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, Pere Santamaria and Teresa P. DiLorenzo

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Type 1 diabetes is an organ-specific autoimmune disease in which T cell-mediated elimination of pancreatic islet β cells results in insulin insufficiency. Although the strong genetic determinant for disease susceptibility is the expression of predisposing class II MHC molecules (1), a series of studies have also uncovered an association with particular class I MHC alleles, including the common HLA-A*0201 (2–11). This class I MHC association is not merely reflective of linkage disequilibrium with disease-promoting class II genes (4, 6, 9). Consistent with the idea that particular class I MHC molecules can foster diabetes development is the finding that CD8+ T cells specific for β cell Ags are present in the peripheral blood of type 1 diabetes patients (12). CD8+ T cells are also seen infiltrating the islets of new-onset and graft-recurrent type 1 diabetes patients, suggesting their contribution to β cell elimination (13–17). In the NOD mouse model of the disease, CD8+ T cells specific for β cell Ags are required pathogenic effectors (18) that have begun to demonstrate potential as therapeutic targets (19–24).

Peptide-based CD8+ T cell assays are showing promise as tools for the detection of β cell autoimmunity in recent-onset type 1 diabetes patients and islet transplant recipients, particularly when multiple T cell epitopes are simultaneously examined (25–27). Although it is true that the specific epitopes targeted can differ from one individual to another, it is clear from both NOD mouse models (28–30) and type 1 diabetes patients (25–27) that peptides derived from the β cell Ags insulin (Ins) and islet-specific peptide-based CD8+ T cell assays are showing promise as tools for the detection of β cell autoimmunity in recent-onset type 1 diabetes patients and islet transplant recipients, particularly when multiple T cell epitopes are simultaneously examined (25–27). Although it is true that the specific epitopes targeted can differ from one individual to another, it is clear from both NOD mouse models (28–30) and type 1 diabetes patients (25–27) that peptides derived from the β cell Ags insulin (Ins) and islet-specific
Our characterization of these new strains has revealed the insulin recognized in the context of these human class I MHC molecules. HLA-B*0702 and used these mice to identify β2m domains from H-2Kb) or a fully human HLA-B*0702 H chain were ob-

expressing human patients (25–27, 34–38). In our efforts to look beyond HLA-IGRP228–236, IGRP265–273, Ins B5–14, and Ins B10–18) that were also found to be recognized in HLA-A*0201-positive type 1 diabetes (30, 33), and this strategy successfully identified epitopes (e.g., 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., IGRP228–236, IGRP265–273, INS B5–14, and INS B10–18) 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 expressing human β2-microglobulin (β2m) and HLA-A*1101 or HLA-B*0702 and used these mice to identify β cell peptides recognized in the context of these human class I MHC molecules. Our characterization of these new strains has revealed the insulin C-peptide to be a rich source of CD8 T cell epitopes. Responses to these epitopes should be of particular utility for immune monitoring of type 1 diabetes patients and islet-transplant recipients, as they cannot reflect an immune reaction to exogenously administered insulin, which lacks the C-peptide. Furthermore, HLA-A*1101 and HLA-B*0702 are representative members of two different HLA supertypes, which are groupings of MHC molecules that have been defined on the basis of structural similarities in the peptide-binding groove (39–41). Members of a supertype show overlap in peptide-binding repertoires (40, 42), and the β cell peptides we identified using one supertype member were found to also bind certain other members of that supertype, potentially resulting in monitoring and therapeutic reagents relevant to large numbers of patients and at-risk individuals.

Materials and Methods

**Derivation of NOD.m2bmnull.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 ob-
tained 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 in-
troduced by intercrossing with the NOD.m2bmnull.hβ2m strain (45), yielding the two new strains designated NOD.m2bmnull.hβ2m.HLA-A11 and NOD.m2bmnull.hβ2m.HLA-B7.

**Derivation of NOD.hCD8 mice**

C57BL/6 mice expressing human CD8α and human CD8β under the control of the murine p56<sup>+</sup> 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.hCD8 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.m2bmnull.hβ2m.HLA-A11, NOD.m2bmnull.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-2K<sup>+</sup> (SF1-1.1), and H-2D<sup>+</sup> (KH95). Splenocytes from NOD.hCD8 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 urine analysis (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. Concen-

trated peptide stocks (2.75 mM) were prepared in 50% acetonitrile/H_2O, 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 supple-
mented with 10% FBS (HyClone), 1 mM sodium pyruvate, nonessential amino acids, 28 μM 2-ME, and 50 U/ml recombinant human IL-2 (PeproTech).

**INF-γ ELISPOT**

INF-γ ELISPOT was performed as described (49) using 2 × 10<sup>4</sup> islet-infiltrating T cells as responders and 2 × 10<sup>4</sup> 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<sup>+</sup> T cells were first pu-
nified 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-2K<sup>+</sup>) 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 de-
tailed elsewhere (51). Briefly, EBV-transformed homozgyous 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 con-

njugated 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
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-2K\(^d\) and H-2D\(^b\) 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 mice, which had a diabetes incidence of 90% (Fig. 2C), 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 8–11-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 IGRP\(_{272-280}\), having the sequence AINSEMFLR, 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-
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.β2mnull.β2m.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 (TLALEVASQQK) was found to be the minimal epitope present in mixture Ins281 (Fig.

**FIGURE 2.** NOD.β2mnull.β2m 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.

**FIGURE 3.** Islet-infiltrating T cells from NOD.β2mnull.β2m.HLA-A11 mice recognize IGRP and insulin peptides. (A) Islet-infiltrating T cells from a 17-wk-old nondiabetic female NOD.β2mnull.β2m.HLA-A11 mouse were used to screen a murine IGRP peptide library by IFN-γ ELISPOT using syngeneic splenocytes pulsed with 1 μM peptide as APCs. (B and C) Islet-infiltrating T cells from (B) two 15-wk-old nondiabetic or (C) five 13- to 27-wk-old diabetic female NOD.β2mnull.β2m.HLA-A11 mice were used to screen Ins1 and Ins2 peptide libraries as in (A). Numbers corresponding to the reactive mixtures are indicated.
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...
FIGURE 5. Ins1 C21–30 and Ins1 C22–31 are independent C-peptide–derived T cell epitopes in NOD.mβ2mnull·β2m·HLA-A11 mice. Islet-infiltrating T cells from a diabetic female NOD.mβ2mnull·β2m·HLA-A11 mouse were tested by IFN-γ ELISPOT for reactivity to peptide mixtures Ins130 or Ins140 as well as the individual peptides making up those mixtures. The peptide sequences are shown next to their corresponding bars, and the minimal epitopes for each mixture are shown in bold. Similar results were obtained using islet-infiltrating cells from nondiabetic mice (data not shown).

10-mer was the minimal epitope responsible for the reactivity observed to mixture Ins180 (Fig. 5). Taken together with our peptide library screening results (Fig. 3B, 3C), these findings confirm the 10-mer peptide Ins1 C22–31 as an independent epitope.

T cells cultured from islets of NOD.mβ2mnull·β2m·HLA-A11 mice according to our protocol generally contained nearly 90% CD8+ T cells (Fig. 4D). Nonetheless, to confirm that CD8+ T cells were indeed responsible for the IFN-γ responses we observed, we magnetically separated CD8+ and CD8- islet-infiltrating cell populations and tested them for reactivity to the predominant epitopes IGRP272–280 and Ins1 C21–30. We found that reactivity was enriched 3- to 7-fold in the CD8+ population (Fig. 4C). Finally, we wanted to verify that these peptides were recognized by CD8+ T cells restricted to the transgenic HLA-A11 molecules rather than to the endogenous NOD class I MHC molecules H-2Kd and H-2Dd, which are also expressed in the NOD.mβ2mnull·β2m·HLA-A11 mice (Fig. 1A). When we stained islet-infiltrating T cells from these mice with peptide/HLA-A11 tetramers (containing the α3 domain from H-2Kd as found in the HLA-A11–transgenic mice), a clear population of CD8+ T cells bound the IGRP272–280/HLA-A11 or the Ins1 C21–30/HLA-A11 tetramers, whereas this was not the case for the irrelevant HIV nef/HLA-A11 reagent (Fig. 4D). In addition to confirming MHC restriction, this flow cytometry analysis also confirmed that the CD8+ T cell population was responsible for the peptide reactivities we observed.

Table I provides a summary of the peptides we found to be recognized by HLA-A11–restricted islet-infiltrating T cells in NOD.mβ2mnull·β2m·HLA-A11 mice. Importantly, these experiments have identified the insulin C-peptide as a rich source of CD8+ T cell epitopes.

**Epitope mapping using HLA-B7–transgenic mice**

Because the low spontaneous disease incidence observed in the HLA-B7–transgenic NOD.mβ2mnull·β2m mice (Fig. 2B) made it difficult to collect sufficient numbers of islet-infiltrating T cells, it was not possible to do the extensive screens and characterizations carried out for the HLA-A11–transgenic mice. However, given our identification of three C-peptide–derived epitopes in the HLA-A11–transgenic mice, we used islet-infiltrating T cells from the HLA-B7–transgenic mice to perform a limited screen of the Ins1 peptide library that was restricted to the C-peptide and its flanking regions. Remarkably, mixture 73 elicited a positive T cell response (Fig. 6). Based on the peptide binding motif described for HLA-B*0702 (P at position 2, and L at the C terminus) (58), the 10-mer peptide in this mixture (Ins1 C15–24; SPGDQLTLAL) is most certainly responsible for the activity observed. Consistent with this idea is our finding that the 10-mer peptide Ins1 C15–24 bound very well to HLA-B*0702 (IC50 of 4.4 nM in a competitive binding assay; Table II).

The identified epitopes bind multiple members of the corresponding HLA supertype

Members of an HLA supertype show overlap in peptide-binding repertoires (40, 42). To determine whether the epitopes we identified using HLA-A11–transgenic NOD mice were capable of binding other alleles of the A3 supertype, we performed competitive peptide-binding assays under conditions where the measured IC50 is a reasonable approximation of the true KD (Table I). Murine IGRP272–280 exhibited strong to intermediate binding (IC50 < 500 nm) not only to HLA-A*1101, but also to three other A3 supertype members (A*0301, A*3101, and A*6801). Additionally, binding to other A3 supertype alleles was detectable, albeit with weak affinity (IC50 between 500 and 5000 nM; A*3301 and A*6601). Importantly, human IGRP272–280 also showed binding to A*1101 and to certain other members of the A3 supertype, albeit to varying degrees and generally lower affinity, perhaps reflective of the fact that it bears a less optimal A3 supertype motif. In the case of the murine insulin epitopes we identified and their human equivalents, some of these bound multiple members of the corresponding HLA supertype also (Tables I, II).

**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>AINSEMFLLR</td>
<td>209</td>
<td>428</td>
<td>49,752</td>
<td>67</td>
<td>1,081</td>
<td>4,547</td>
<td>109</td>
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<tr>
<td>Human</td>
<td>IGRP272–280</td>
<td>AINSEMFLLL</td>
<td>2,824</td>
<td>1,258</td>
<td>6,776</td>
<td>1,071</td>
<td>12,784</td>
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<td>Mouse</td>
<td>Ins1 C21–30</td>
<td>TLEALEVARQK</td>
<td>3,327</td>
<td>605</td>
<td>23,280</td>
<td>13,026</td>
<td>25,455</td>
<td>—</td>
<td>13,432</td>
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<td>Mouse</td>
<td>Ins2 C23–32</td>
<td>tLEALEVARQK</td>
<td>833</td>
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<td>4,912</td>
<td>492</td>
<td>9,028</td>
<td>11,047</td>
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<td>10,808</td>
<td>9,145</td>
<td>12,966</td>
<td>12,582</td>
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*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).
NOD, hCD8 mice are diabetes-susceptible

Our mapping of HLA-A11–restricted epitopes using our HLA-
transgenic NOD.mι2mnull.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 chains would thus likely
enjoy reduced competition from those restricted to the murine
class I MHC molecules. This may support the development of
human cell–specific vaccines. Murine class I MHC molecules con-
altered peptide binding, it could instead, or in addition, be due 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.mι2mnull.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.mι2mnull.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.mι2mnull.hβ2m mice is insuf-

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Position</th>
<th>Sequence</th>
<th>Binding to B7 Supertype Members (IC50, nM)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>B*0702</td>
</tr>
<tr>
<td>Mouse</td>
<td>Ins1 C15-24</td>
<td>SPGDLQTLAL</td>
<td>4.4</td>
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<td>Human</td>
<td>Ins1 C15-26</td>
<td>CpGAGSLQTLAL</td>
<td>462</td>
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*Bold and underlined letters indicate amino acid identity between murine and human peptides.

Dash indicates IC50 > 50,000 nM.
HLA-A11 and HLA-B7 are not (4, 6). HLA-A2 is a susceptibility allele for type 1 diabetes in humans, whereas HLA-A11 and HLA-B7 are not (4, 6). Spontaneous CD8\(^{+}\) T cell responses to only a limited number of epitopes entirely contained within the insulin C-peptide (hIns C\(_{20-28}\) for HLA-A2 and HLA-A3, and hIns C\(_{23-32}\) for HLA-A3) have been reported previously (Fig. 8) (25, 26). Our identification of four such peptides in our work (Ins1 C\(_{21-30}\), Ins2 C\(_{23-32}\), and Ins1 C\(_{22-31}\) for HLA-A11, and Ins1 C\(_{15-24}\) for HLA-B7) (Fig. 8) establishes the C-peptide as a rich source of disease-relevant CD8\(^{+}\) T cell epitopes. In humans, reactivities to C-peptide epitopes may be particularly informative as they cannot represent an immune response to exogenously administered insulin.

It is possible that the C termini of some of the peptides identified in this study (Ins1 C\(_{21-30}\) and Ins2 C\(_{23-32}\)) may be generated independently of the proteasome. This is an important point, as proteasomal cleavage is often taken as a prerequisite for the examination of candidate T cell epitopes. Proteasome-independent 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 the cytosolic endopeptidase nardilysin. As nardilysin cleaves before or between dibasic residues, its involvement in the generation of Ins1 C\(_{21-30}\) and Ins2 C\(_{23-32}\) (Fig. 8) should be considered. Similar to HLA-A*0201 and A*1101, B*0702 is also a member of a well-characterized and functionally verified HLA supertype, that is, the B7 supertype (42). Although in this study we were only able to conduct limited epitope mapping studies using the NOD.

The restricting MHC is noted in each case. The frequency of 43–57% (39), further investigation is clearly warranted. We anticipate that introduction of human CD8 from our newly developed NOD.hCD8 strain (Fig. 7) to the HLA-B7–transgenic NOD mice will further increase their utility for Ag discovery experiments (59, 60).

Although we have not yet proven directly that the T cell reactivities we have 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-\(\gamma\) 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-\(\gamma\) 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 75% 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 \(\beta\) 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 \(\beta\) 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-
sequest clinical trials. When the A2, A3, and B7 supertypes are considered together, the population coverage provided ranges from 83 to 88%, depending on the population examined (39). Thus, increased knowledge of the β cell peptides recognized in the context of representative supertype members should result in immunological tools relevant to large numbers of individuals.

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Disclosures

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