice and their human equivalents

Table I. MHC binding capacity of mouse peptides recognized by islet-infiltrating HLA-restricted T cells in HLA-A*1101-transgenic NOD mice and their human equivalents

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<tbody>
<tr>
<td>Mouse</td>
<td>IGRP272-280</td>
<td>AINEEMFLR</td>
<td>209</td>
<td>428</td>
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<td>67</td>
<td>1,081</td>
<td>4,547</td>
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<td>25,455</td>
<td>—</td>
<td>13,432</td>
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<tr>
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<td>—</td>
<td>—</td>
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<td>—</td>
<td>39,403</td>
<td>—</td>
<td>48,509</td>
<td>41,456</td>
</tr>
<tr>
<td>Mouse</td>
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<td>17,521</td>
<td>—</td>
<td>38,387</td>
<td>3,271</td>
<td>515</td>
<td>66</td>
</tr>
</tbody>
</table>

*Bold and underlined letters indicate amino acid identity between murine and human peptides.

Dash indicates IC50 > 50,000 nM.

Recently reported as an HLA-A3-restricted epitope in humans (26).
The number of the reactive mixture is indicated, and the individual pep-
aichic nature of the transgenic HLA-A11 molecule in which the
murine T cell-specific p56lck proximal promoter (46). We used
transgenically expressing human CD8 under the control of the
advantage of a previously described strain of C57BL/6 mice
for fully human class I MHC heavy chains would thus likely
Future epitope mapping studies employing NOD mice transgenic
for development in NOD.hCD8 females was as observed for NOD mice
and diabetes incidence in the newly developed NOD.hCD8 strain.
As reported for the C57BL/6 mice expressing human CD8 (46),
the murine CD4+/murine CD8+ T cell ratio was unaltered by the
diabetes susceptibility is restored when the HLA-A2 H chain is
enjoy reduced competition from those restricted to the murine
specific T cells restricted to the transgenic HLA molecules may
an altered peptide binding, it could instead, or in addition, be due to
an altered T cell repertoire. Murine class I MHC molecules con-
taining human β2m are apparently nondiabetogenic and fail to
support the development of β cell-specific T cell responses (45).
Thus, in the NOD.mβ2mnull.hβ2m.HLA-A11 strain, β cell-
specific T cells restricted to the transgenic HLA molecules may
enjoy reduced competition from those restricted to the murine
class I MHC molecules.

Despite the diabetes-protective effect of hβ2m in NOD.mβ2mnull.hβ2m mice alluded to immediately above (45),
diabetes susceptibility is restored when the HLA-A2 H chain is
also expressed (63). In contrast, expression of either the HLA-A11
or the HLA-B7 H chain in NOD.mβ2mnull.hβ2m mice is insuf-

NOD.hCD8 mice are diabetes-susceptible
Our mapping of HLA-A11–restricted epitopes using our HLA-
transgenic NOD.mβ2mnull.hβ2m mice was likely aided by the
chimeric nature of the transgenic HLA-A11 molecule in which the
α3 domain was derived from the murine class I MHC molecule
H-2Kb. This would be expected to facilitate HLA-A11–restricted
T cell responses, as murine CD8 interacts preferentially with the
murine α3 domain compared with the human α3 domain (59, 60).
Future epitope mapping studies employing NOD mice transgenic
for fully human class I MHC heavy chains would thus likely
benefit from the coexpression of human CD8. To this end, we
took advantage of a previously described strain of C57BL/6 mice
transgenically expressing human CD8 under the control of the
murine T cell-specific p56lck proximal promoter (46). We used
these mice to introduce human CD8 to NOD mice, backcrossed
for at least 10 generations, and then analyzed T cell populations
and diabetes incidence in the newly developed NOD.hCD8 strain.
As reported for the C57BL/6 mice expressing human CD8 (46),
the murine CD4+/murine CD8+ T cell ratio was unaltered by the
expression of human CD8, and human CD8 was expressed on both
murine CD4+ and murine CD8+ T cells (Fig. 7A). Diabetes de-
velopment in NOD.hCD8 females was as observed for NOD mice
(Fig. 7B). This supports the idea that introduction of hCD8 to
NOD models transgenic for fully human class I MHC chains will
increase their usefulness for epitope mapping studies. Such work
is already in progress in our laboratory.

Discussion
Although multiple β cell peptides have been identified as the
antigenic targets of CD8+ T cells in type 1 diabetes patients,
nearly all of these are recognized in the context of HLA-A*0201
(12). Thus, they are only relevant to a subset of patients (32).
Although the gene frequency of HLA-A*1101, used in this study,
is generally lower than that of HLA-A*0201, for example, 7 and
27% among the white population in the United States, respectively
(61), HLA-A*1101 is a representative member of the A3 supertype,
which has a phenotypic frequency of 38–53%, depending on the
ethnic group examined (39). This compares favorably to the
phenotypic frequency of 39–46% for the A2 supertype (39), of
which HLA-A*0201 is a member. In addition to HLA-A*1101,
other A3 supertype members include A*0301, A*3101, A*3301,
and A*6801 (39). The A3 supertype is characterized by a B-
pocket specificity (position 2 of the peptide) for the amino acids
A, I, L, M, V, S, or T, and an F-pocket specificity (peptide C
terminus) for R or K (39). Importantly, this supertype has been
functionally verified, meaning that it has been established that
a peptide that binds one member of the supertype will likely bind
other members as well (40). This led us to hypothesize that an-
tigenic β cell peptides identified using the HLA-A11–transgenic
NOD mice would have relevance to humans expressing other
members of the A3 supertype. This has now been confirmed by
a very recent report that identified the human equivalent of an
epitope we mapped using our mice (i.e., human Ins C23–32,
and having the sequence PLAEGSLQK; Table I) as being recognized
in the context of HLA-A3 in type 1 diabetes patients (26). This
finding, coupled with our MHC binding results (Table I), supports
the idea that the other epitopes we identified using the HLA-A11–
transgenic NOD mice should also be examined for their relevance
in patients.

When the transgenic HLA-A11 molecules expressed in NOD
mice contained murine β2m, diabetes incidence was much higher
than that observed when human β2m was expressed instead (Fig.
1A, 1C). Nonetheless, we chose to conduct our epitope mapping
studies in the context of human β2m, so that the transgenic HLA
molecules would resemble their human counterparts as closely as
possible. For example, HLA class I H chains associated with
murine β2m appear to take on an altered structure (as measured by
Ab reactivity) compared with those associated with human β2m
(62). This suggests that the peptide-binding repertoire might also
be altered. Interestingly, we searched for T cell responses to our
newly identified HLA-A11–binding epitopes IGRP 272–280 and
Ins1 C21–30 using islet-infiltrating T cells derived from 13 NOD
HLA-A11 mice (expressing murine β2m), and we were unable to
detect reactivity (data not shown). Although this might reflect
altered peptide binding, it could instead, or in addition, be due to
an altered T cell repertoire. Murine class I MHC molecules con-
taining human β2m are apparently nondiabetogenic and fail to
support the development of β cell-specific T cell responses (45).
Thus, in the NOD.mβ2mnull.hβ2m.HLA-A11 strain, β cell-
specific T cells restricted to the transgenic HLA molecules may
enjoy reduced competition from those restricted to the murine
class I MHC molecules.

Table II. MHC binding capacity of mouse peptides recognized by islet-infiltrating HLA-restricted T cells in HLA-B*0702-transgenic NOD mice and
their human equivalents

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<th>Organism</th>
<th>Position</th>
<th>Sequence</th>
<th>Binding to B7 Supertype Members (IC50, nM)</th>
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<td>B*0702</td>
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<td>Mouse</td>
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<td>Human</td>
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<td>CGAGSLQTLAL</td>
<td>462</td>
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</table>

*Bold and underlined letters indicate amino acid identity between murine and human peptides.

*Dash indicates IC50 > 50,000 nM.
whereas HLA-A11 and HLA-B7 are not (4, 6). HLA-A2 is a susceptibility allele for type 1 diabetes in humans, this intriguing phenomenon may be related to the finding that the C terminus of Ins1 C21–30 and Ins2 C23–32 (Fig. 8) should be considered. Fore or between dibasic residues, its involvement in the generation of the cytosolic endopeptidase nardilysin. As nardilysin cleaves before or after this residue, its involvement in the generation of the C terminus of an HLA-A3–restricted viral epitope (ELFSYLIEK) has recently been reported (64). In that study, the peptide C terminus was instead found to be generated by viral cleavage sites are denoted by arrows, and residues trimmed by carboxypeptidase E are depicted in black. C-peptide epitopes identified in this study are pathogenic, their presence within the pancreatic islets prior to disease development (Figs. 3A, 3B, 4A–C, 6 and data not shown) certainly supports this idea, as does their secretion of IFN-γ in response to Ag stimulation. Also, we have previously shown, using both standard and HLA-A2–transgenic NOD mice, that T cell specificities identified using islet-infiltrating cells and IFN-γ ELISPOT assays do indeed exhibit cytotoxicity in vivo (28). In addition to using islet-infiltrating cells to identify disease-relevant Ags, our MHC binding results suggest that it is also important to look for candidate epitopes in an unbiased way, as we have done, rather than limit candidates to those that bind well to MHC. As seen in Tables I and II, the mouse epitopes we identified bound to MHC with a wide range of affinities. Despite these advantages of our epitope mapping approach, one drawback is that some of the peptides identified may not be relevant to the human disease due to sequence differences between the mouse and human versions. For example, human preproinsulin and IGRP are only 80% identical to their mouse counterparts. Ideally, then, human autoantigens would be introduced into HLA-transgenic NOD mouse models in the future. The establishment of NOD mice transgenically expressing human IGRP in β cells is an important first step toward this goal (65).

T cell epitope mapping continues to be a major research effort for a number of laboratories, and this interest is due, at least in part, to the potential of such work to identify peptides that could be useful for the development of preventive or therapeutic strategies. As suggested by a recent report (21), it is possible that manipulation of multiple specificities will be beneficial, if not required, so the more that is known about the relevant epitopes, the more successful the field is likely to be. Identification of new epitopes will also increase the utility of T cell assays, which have begun to show promise as tools for immune monitoring in type 1 diabetes, for example, to identify β cell autoimmunity in patients (25), to predict islet graft rejection (26, 27), or to confirm the immunological efficacy of intervention therapies regardless of their clinical outcome (66), information that could be used to guide sub-
from insulin-dependent diabetes mellitus; changes in HLA, adhesion molecules and autoantigens, restricted T cell receptor V β usage, and cytokine profile. J. Immunol. 153: 1360–1367.


