The Insulin-Specific T Cells of Nonobese Diabetic Mice Recognize a Weak MHC-Binding Segment in More Than One Form

Matteo G. Levisetti, Anish Suri, Shirley J. Petzold and Emil R. Unanue

*J Immunol* 2007; 178:6051-6057; doi: 10.4049/jimmunol.178.10.6051

http://www.jimmunol.org/content/178/10/6051
The Insulin-Specific T Cells of Nonobese Diabetic Mice Recognize a Weak MHC-Binding Segment in More Than One Form

Matteo G. Levisetti,*† Anish Suri, † Shirley J. Petzold, † and Emil R. Unanue‡

Several naturally occurring anti-insulin CD4 T cells were isolated from islet infiltrates of NOD mice. In accordance with the results of others, these T cells recognized the segment of the β-chain from residues 9–23. Peptides encompassing the Br(9–23) sequence bound weakly to I-A\(^{\text{b}}\) in two main contiguous registers in which two residues at the carboxyl end, P20Gly and P21Glu, influenced binding and T cell reactivity. Naturally occurring insulin-reactive T cells exhibited differing reactivities with the carboxyl-terminal amino acids, although various single residue changes in either the flanks or the core segments affected T cell responses. The insulin peptides represent another example of a weak MHC-binding ligand that is highly immunogenic, giving rise to distinct populations of autoimmune T cells. *The Journal of Immunology, 2007, 178: 6051–6057.

T

here is extensive experimental evidence supporting an important role for insulin as an autoantigen in the pathogenesis of autoimmune diabetes (reviewed in Refs. 1 and 2). Wegmann and collaborators (3, 4) first reported that insulin-reactive CD4\(^{+}\) T cells constituted a large component of the early islet infiltrate and that these insulin-reactive T cells accelerated or transferred disease into young NOD or NOD.scid recipients. Furthermore, they showed that most of these insulin-reactive T cells recognized the 9–23 peptide of the insulin B chain (5). These findings led to interventions aimed at inducing insulin-specific tolerance, such as intranasal, s.c., or intrathymic administration of the B(9–23) peptide, which were largely successful at preventing or reducing the incidence of diabetes in NOD mice (6–8). Recent investigations confirmed the important role for insulin as an autoantigen in the pathogenesis of autoimmune diabetes. First, NOD mice rendered tolerant to insulin by transgenic overexpression of the preproinsulin molecule in APCs had a greatly reduced incidence of diabetes (9, 10). And second, NOD mice in which the dominant epitope of insulin was mutated by genetic manipulation had a greatly reduced incidence of disease (11). Third, an anti-insulin B(9–23) TCR-transgenic mouse developed disease when crossed onto a RAG-deficient background (12). There is also evidence that insulin-reactive T cells regulate diabetogenic cells and protect mice from spontaneous disease or disease caused by the adoptive transfer of pathogenic clones (13–15).

In contrast to the many cellular studies, the binding interaction of the insulin B chain with the I-A\(^{\text{b}}\) molecule has been studied to a limited extent. Indeed, the insulin B:(9–23) peptide was shown to bind to I-A\(^{\text{b}}\) (16–19); however, the detailed nature of its binding to the MHC molecule was not evaluated, although the issue of a weak interaction has been alluded to as evidenced by its fast dissociation rate (18).

In this study, we isolated insulin-reactive CD4 T cells from prediabetic NOD mice, all of which were reactive with the 9–23 segment of the insulin B chain, confirming the studies of Wegmann and colleagues (3–5), Abiru et al. (20), and Halbout et al. (21). We correlated their activation with the interactions of the B chain 9–23 segment to the I-A\(^{\text{b}}\) class II MHC molecule. The insulin B(9–23) peptide interacts weakly with the I-A\(^{\text{b}}\) molecule and contains at least two potential binding registers. Two amino acids at the carboxyl end, P20Gly and P21Glu, influenced binding and T cell reactivity.

Materials and Methods

Animals

The NOD mice were originally obtained from The Jackson Laboratory. All mice were housed and cared for in accordance with the guidelines of the Washington University Committee for the Humane Care of Laboratory Animals and with National Institutes of Health guidelines on laboratory animal welfare.

Antigens

Peptides were synthesized by F-moc techniques, and their identity was verified by MALDI time-of-flight mass spectrometry (Applied Biosystems). Human insulin was purchased in solution (10 mg/ml) from Sigma-Aldrich.

Peptide-binding assays

Soluble I-A\(^{\text{b}}\) was produced using the recombinant baculovirus system as previously described (19). Peptide-binding assays were done under acidic (pH 5.5) or neutral (pH 7.5) conditions. Briefly, 0.5–1 μg of I-A\(^{\text{b}}\)/class II-associated invariant chain peptide was treated with 0.1 U of thrombin to cleave both the zipper tails and peptide linker (Novagen) and simultaneously incubated with 0.125 pmol of \(^{125}\)I-radiolabeled mimotope reference peptide (GKKVATTVHAGYG) (19) and increasing doses of unlabeled peptides in 200 mM Tris (2-carboxyethyl)phosphine hydrochloride, 20 mM MES, and 150 mM sodium chloride. Binding reactions were incubated overnight at 25°C in 30-μl volumes. Complexes were purified from free peptide by gel filtration Bio-spin columns (Bio-Rad). The percent of bound peptide was evaluated by gamma counting. Usually ~25–35% of input peptide was bound, whereas <0.5% of peptides non-specifically passed through the Bio-spin columns. The IC\(_{50}\) value is very close to the binding equilibrium constant. For each variable, binding assays were done at least twice, but usually four to six times. Individual binding
results varied <20% from the averaged value. Within experiments, variations did not exceed 15%. Among experiments using different batches of I-A^b, variations in binding of the reference peptide varied ~25%; the IC_{50} for the B:(9–23) insulin peptide binding varied from ~1 to 3 μM. Dissociation rate was done following binding of the 125I-labeled B:(9–23) peptide of insulin-1 or insulin-2 to I-A^b. For labeling, the peptides contained a tyrosine either at the amino or carboxy termini attached by a double alanine linker to the 9–23 peptide. After binding for 24 h, the complex was isolated by gel filtration on Bio-spin columns and incubated in the presence of a 1000-fold excess of unlabelled reference peptide at room temperature or 37°C. After 1, 2, 4, and 24 h, the amounts of peptide complexed to I-A^b were determined by purifying the complex through Bio-spin columns.

**Generation of T cell hybridomas**

Infiltrated islets of Langerhans were isolated from NOD mice of various ages (12–24 wk) and dispersed to a single-cell suspension by standard methods (22). Dispersed islet cultures, containing β cells, T cells, and APCs were stimulated in culture for 72 h in the presence of IL-2 (200 U/ml), and T cells were fused to the BW5147 thymoma partner cell line as described in Materials and Methods. The response of each clone is presented relative to its reactivity with the insulin B:(9 –23) segment (or the 12–20 or 13–21 peptides shown below) was profoundly affected whether conducted at neutral pH (data not shown; all binding results indicated below were made at pH 5.5).

The whole human insulin molecule did not bind in its native state. But binding was found when the assays were done using reducing agents (0.58 μM), an indication that the insulin molecule, like most proteins, cannot bind in its native conformation. (Human insulin and the mouse insulin-2 share identity at the B:(9–23) segment.) Truncation of the B:(9–23) segment by one or two amino-terminal residues, to 10–23 or 11–23, did not affect the binding. The dissociation of the B:(9–23) peptide was found to be relatively fast with loss of 45–75% of the bound peptide in 2 h, in agreement with the data previously published by Wucherpfennig’s laboratory (18), which also demonstrated the poor binding of insulin peptides.

Based on previous binding studies (16–19), the insulin B:(9–23) segment is predicted to contain two favorable binding core segments: 13–21:EALYLVCGE and 12–20:VEALYLVCG. A core segment comprises the nine-residue stretch of a peptide from the B:(9 –23) insulin peptide (or the 12–20 or 13–21 peptides shown below) was prepared by substituting lysine at P9 in which the MHC anchor amino acids mainly include P1, P4, P6, and P9, whereas the solvent-exposed residues that contact the TCR include P2, P3, P5, and P8. The P9 residue can interact with the unpaired Arg76 of the I-A^b α-chain. It can be identified by examining the effects of substituting lysine at P9. A lysine at P9 hinders the binding of the peptide (23, 24, 27). Single mutation of either Glu21 or Gly20 to lysine in the full-length B:(9–23) peptide affected binding slightly or not at all. However, double mutations of Glu20 and Gly21 to lysines resulted in loss of binding, to 12–20 or 13–21 peptides from 2.4 μM (Table II). We interpreted this result to indicate that both P20Gly and P21Glu contributed to the binding at the carboxyl end of the peptide. Either the peptide was binding by one of two contiguous binding registers, i.e., 12–20 and 13–21; or there was

---

**Table I. Human and murine insulin sequences**

<table>
<thead>
<tr>
<th>B Chain</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>F V N Q H L C G S H L V E A L Y L V C G E R G F F Y T P K T</td>
</tr>
<tr>
<td>Murine INS-1</td>
<td>F V K Q H L C G P H L V E A L Y L V C G E R G F F Y T P K S</td>
</tr>
<tr>
<td>Murine INS-2</td>
<td>F V N Q H L C G S H L V E A L Y L V C G E R G F F Y T P M S</td>
</tr>
</tbody>
</table>

---

---

**Table II. Binding of B chain peptides**

<table>
<thead>
<tr>
<th></th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>IC_{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>INS-1</td>
<td>P H L V E A L Y L V C G E R G</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INS-2</td>
<td>S H L V E A L Y L V C G E R G</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
cooperativity between P20 and P21 with P20 being P9 of the peptide and P21, the P10 (Table II). Such cooperativity was predicted in the crystallographic analysis by Corper et al. (25) and found later to hold true (23). Another example of such cooperativity was reported with HLA-DR1 (29).

Evaluation of the 13–21 and the 12–20 segments of B:(9–23) peptide

To evaluate further the two putative registers, the corresponding 9-mer minimal peptides were examined. A peptide containing the 13–21 segment is predicted to bind by way of Glu21 at P9, whereas a peptide containing the 12–20 will have Gly20 at P9 (Tables I–III).

As shown in Tables III and IV, each of the minimal nine-residue peptides bound to I-A^B7, 13–21: EALYLVCGE, and 12–20: VEALYLVCG. Tables III and IV show the variant peptides examined and their binding: the peptides have been positioned in the most likely registers.

The 13–21 peptide bound less well than the 12–20 and a lysine substitution of P21Glu markedly reduced binding, confirming the usage of the terminal glutamic acid (Table III, peptide 2). In the 13–21 peptide, the P4Tyr is expected to be unfavorable. Indeed, a peptide in which the P4Tyr was changed to alanine showed a modest improvement in binding (Table III, peptide 4). However, changing the P4Tyr to lysine did not hinder the interaction:

Table III. Evaluation of 13–21 segment of the B:(9–23) peptide

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Peptide Sequence</th>
<th>IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>13–21:</td>
<td>EALYLVCGE</td>
<td>5.9</td>
</tr>
<tr>
<td>13–21, P9K:</td>
<td>EALYLVCG</td>
<td>16.9</td>
</tr>
<tr>
<td>13–21, P4K:</td>
<td>EALYLVCGK</td>
<td>4.7</td>
</tr>
<tr>
<td>13–21, P4A:</td>
<td>EALYLVCG</td>
<td>3.6</td>
</tr>
<tr>
<td>13–21, P1A:</td>
<td>EALLYLVCG</td>
<td>3.2</td>
</tr>
<tr>
<td>13–20:</td>
<td>EALYLVCG</td>
<td>14.2</td>
</tr>
<tr>
<td>13–20, P4A:</td>
<td>EALYLVCG</td>
<td>6.6</td>
</tr>
<tr>
<td>13–20, P4K:</td>
<td>EALLYLVCG</td>
<td>6.2</td>
</tr>
<tr>
<td>AA-13–21:</td>
<td>AA EALYLVCGE</td>
<td>0.7</td>
</tr>
<tr>
<td>AA-13–21, P9K:</td>
<td>A EALLYLVCGE</td>
<td>5.0</td>
</tr>
</tbody>
</table>

^a Indicated are the peptides examined (IC50 values, μM). For peptides 1, 3, and 4, the A and B refer to the two possible binding registers. The A in each indicates our preferred binding register.

Downloaded from http://www.jimmunol.org/ by guest on January 21, 2018
anchor site of I-A$^\beta$ prefers small to medium size hydrophobic residues, whereas charged residues are not favored (Table III, peptide 3). Conceivably, the 13–21 P4Lys peptide could register shift in the core-binding segment of the B chain peptide to I-A$^\beta$, and is helped by the P10Glu, leaving an empty P1. An alternative suggestion is that the strong P1 and P9 glutamic acid residues tolerate an unfavorable P4. In support of this interpretation are results with the 13–21 peptide in which the P1Glu was changed to alanine: note peptides 5, 5, and 7 where a negative effect is found when a lysine is placed at P4. Finally, eight amino acid residue peptides bound weakly. Such 8-mers in which either the P1 or the P9 were removed did not bind if lysine was placed at the putative P4, compare peptides 8 and 9 and 10 to 11 and 12.

The 12–20 peptide bound at 2.5 $\mu$M but changing the P20Gly to a lysine markedly reduced the binding, an indication that the P20Gly is indeed a MHC anchor residue (Table IV, cf peptides 1 and 2). Changing the P5Tyr to lysine affected the binding, which dropped to 5.7 $\mu$M, although a change to alanine had no effect (2.6 $\mu$M). The prediction was that the P5 Lys would not inhibit a strict 12–20 peptide interaction because the P5 would be a TCR contact residue. One explanation is that register shifting is taking place within the 12–20 peptide as indicated by two alternatives shown as 1B and 1C in Table IV.

Addition of two alanines at the amino terminus of the two core segments improved binding: to 0.7 $\mu$M for the 13–21 peptide (Table III, peptide 13) and to 0.8 $\mu$M for the 12–20 peptide (Table IV, peptide 6). The Ala-Ala–12–20 peptide lost binding strength when a lysine was substituted for the terminal Gly20, to 11.1 $\mu$M (Table IV, peptide 7). For the Ala-Ala–13–21 peptide, the loss, although pronounced, was not as much, to 5 $\mu$M (Table III, peptide 14), which could be explained by register shift to the alternative 12–20 register.

In toto, the data suggest that there are two primary binding regist- ers in the B (9–23) segment and that shifting between the two can take place. The two major carboxyl-terminal residues, Gly20$^\beta$ and Glu21$^\beta$, influence the binding and as indicated below the reac- tivities of T cells. Admittedly, the precise identification of the core-binding segment of the B chain peptide to I-A$^\beta$ will be de- finitively established only if each is crystallized, an approach that has been difficult thus far.

Other binding segments were examined, but no others were identified binding to I-A$^\beta$ in the low micromolar concentration range. The 15–23 segment is an example of one, which did not bind, but addition of the two consecutive alanines at the amino terminus terminated a weak binding, 10.6 $\mu$M (Table IV). As with the 13–21 segment, the additions of lysine helped identify two binding segments when two alanines were placed on the amino terminus, one as part of register-1 (AALYLVCGE) and the second containing the core 15–23: LYLVCGERG (Table IV, peptides 10–12).

### Table V. Anti-insulin T cell reactivities

<table>
<thead>
<tr>
<th>Register 2</th>
<th>I43</th>
<th>I90</th>
<th>AS91</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Register 1</th>
<th>I3</th>
<th>I85</th>
<th>I155</th>
<th>AS150</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PHILEALYLVCGERG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*The specificities of insulin-reactive T cells appear to fall into two broad cate- gories. One group, I43, I90, AS91, recognized a register 2 core segment. A fifth T cell of the 11 isolated responded the same as I.3. Three other T cells responded as I.43, I.55, AS150, recognized a register 1 core segment. A fifth T cell, such as AS91, recognized a register 2 core segment (VEALYLVCG).*

### Specificity of insulin-reactive islet-infiltrating T cells

Islet-infiltrating T cells were isolated and examined for reactivity with the insulin B(9–23) peptide. Consistent with published reports (3), insulin-reactive T cells were relatively abundant in the islet infiltrates of NOD mice between 12 and 24 wk of age. Most T cells identified one as part of register-1 (AALYLVCGE) and the second containing the core 15–23: LYLVCGERG.
Amino acid changes in either the core segment or the flanks affected the T cell response. Truncations of both amino and carboxyl termini had a marked influence despite the finding that some of the truncations did not change the binding to I-A\(^{b}\). For example, all T cells reacted poorly or not at all to peptides lacking the histidine at residue 10: peptide 11–23 for the most part was non-stimulatory, indicating that His\(^{10}\) was an important residue in the flanking region that influenced T cell recognition (Table V). However, peptide 11–23 bound as well as 10–23 (data not shown). The same effects were found at the carboxyl termini where loss of the flanking region that influenced T cell recognition (Table V).

Changes in residues of the core segments likewise affected all of the T cells: Glu\(^{13}\) to lysine, Leu\(^{15}\) to alanine, Tyr\(^{16}\) to alanine, and Leu\(^{17}\) to alanine. None of these changes affected their binding to I-A\(^{b}\) molecules (data not shown).

These results indicate that several amino acids in a long peptide stretch of as much as 14 residues, from His\(^{10}\) to Gly\(^{23}\), have an influence on T cell reactivity independent of the core segment that binds to the I-A\(^{b}\) molecule. Previous studies (30, 31) of T cell recognition of MHC-bound peptides showed effects of P-1 and P-2 or P-10 and P-11, although these were usually restricted to certain sets of T cells. In contrast, all seven clones examined here were profoundly affected by changes on both sides of the core nonamer. Similar observations were made in our previous report on the structural analysis of I-A\(^{b}\) bound to a lysosome peptide. In that case, changing the peptide anchors, particularly P1, did not affect binding but markedly affected T cell recognition (19).

Regardless, the T cells could be grouped into two major sets based on their various responses to truncated or mutated peptides. Recognition of peptides by one set of T cells depended on the recognition of P20Gly (Table V, register 2). These T cells were all unresponsive to a peptide containing a Lys\(^{20}\). These T cells were affected, but to a lesser extent, by a lysine substitution at residue 21. Of note, truncations of the Gly\(^{23}\) and Arg\(^{22}\) had an effect. This set of T cells was also highly influenced by residues at position 10 of the B:(9–23) sequence, which presumably is P-2. Likewise, all changes in residues within the core segment had a major effect.

Another group of T cells depended on the recognition of Gly\(^{20}\), Glu\(^{23}\), and Arg\(^{22}\). Truncation of the peptide at either Arg\(^{22}\) or Glu\(^{23}\) resulted in a decrease or loss of reactivity, as did lysine substitution at Glu\(^{21}\) or Gly\(^{20}\) (Table V, register 1). These results are in sharp contrast to the first set where there is a main residue change that distinguishes them. These T cells were also affected by amino acid changes in the core segment and changes in the flanking residues.

A previous report by Abiru et al. (20) examined five clones, isolated as done here, all reactive with the B:(9–23) segment. Although it is difficult to make strict comparisons between both studies, some common features are apparent: several alanine substitutions affected recognition and some of these were also spread throughout the peptide. The pattern of reactivity of four of them is compatible with register-1 recognition of the 13–21 segment, but also very sensitive to the Gly\(^{23}\) and Arg\(^{22}\) residues. One clone, however, appeared to recognize a 9–16 segment. We tested such a sequence and found it to bind very poorly to I-A\(^{b}\) at 31 \(\mu M\). In another report, the sensitivity of five of the anti-insulin clones, isolated by Wegmann and colleagues (32), to alanine substitutions in the B:(9–23) segment was further characterized. Changes in residues at both flanks and core segments affected the response: two of them were compatible with recognition of the 13–21 segment, i.e., a loss of responses to Glu\(^{21}\), but not to Gly\(^{20}\), and one was compatible with the 12–20 reactivity. Different studies (13, 15) reported on an insulin-reactive clone recognizing the 12–25 segment. Together, our studies and those cited above indicate a great heterogeneity in the T cell recognition of the B:(9–23) segment of insulin.

**Discussion**

The main findings from these studies are 1) the relatively poor binding of insulin peptides to I-A\(^{b}\), as exemplified by their high IC\(_{50}\) and fast dissociation rates; 2) two possible contiguous binding segments, one using a glycine as its major MHC anchor residue, the other having a glutamic acid; plus others of very low affinity; 3) a broad effect on the T cell responses of single amino acid substitutions in the peptides; this effect is noted both in the flanks and in the core segments, and in the latter, either in MHC or TCR contact residues as predicted by their binding core segment; and 4) two sets of T cells that, despite the global changes, are preferentially affected by changes in the Gly\(^{20}\) or Glu\(^{21}\), albeit to various degrees.

Insulin represents an autoantigen in which its binding strength to a class II molecule is weak and yet it correlates with a high representation of reactive clones and a strong influence on the development of the disease. As shown here, and by the extensive work of others, the B:(9–23) segment appears to be a "hot spot" fostering the development of different sets of T cells.

The first documentation of such a phenomenon, i.e., weak binding with strong biological reactivity, was made in the studies of Wraith and colleagues (34, 35) in which they noted that a myelin basic protein peptide induced disease despite a notably weak interaction with the I-A\(^{b}\) class II molecule (33, 34). We had speculated that in the case of the NOD mouse, the high percentage of SDS-unstable MHC class II molecules might indicate weak peptide binding, and that such weak interactions may be a factor contributing to poor thymic-negative selection (35). In retrospect, the correlation between SDS stability and peptide affinity and dissociation rate in the I-A\(^{b}\) molecule remains unexplained from a biochemical and structural standpoint. The weak interaction of peptide with a class II MHC molecule was interpreted to result in weak-negative selection leading to the escape from the thymus.
the autoreactive T cells. In the case of the diabetic NOD mouse, previous studies (36, 37) identified a high number of autoreactive T cells. These were attributed to the I-A^B7 molecule, indicating that this MHC molecule in general had a propensity for high autoreactivity.

The weak binding interaction of the B chain 9–23 segment raises a number of questions. First, why is the 9–23 segment of the insulin B chain a central focus of the T cell response? An explanation may lie in the studies from Goverman’s laboratory (38) on the response to myelin basic protein. Their data suggest that a different segment of the protein binds with high affinity and the T cells to it are negatively selected. In contrast, the weak binding segment (encompassing the 1–11 residue) escapes negative selection. Of note, a peptide from mouse prosinulin, B24-C36 has been reported to bind to soluble I-A^B7 with relatively good binding affinity (39).

Second, why, if the interaction is weak, are such T cells activated in peripheral sites resulting in autoimmunity? The concentration of insulin in blood and extracellular fluid is in the nanomolar range and yet peptide binding occurs in the micromolar range. One could postulate that the insulin receptor on APCs would be required to achieve the concentration needed for binding (40); however, the high IC_50 value (30 µM) for binding of the β-chain makes this unlikely. A different explanation is to posit that the islet-resident APCs, which are in intimate contact with β cells, may take up insulin granules directly and are therefore exposed to high levels of insulin.

And, third, very much related to the aforementioned question, is how to explain the discrepancy between the biological assays in which T cells are being activated at low micromolar concentration and the peptide binding results? The biological assays must contain compensatory mechanisms that improve the quality of these interactions in a way that is not detected by the straight chemical assays. Perhaps the concentration of peptide-MHC in the synapse, cooperativity among different peptide-MHC, and the dynamics of the APC presentation favor the presentation of these low-affinity epitopes.

Regardless, the weak interaction of the insulin peptides with I-A^B7 could explain the results of multiple residue changes affecting the T cell response. These changes suggest that the insulin peptides may have more conformational flexibility as a result of their weak interaction with I-A^B7. Weak MHC anchor sites in the peptide may translate into a loose flexible structure. Changes in flanks or in MHC contact residues have been found to affect TCR recognition and, in some instances, the flank residue may serve as a TCR contact, although this is not always the case. Perhaps, as indicated by the three reports on the structures of T cell receptors bound to myelin basic protein peptide-MHC complexes, it points to the need for broader contacts between both the peptide-MHC and the T cell receptors (41–43).

The binding features of the insulin B(9–23) peptide to the diabetes susceptibility allele, I-A^B7, in addition to the characterization of various sets of T cells adds a different perspective to our understanding of the role of insulin as an autoantigen in NOD mouse diabetes. Whether or not such features are true for the binding of the insulin peptides to human diabetes-risk alleles, such as DQ8, and for the repertoire of anti-insulin T cells in patients with type 1 diabetes, needs to be examined. Given the striking similarities in the motif of peptide selection between I-A^B7 and DQ8, these features may apply to the human disease (24).

Acknowledgments

We thank Patrice Bittrner, Kevin Clark, and Kimberley Unger for technical assistance.

Disclosures

The authors have no financial conflict of interest.

References

deciphering the link between I-Ag7 and autoimmune diabetes. Science 288: 505–511.


