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DO THE PEPTIDE-BINDING PROPERTIES OF DIABETOGENIC CLASS II MOLECULES EXPLAIN AUTOREACTIVITY?

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Abstract

One seminal aspect in autoimmune diabetes is antigen presentation of beta cell antigens by the diabetes-propensity class II histocompatibility molecules. The binding properties of I-A^{g7} molecules are reviewed here and an emphasis is placed on their selection of peptides with a highly specific sequence motif, in which one or more acidic amino acids are found at the carboxy end interacting at the P9 anchoring site of I-A^{g7}. The reasons for the central role of I-A^{g7} in the autoimmune response are analyzed. The insulin B chain segment 9-23 is a hot spot for T cell selection and a striking example of a weak MHC binding peptide that triggers autoreactivity.

Introduction

The direct association of Major Histocompatibility Complex (MHC) molecules in the onset of various autoimmune disorders, including type 1 diabetes mellitus (T1DM), points a central role to their peptide binding features. Several attempts have been made to identify the natural peptides bound to MHC molecules, starting from the pioneering studies of Rammensee [1-3], and continuing with the introduction of mass spectrometry [4,5]. In our own experience, we have evaluated naturally processed peptides selected by various murine (I-A^k, I-A^d, I-E^k, I-E^p, I-A^{g7}, I-A^{g7PD} and K^d) and human (DQ8) MHC molecules. A conclusion from the identification of large numbers of natural peptides is that each MHC molecule selects for a unique repertoire of peptides in an allele-specific manner.

Biochemical, Structural and Functional Properties of diabetes-associated class II MHC molecules

A notable feature shared between the murine (I-A^{g7}) and human (DQ2 or DQ8) diabetogenic class II MHC molecules is the expression of a non-Asp amino acid at position 57 of the β chain [6]; most other class II MHC molecules contain a conserved Asp at β 57, which forms an ion-pair with an opposing Arg at α 76 that defines the rim and the structural features of the P9 pocket of the binding groove [7,8]. The absence of this salt bridge in the case of diabetogenic class II MHC molecules generated a P9 pocket that was wider, shallower and open towards the C-terminus [9-11]. However, the effect of these unique structural features on peptide selection remained unclear: earlier reports that analyzed binding interactions with synthetic peptides or

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peptide libraries suggested that diabetogenic MHC molecules bound peptides promiscuously with little or no specificity [12-15]. These conclusions were further bolstered by previous work from different laboratories, demonstrating that the diabetogenic class II MHC molecules, were unstable in SDS, i.e. they dissociated into the α - and β -subunits under non-denaturing conditions [13,16-21]. Prior work with other alleles, such as I-A^k or I-A^b, had established a clear correlation between SDS stability and peptide quality: high affinity peptides formed SDS-stable heterodimers, while low affinity peptides did not [16,22,23]. Since I-A^{g7} and DQ8 were noticeably SDS-unstable it was proposed that they must bind promiscuously and to low affinity peptides [16]. However, mass-spectrometry based identification of naturally processed peptides selected by I-A^{g7} and DQ8 generated very different results: both the murine and human alleles selected for similar peptides with a very well defined binding motif characterized by multiple C-termini acidic amino acids [17,24-26].

Binding studies demonstrated that the acidic amino acids interacted with the P9 pocket and contributed significantly towards binding affinity [24,25]. And moreover, the specificity of the P9 pocket, composed of the non-Asp β 57 residue, was crucial in determining selection of peptides [24,26]. Table 1 depicts an example of a peptide family from the E2B integral membrane protein highly selected by APCs expressing either I-A^{g7} or DQ8. Note that every member contains a stretch of 3 acidic amino acids ("EED") towards the C-terminus. Binding assays to soluble I-A^{g7} or DQ8 identified the same 9-mer binding core (shown in bold and underlined in Table 1) that contained an acidic P9 anchor [24,25]. Hence, SDS-instability is an intrinsic feature of diabetogenic class II MHC molecules, which is not reflective of the quality or specificity of peptides that are bound by these molecules. It is important to note that I-A^{g7} binds to many of its ligands in the low micro-molar range, which is relatively weaker when compared to other murine alleles such as I-A^k that bind to its peptides in the nano-molar range.

Recently, we utilized the vast datasets of natural peptides to develop a computational program that efficiently predicts epitopes selected by diabetogenic class II MHC molecules [27]. This approach had several unique aspects: (i) only the naturally selected peptides isolated from APCs were used in the development of this algorithm; (ii) the physical and structural characteristics of the binding pockets were used to refine the preferred amino acid anchors; and (iii) hindering residues, i.e. amino acids that are disfavored at a particular position, were used to screen potential binding peptides. All these factors, when applied in concert, enabled this algorithm to identify the preferred binding motif for I-A^{g7}, as shown in Figure 1. When tested against many natural peptides, the algorithm successfully assigned high scores to high affinity binders and lower scores to peptides that bound poorly [27]. Moreover, the algorithm successfully predicted I-A^{g7} peptides from hen egg lysozyme that were recognized by T cells in NOD mice [27]. Hence, in this case, predictive algorithms that have evolved from analysis of naturally processed peptides from class II MHC molecules can be powerful tools for future analysis of unknown epitopes from target islet beta cell antigens. On the other hand, programs that have been developed for class I MHC epitope prediction [28] have been successful because of the fact that class I MHC molecules select for a very homogenous repertoire of peptides, i.e. they are all between 8-10 amino acids in length and exhibit a very well-defined binding motif. In contrast, class II MHC molecules select for peptides with varying length and many are selected as families (Table 1), which makes it difficult to identify the 9-mer binding core. This is further complicated by the possibility that some peptides can bind in multiple registers (as discussed later for the dominant insulin epitope in T1DM) and that class II bound peptides are edited by H-2 DM.

What are the consequences of the peptide binding properties of class II MHC molecules on T cell selection and peripheral activation? Earlier studies by Kanagawa et al and Ridgway et al noted that the expression of I-A^{g7} alone, independent of the genetic background, was sufficient

for escape of autoreactive T cells - i.e. these were CD4⁺ T cells that reacted with syngeneic APCs alone with no need for any exogenous antigens [29-31]. Note in Table 2 from the experiments of Kanagawa et al [29], the frequencies of autoreactive T cells was significantly higher in I-A^{g7}-expressing strains and independent of the NOD background genes.

More recently, Stratmann et al generated I-A^{g7} tetramers using a synthetic 'mime' peptide known to stimulate diabetogenic CD4⁺ T cells, including the well-characterized BDC T cell clone. Here again, tetramer-positive CD4⁺ T cells were identified in the periphery in both NOD and B6.G7 (congenic B6 mice expressing H-2^{g7}) mouse strains [32]. However, whether escape of these T cells is a result of inadequate amounts of thymic self-peptides due to expression levels or low-affinity binders, or both, remains to be determined. In another study, Ferlin et al used transgenic NOD mice that expressed the Leishmania Antigen receptor for C Kinase (LACK) as a self protein to study T cell tolerance [33]. In this report it was noted that the LACK peptides that bound to I-A^{g7} with low-affinity induced T cell tolerance [33]. However, in this case a high level of expression of this low-affinity ligand may also be a factor in tolerance induction. The relationship between peptide binding and specific beta cell antigens has best been studied against peptides from the insulin molecules, as described next.

While these studies have pointed to a MHC-centric role for I-A^{g7} in thymic selection, other reports have identified a role for the NOD background genes in generating a compromised thymic selection milieu [34-36]. Kishimoto et al noted that NOD thymocytes were more resistant to deletion upon crosslinking of the TCR via monoclonal antibodies [34], while other studies with CD4⁺ TCR transgenic mice concluded that antigen encounter resulted in inefficient deletion of thymocytes on the NOD background when compared to non-NOD strains [35,36]. It is likely that both MHC and non-MHC background genes contribute towards selection of a self-reactive T cell repertoire.

Another feature of I-A^{g7} that may contribute towards autoimmunity relates to its unique properties of peptide selection. We and others have noted that I-A^{g7}-bound peptides contain multiple C-termini acidic residues that contribute towards binding affinity [17,24]. More importantly, >80-90% of peptides isolated from I-A^{g7} contain C-terminal stretches of two or three acidic residues [24,25] (Figure 1). A consequence of this highly selective repertoire may mean that the diversity of peptides selected by I-A^{g7} in the thymus may be narrower when compared to other class II MHC molecules. If so, then this would directly impact T cell selection. Similarly, the unique features of peptide selection by I-A^{g7} may allow for this molecule to efficiently select for islet β -cell-derived peptides that may be targets of autoreactive T cells. Indeed, many of the known epitopes from autoantigens in T1DM (including insulin) exhibit the preferred I-A^{g7} binding motif. The role for the non-Asp β 57 (β 57Ser) in selection of these peptides is foremost: APCs expressing a modified I-A^{g7} wherein the β 57 was changed to the conserved Asp (termed I-A^{g7PD}) selected for entirely different sets of peptides that did not exhibit the presence of c-terminal acidic amino acids [24,26]. These results support the observations of Kanagawa et al who followed the thymic selection and peripheral activation of the diabetogenic CD4⁺ BDC T cell in mice expressing either I-A^{g7} or I-A^{g7PD}. Results from this study demonstrated that while BDC T cells were selected by both I-A^{g7}- and I-A^{g7PD}-expressing thymi, only I-A^{g7} APCs in the periphery were able to activate this pathogenic T cell [37].

Weak MHC binding peptides and autoreactivity: the case of the dominant insulin T cell epitope

Insulin, and particularly the 9-23 peptide of the insulin beta chain, is a primary target of T cell reactivity in both the human disease and the NOD mouse [38,39] (in this issue, contribution by Eisenbarth). Initial binding studies of the B:(9-23) peptide to soluble I-A^{g7} in detergent free

conditions revealed poor binding and fast dissociation rates [10,13,14,40]. The IC₅₀ values for the B:(9-23) peptide were measured in the 5-10 μ M range at endosomal pH and the half-life of the complexes was less than two hours. Recently, a detailed analysis of the binding of this epitope to I-A^{g7} was made which confirmed these general findings and revealed several interesting new features [41]. First, the B:(9-23) peptide bound weakly to I-A^{g7} in two contiguous registers, using two neighboring binding core segments, as shown in Table 3. Note that one register utilized the preferred glutamic acid as the P9 anchor, while the other register utilizes a glycine, which can also be accommodated at this position. Changing either of these to a lysine, which should result in a loss of binding, had no effect on binding. However, a noticeable loss of binding was evident when both the glycine at 20 and glutamic acid at 21 were mutated to lysines (Table 3). To confirm the presence of two registers, we demonstrated that the minimal 9-mer binding core of each segment did indeed bind to I-A^{g7} (Table 3).

The presence of two binding registers had a biological correlate: naturally occurring insulin-reactive T cells isolated from pancreatic nodes and infiltrated islets of NOD mice recognized either one or the other register, as shown by the truncation analysis of the insulin epitope to identify the minimal T cell epitope (Figure 2). Moreover, insulin T cells were sensitive to amino acid changes in either the flanks or the core segments of the peptide-affected binding and T cell responses [41].

Taken together, these data suggest that the B:(9-23) peptide and the I-A^{g7} molecule form a loose and flexible complex. Furthermore, these results established a direct relationship between a weak peptide-MHC and the development of an autoimmune T cell repertoire. The great variability in sensitivity to amino acid changes of the anti-(9-23) T cells described in the report may be a reflection of the many conformations that the peptide-MHC exhibits. Whether this great heterogeneity in TCR recognition of single peptide-MHC complexes is a universal feature of autoimmune specificities for weakly interacting peptides will have to be further examined when other epitopes are fully characterized. Nevertheless, insulin appears to be an autoantigen in which the binding of the immunodominant epitope, B:(9-23), to the class II MHC molecule is weak, and yet it correlates with a high representation of autoreactive T cells and a strong influence on the development of disease.

If weak peptide-MHC complexes allow for the escape of autoreactive T cells from the thymus, the obvious question remains, how are these T cells activated in the periphery? One explanation may simply be related to the anatomic distribution and relative concentrations of the given autoantigen. In the case of beta cell specific autoantigens, such as insulin, the large quantity of these secreted proteins in a local milieu may be sufficient to overcome the inherent weakness of the peptide-MHC interaction. Intra-islet dendritic cells must encounter very large quantities of insulin, both secreted and contained in granules of beta cells, such that the number of processed and presented peptides is of a sufficient threshold to activate insulin reactive T cells. This idea is supported by evidence that early diabetogenic T cell priming occurs in the draining peri-pancreatic lymph node, where naïve T cells encounter APCs charged with abundant beta-cell antigens [42,43]. A second component that most likely plays a role in resolving the apparent paradox between the failure of negative selection and peripheral activation is that of costimulation. Presumably, autoimmune T cells recognizing weak peptide-MHC complexes that escape the thymus receive additional signals in the periphery that push them over a threshold for activation. However this idea alone, in the case of the autoantigen insulin which circulates throughout the body, cannot explain the localization of T cell priming to the peri pancreatic lymph nodes, but costimulation, in concert with the presence of a quantitative excess of antigen seems a plausible mechanism for the loss of tolerance to weakly interacting self peptides. In this manner, protein and peptide secreting endocrine glands, such as the pancreatic islets seem to represent prime targets for weak peptide-MHC targeted T cell mediated autoimmunity. Moreover, the T cell activation thresholds for such weak ligands may be

enhanced by the repertoire of self-peptides present on an APC. Recent reports with both CD4⁺ and CD8⁺ T cells have suggested that normal self peptides contribute towards the formation of an immunological synapse and T cell activation [44,45]. The extent and specificity of these self peptide-MHC complexes in facilitating activation of antigen-specific T cells needs to be examined in greater detail. As a side note, earlier work in the experimental autoimmune encephalomyelitis model had also suggested a relationship between low affinity peptides and T cell autoreactivity [46,47]. In this inducible disease model, the autoantigenic peptide, Ac1-11 of myelin basic protein, bound poorly to the class II MHC molecule, I-A^u, and autoreactive T cells were shown to escape negative selection in the thymus [46,47].

Concluding remarks

An understanding of the biochemical basis of peptides selected by diabetogenic class II MHC molecules is key to gaining insights into the identity and nature of islet β cell antigens that are targeted in diabetes. Analysis of natural peptides has provided useful information with regards to the sequence specificity that can now be applied to predictive algorithms. However, further work needs to clarify the role of the highly specific binding motif of I-A^{g7} peptides and thymic selection of the T cell repertoire. On the other hand, a role for weak binding ligands such as the insulin peptides in autoimmune diabetes, is also emerging. Whether such peptides form a significant fraction of the repertoire of islet β cell epitopes that are targeted by diabetogenic T cells needs to be examined in finer detail.

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Figure 1.

I-Ag7 binding motif as determined by the alignment results from the expectation-maximization alignment algorithm of Chang et al [27] and shown by WebLogo. Preferred amino acids from positions P1 to P9 are indicated by their one letter code.

Figure 2.

Examples of two natural T cell clones from NOD mice that recognize different registers of the insulin peptide. The optimal register encompassed the entire 9-23 segment.

Table 1

An example of a dominant peptide family from the E2B integral membrane protein that is selected by APCs expressing I-Ag7 or DQ8. Note the presence of acidic amino acids towards the c-terminus of each family member. The 9-mer binding core is shown in bold and underlined. In other results, the Glu124 was identified as the P9-MHC anchor residue

<u>Peptide</u>	<u>Amino Acid Sequence</u>
107-126:	A P A A R Y Q T I <u>E E N I K I F E E</u> D A
110-126:	A R Y Q T I <u>E E N I K I F E E</u> D A
110-128:	A R Y Q T I <u>E E N I K I F E E</u> D A V E
111-128:	R Y Q T I <u>E E N I K I F E E</u> D A V E
112-125:	Y Q T I <u>E E N I K I F E E</u> D
112-126:	Y Q T I <u>E E N I K I F E E</u> D A
112-127:	Y Q T I <u>E E N I K I F E E</u> D A V
112-128:	Y Q T I <u>E E N I K I F E E</u> D A V E
113-126:	Q T I <u>E E N I K I F E E</u> D A
114-126:	T I <u>E E N I K I F E E</u> D A
114-127:	T I <u>E E N I K I F E E</u> D A V
114-128:	T I <u>E E N I K I F E E</u> D A V E

Table 2Frequency of Autoreactive T cells^a

Mouse Strain	Background	Class II MHC	Frequency of Autoreactive T cells
NOD	NOD	I-A ^{g7}	~1:1174
NOD.H2b	NOD	I-A ^b	<1: 10,000
B6	B6	I-A ^b	<1:10,000
B6.G7	B6	I-A ^{g7}	~1:1216

^a Adapted from the published study of Kanagawa et al [29]

Table 3
Binding of Insulin B Chain Peptides^b

	9 ————— 23	IC₅₀ (μM)
INS 9-23	PHLVEALYLVCGERG	2.4
	PHLVEALYLVCG K RG	3.7
	PHLVEALYLV C ERG	1.1
	PHLVEALYLV C K RG	12.0
INS 13-21 (Register 1)	EALYLVCGE	5.9
INS 12-20 (Register 2)	VEALYLVCG	2.5

^b Binding analysis of synthetic peptides to soluble I-A^{g7}. Binding studies were performed at pH 5.5 [41]. Replacement of the natural residues with lysines allowed for the identification of the P9-MHC contact amino acid.