Insights into autoimmunity gained from structural analysis of MHC–peptide complexes

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The structural and functional properties of HLA-DQ and -DR molecules that confer susceptibility to several common autoimmune diseases, such as type 1 diabetes, rheumatoid arthritis and multiple sclerosis, have been defined. The relevant polymorphisms directly affect interaction with peptides, which provides strong support for the hypothesis that these diseases are peptide-antigen driven. Several studies indicate that structural modifications of peptides can affect MHC class II binding and/or TCR recognition and should be considered in the analysis of T cell responses in autoimmune diseases.

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Abbreviations

APC	antigen-presenting cell
GAD	glutamic acid decarboxylase
HEL	hen egg lysozyme
MBP	myelin basic protein
MS	multiple sclerosis
NOD	nonobese diabetic
PAD	peptidylarginine deiminase
RA	rheumatoid arthritis

Introduction

The MHC, on human chromosome 6p21, is a principal susceptibility locus for many human autoimmune diseases. Susceptibility to a number of these diseases, including rheumatoid arthritis (RA), multiple sclerosis (MS) and type 1 diabetes, is associated with particular alleles of HLA-DR and/or -DQ genes, providing strong evidence for a significant role of MHC class II restricted antigen presentation in these disease processes [1–3]. In several DR-associated autoimmune diseases it has been possible to define structural features of the relevant MHC class II molecules that determine their interaction with peptides. For example, susceptibility to RA is associated with the 'shared epitope', a segment of the DRβ chain (β67–β74) that is very similar in sequence among the disease-associated subtypes of DR4 (DRB1*0401 and 0404) and DR1 (DRB1*0101) [4]. In structural terms, this shared epitope primarily defines the shape and charge of the P4 pocket [4–6]. The P4 pocket has a positive charge in the RA-associated DR1 and DR4 subtypes, but a negative charge in a DR4 subtype (DRB1*0402) that is associated with susceptibility to pemphigus vulgaris (PV), an autoimmune disease of the skin. Susceptibility to RA or PV is therefore dependent on the structural properties of a key pocket of the DR peptide-binding site [3–7]. The analysis of DR molecules in autoimmune diseases has therefore greatly benefited from the crystal structure of HLA-DR1, which allowed analysis of such polymorphisms in structural terms [8].

This review focuses on recent progress on the structure and function of DQ molecules in the pathogenesis of type 1 diabetes and celiac disease. In addition, the emerging role of enzymatic modifications of target antigens will be discussed, with an emphasis on the role of modified antigens in human diseases.

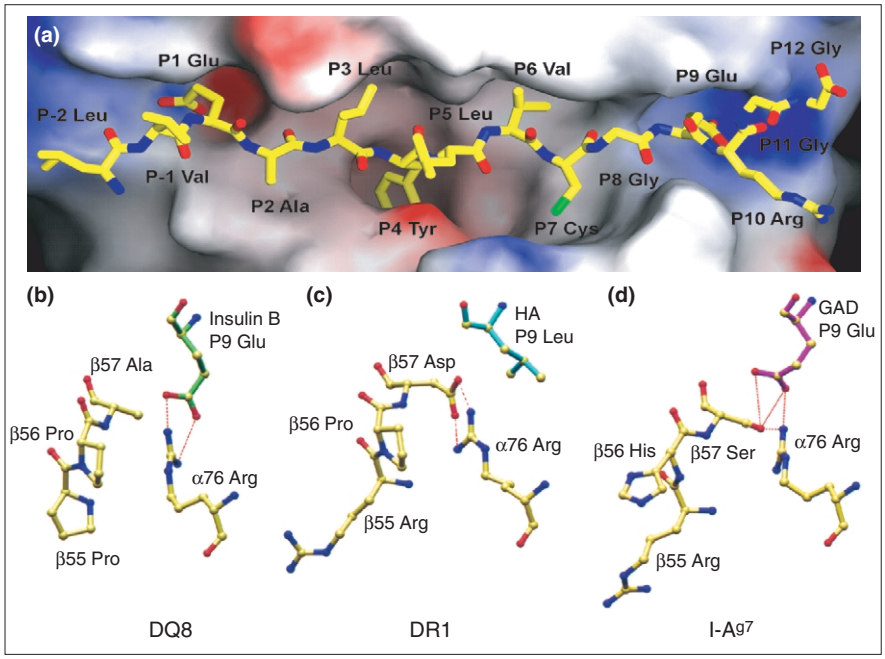
The structure of human and murine MHC class II molecules in type 1 diabetes

HLA-DQ molecules are important in the pathogenesis of type 1 diabetes and celiac disease, but until recently no crystal structure of DQ was available. In addition, the interaction of peptides with DQ molecules has been more difficult to define than for DR molecules. DR-binding peptides have a prominent anchor residue for the hydrophobic P1 pocket and this pocket has limited variability among DR molecules [8,9]. In contrast, both DQα and β chains are highly polymorphic, which made it more difficult to determine the 'binding frame' of peptides for DQ molecules based on an unambiguously defined anchor residue.

The highest risk for type 1 diabetes is conferred by alleles of DQ genes that encode DQ8 (DQA1*0301, DQB1*0302) and DQ2 (DQA1*0501, DQB1*0201) [1,2,10]. These MHC class II molecules are thought to be important in the T cell mediated autoimmune response that results in destruction of insulin-producing β cells in the pancreas. Susceptibility to type 1 diabetes has been correlated with the β57 polymorphism in DQ in humans and I-A in nonobese diabetic (NOD) mice [1,2,10,11], indicating that certain structural features of these MHC class II molecules could be important in antigen presentation.

DQ8 was crystallized with a peptide from human insulin (B chain, residues 9–23) that represents a prominent T cell epitope for islet-infiltrating $CD4^+$ T cells in NOD mice [12•,13]. In addition, an epitope for $CD8^+$ T cells (residues 15–23) from NOD mice is contained within the same segment of the insulin B chain [14]. Recently, a T cell response to the insulin B (9–23) peptide has been documented in patients with recent onset of type 1 diabetes and in prediabetics [15]. The insulin B (9–23) peptide binds with high affinity to DQ8 and the complex has a long half-life ($t_{1/2}$ >72 hours) [16]. The crystal structure demonstrates particular features of DQ8 that allow presentation of this insulin peptide. Three side-chains of the insulin peptide are buried in deep pockets of the DQ8 binding site, and two of these peptide residues carry a negative

Figure 1



Disease-associated polymorphisms determine the interaction of MHC class II molecules with peptides. **(a)** Crystal structure of the complex of HLA-DQ8 and the human insulin peptide (B chain, residues 9–23; shown as a stick structure). Peptide side-chains P1 Glu, P4 Tyr and P9 Glu are buried in deep pockets, whereas P6 Val and P7 Cys lie on shelf-like surfaces. The P4 pocket of DQ8 is nonpolar (white), whereas the P9 pocket is positively charged (blue); negative charges are indicated in red. Negatively charged peptide side-chains of the insulin peptide (glutamic acid) serve as anchor residues for both the P1 and the P9 pocket. **(b–d)** Views from the carboxy-terminal end of the peptide (equivalent to the right-end of part [a]); class II β chain helix residues 55–57 are shown on the left of (b–d), class II α chain residue 76 is shown at the bottom right and peptide P9 residues are shown shown at the upper right. (c) In MHC class II molecules that carry aspartic acid at β57, such as DR1, a salt bridge is formed between β57 Asp and α76 Arg. Due to the salt bridge between β57 Asp and α76 Arg, the P9 pocket of DR1 is electrostatically neutral. In the crystal structure of DR1, a hydrophobic peptide side-chain (Leu) of the influenza HA peptide occupies the P9 pocket. (b) In DQ8, β57 is an alanine and a salt bridge is instead formed between P9 Glu of the insulin B (9–23) peptide and α76 Arg. (d) In the crystal structure of I-A^{g7} with the GAD (206–220) peptide, P9 Glu of the GAD peptide forms hydrogen bonds with β57 Ser and α76 Arg. DQ8 differs from I-A^{g7} at β chain residues 55–57 (Pro–Pro–Ala in DQ8; Arg–His–Ser in I-A^{g7}). Reproduced, with permission, from [12•].

charge (glutamic acid at P1 and P9). A tyrosine residue is bound in the P4 pocket, which is very deep and hydrophobic (Figure 1) [12•]. The observation that acidic residues can be accommodated in two pockets of DQ8 has implications for the pathogenesis of type 1 diabetes and celiac disease, as discussed below.

Particularly important are the structural features of the P9 pocket of DQ8, which is in part shaped by residue β57. Both DQ8 and DQ2 carry an alanine at β57, rather than an aspartic acid residue observed in alleles that do not confer susceptibility to type 1 diabetes (Figure 1b,d) [1,2,10]. In MHC class II molecules with aspartic acid at this position, the P9 pocket is electrostatically neutral since aspartic acid at β57 forms a salt bridge with arginine at α76 (as shown in Figure 1c for DR1) [8]; in contrast, the P9 pocket of DQ8 has a positive charge (blue color in Figure 1a), due to the absence of a negatively charged residue at β57. In the DQ8–insulin-peptide complex, a salt bridge is instead formed between the glutamic acid side-chain of the peptide and arginine at α76 (Figure 1b) [12•]. The formation of a salt bridge between the peptide and α76 accounts for the observed preference of the P9 pocket of DQ8 for negatively charged amino acids, and may contribute to the long half-life of the insulin peptide with DQ8 [16]. However, it is important to note that other residues can also be accommodated in the P9 pocket of DQ8, albeit with a reduced affinity [16–18]. In contrast, negatively charged peptide residues are strongly disfavored in the P9 pocket of MHC class II molecules with an aspartic acid at β57.

The crystal structure of I-A^{g7}, the MHC class II molecule that confers susceptibility to diabetes in NOD mice, has also been determined, allowing direct structural comparison of these diabetes-associated MHC molecules [11,19,20•,21•]. An important similarity between these structures is that the P9 pocket of both DQ8 and I-A^{g7} is basic [12•,20•,21•]. Peptide-binding studies demonstrated that the P9 pocket of I-A^{g7} has a preference for negatively charged residues, as observed for DQ8 [22–24]. In the complex of I-A^{g7} with peptide from glutamic acid decarboxylase (GAD), a glutamic acid side-chain occupies the P9 pocket and forms hydrogen bonds with arginine at α76 and serine at β57 (Figure 1d). Despite these important similarities, most of the polymorphic residues that shape the P9 pocket actually differ between DQ8 and I-A^{g7}, including residues β55–β57 (Pro–Pro–Ala in DQ8 and Arg–His–Ser in I-A^{g7}, as shown in Figure 1b,d) [12•,20•,21•]. The difference in the residues that shape the P9 pocket indicates that the alleles that encode the β chain of the diabetes-associated DQ and I-A molecules evolved independently from each other [12•].

Due to the structural similarities, DQ8 and I-A^{g7} can present the same peptides [16]. The majority of peptides that were identified as T cell epitopes of insulin, GAD65 and heat shock protein 60 (HSP60) in NOD mice also bind to DQ8 [13,16,25,26]. As discussed above, the P9 pocket of DQ8 and I-A^{g7} has a preference for negatively charged residues and, in addition, the P4 pocket of both molecules is large and hydrophobic. Differences are observed in the detailed

architecture of the P1 pocket, which can accommodate a number of different amino acid side-chains in both DQ8 and I-A^{g7}. The structural data suggest that the insulin B (9–23) peptide could bind to I-A^{g7} with the same anchor residues in the P1, P4 and P9 pockets as observed for DQ8 [12•], even though the I-A^{g7}–insulin-peptide complex has a shorter half-life than the DQ8–insulin-peptide complex [16]. The GAD65 (206–220) peptide that is immunodominant in NOD mice binds with high affinity to both I-A^{g7} and DQ8 [16,24]. The crystal structure of I-A^{g7} was determined with this GAD65 peptide and available motif data indicate that the same residues (P1 Ile, P4 Val and P9 Glu) are likely to represent anchors for binding to DQ8.

The crystal structures demonstrate that β57, a key polymorphic residue, directly affects the interaction of these MHC class II molecules with peptides. The structural and functional similarities between DQ8 and I-A^{g7} suggest that similar antigen-presentation events are involved in the development of type 1 diabetes in humans and in NOD mice [12•].

Presentation of deamidated gliadin peptides by DQ8 and DQ2 in celiac disease

Susceptibility to celiac disease, a relatively common inflammatory disease of the small intestine, is associated with the same MHC class II molecules — DQ2 and DQ8 — that confer susceptibility to type 1 diabetes. The majority of patients with celiac disease express DQ2 (>90% in most ethnic groups) and/or DQ8 [27]. Celiac disease is one of the few HLA-associated diseases in which the critical antigen is known. The disease is caused by ingestion of cereal proteins, in particular wheat gliadins, and removal of these proteins from the diet results in clinical remission [28]. Celiac disease is much more prevalent in patients with type 1 diabetes (there is an incidence of 7.7%–8.7% of biopsy-confirmed cases) than in the general population (incidence of 0.2%–0.5%). Antibodies to transglutaminase, a marker for celiac disease, are particularly common in type 1 diabetics who are homozygous for DQ2 (32.4% of patients are antibody positive) [29,30]. The increased risk for celiac disease in patients with type 1 diabetes is, at least in part, due to the shared MHC class II genes.

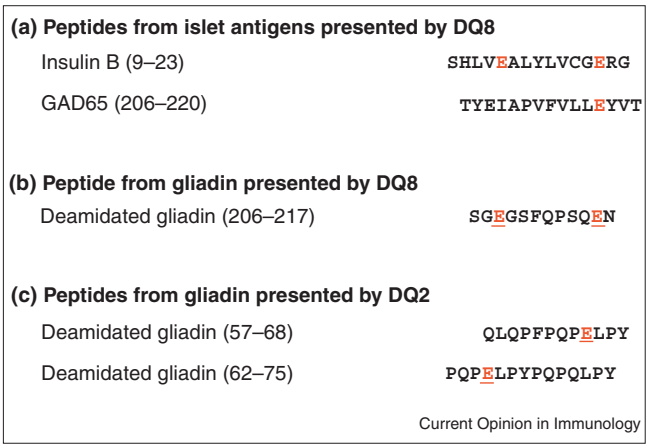
T cell clones specific for gliadins have been isolated from intestinal biopsies of patients with celiac disease and these T cell clones are DQ2- or DQ8-restricted and proliferate in response to gliadins that have been proteolytically cleaved by pepsin or chymotrypsin [31–33,34•]. Patients with celiac disease also develop antibodies to tissue transglutaminase, an enzyme in the intestinal mucosa that can deamidate glutamine residues to glutamic acid when limiting amounts of primary amines are present. The enzyme also catalyzes selective cross-linking of proteins between glutamine and lysine residues. Gliadins are very rich in glutamine and proline residues, and treatment of gliadin with transglutaminase dramatically increases its stimulatory activity in T cell assays, both for DQ2- and DQ8-restricted T cell clones. These findings indicate that transglutaminase converts certain glutamine residues of gliadin to glutamic acid [32,33].

A DQ8-restricted T cell epitope of gliadin was mapped to residues 206–217 within a natural pepsin fragment using T cell clones isolated from intestinal biopsies of two patients [31]. Mass-spectrometric analysis of proteolytic gliadin fragments treated with transglutaminase demonstrated deamidation of glutamine 208 and 216. Synthetic peptides in which one or both of these residues were replaced by glutamic acid had a greatly increased stimulatory capacity for these DQ8-restricted T cell clones (Figure 2b, underlined). The two glutamine/glutamic-acid residues are spaced such that they could represent P1 and P9 anchors of the peptide, which would place phenylalanine 211 in the P4 pocket [35]. When both glutamines are converted to glutamic acid, this gliadin peptide therefore has DQ8 anchors that are strikingly similar to the insulin B (9–23) peptide: glutamic acid at P1 and P9, and an aromatic residue (phenylalanine instead of tyrosine) at P4 (Figure 3a). This binding mode has been modeled using these residues as P1, P4 and P9 anchors for DQ8, and is also consistent with the structure of DQ8 [12•,35].

Conversion of a single glutamine to glutamic acid (residue 65) is critical for the DQ2-restricted T cell response to gliadin [34•,36•]. This gliadin segment (residues 57–75) contains two overlapping T cell epitopes, residues 57–68 and 62–75, centered around residue 65 (Figure 2c). For both peptides, conversion of glutamine 65 to glutamic acid greatly increases the stimulatory capacity for DQ2-restricted T cell clones isolated from the intestine. Peptide-binding studies demonstrated enhanced binding of peptides 57–68 and 62–75 to DQ2 when glutamine 65 was replaced by glutamic acid [34•].

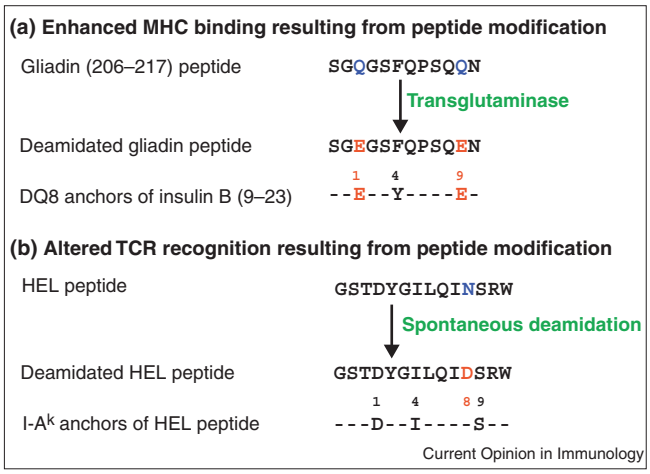
The sequence of DQ2 indicates similarities to DQ8 in the P1 and P9 pockets. The P9 pocket may be larger in DQ2 than in DQ8, due to substitution of β37 by isoleucine (tyrosine in DQ8) [12•]. Position 71 of the DQβ chain is a lysine in DQ2 (threonine in DQ8), which introduces a positive charge into the P4 pocket. This polymorphic residue may also influence the charge of the P7 pocket of DQ2, which appears to have a preference for negatively charged residues [37–39]. Since at least two pockets of the DQ2 binding site can accommodate a glutamic acid residue, deamidation of a single glutamine (residue 65) allows binding of the gliadin peptide in two different binding registers, which are recognized by different T cell clones. For a different T cell epitope derived from γ-gliadin, deamidation of two glutamine residues at P7 and P9 greatly increased DQ2 binding and T cell stimulatory activity [39]. Another study indicated that the P9 pocket of DQ2 could accommodate bulky hydrophobic residues [40]. Certain hydrophobic residues can also be bound in the P9 pocket of DQ8 and I-A^{g7}, but with reduced affinity [18,23]. As discussed above, the 'binding frame' of peptides for DQ molecules can be difficult to determine, and further work will be required to fully define the DQ2 binding motif and the precise positioning of critical deamidated residues of gliadin in the DQ2 binding site.

Figure 2



Peptides presented by DQ8 and DQ2. **(a)** The insulin B chain (9–23) and GAD65 (206–220) peptides are immunodominant T cell epitopes for I-A^{g7}-restricted T cells in NOD mice [13,25,26]. Both peptides bind to DQ8 with high affinity [16]; the P1 and P9 anchors of the insulin peptide are highlighted in red. Available motif data indicate that a glutamic acid residue (E, highlighted in red) of the GAD (206–220) peptide occupies the P9 pocket of DQ8. **(b)** Deamidation of gliadin peptides is important in the pathogenesis of celiac disease. In the gliadin (206–217) peptide presented by DQ8, two glutamine residues can be modified by transglutaminase to glutamic acid (E, underlined) [33]. When glutamic acid is present at both positions, the peptide has DQ8 anchor residues that are similar to the insulin B (9–23) peptide. **(c)** In the gliadin peptides presented by DQ2, conversion of a single glutamine to glutamic acid (E, underlined) increases the affinity for DQ2. Two overlapping peptides of gliadin (residues 57–68 and 62–75) are recognized by different T cell clones [34•].

Figure 3



Structural modifications of peptides that can enhance MHC binding or alter TCR recognition. **(a)** Deamidation of glutamine residues (Q, blue) in the gliadin (206–217) peptide by transglutaminase creates a peptide with an increased affinity for DQ8 (also see Figure 2b) [33]. The peptide in which both glutamine residues have been modified to glutamic acid (E, red) has P1, P4 and P9 anchors that are similar to the insulin B (9–23) peptide. **(b)** Spontaneous deamidation of an asparagine (N, blue) residue in an immunodominant epitope of HEL peptide affects TCR recognition [41•]. This residue is located at the P8 position and can therefore interact with the TCR [44]. Deamidation is dependent on the primary sequence and the position of the asparagine in the three-dimensional structure of the protein. It is promoted by prolonged incubation at 37°C, an elevated temperature or a low pH and introduces a negative charge (D, red) at this TCR-contact residue.

Why is susceptibility to celiac disease associated with DQ2 and DQ8? Both MHC class II molecules can accommodate negatively charged amino acids as anchor residues, as shown for DQ8 in the structure with the insulin B (9–23) peptide. Deamidation of critical glutamine residues in gliadin creates glutamic acid residues that can serve as anchors for binding to DQ2 or DQ8. The example of the DQ8-restricted gliadin peptide illustrates structural features of a MHC class II molecule relevant for antigen presentation in this disease.

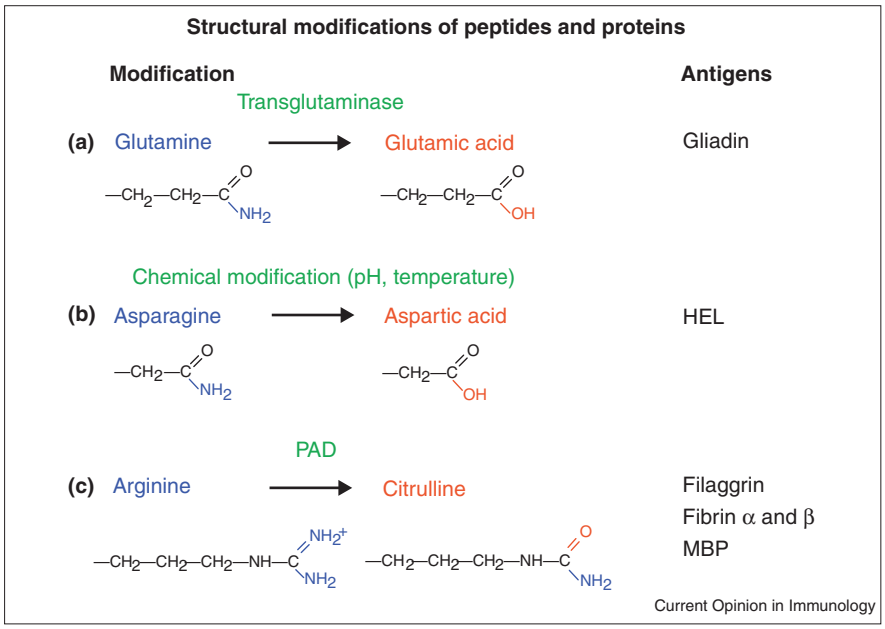
The potential relevance of peptide modifications in T cell mediated immune responses

The studies on celiac disease illustrate that it is relevant to consider potential structural modifications of peptides that could affect binding of peptides to disease-associated MHC class II molecules and/or TCR recognition. Of particular interest are modifications that change the charge properties of a peptide, such as deamidation of glutamine to glutamic acid or asparagine to aspartic acid (gain of a negative charge), and deimidation of arginine to citrulline (loss of a positive charge) (Figures 3 and 4).

Such modifications can occur at peptide residues that are important for TCR recognition. The effect of peptide modification on TCR recognition has been defined in detail for I-A^k-restricted T cell hybridomas reactive with an immunodominant peptide of hen egg lysozyme (HEL) (Figure 3b) [41•]. The majority of T cells from peptide-immunized mice do not secrete IL-2 in response to antigen-presenting cells (APCs) pulsed with the HEL protein. However, these T cells respond to APCs pulsed with trypsin-digested HEL, as well as APCs pulsed with HEL peptides eluted from I-A^k molecules, indicating TCR recognition of a structurally modified peptide [42,43].

In the crystal structure of the HEL peptide bound to I-A^k, asparagine 59 is located at the P8 position and is therefore available for TCR recognition [44] (Figure 3b). Mass-spectrometric analysis of the HEL peptide demonstrated spontaneous deamidation of asparagine 59 to aspartic acid following prolonged incubation at 37°C at a neutral pH [41•]. This type of asparagine deamidation is the most common nonenzymatic protein modification that occurs under physiological conditions [45]. The T cell hybridomas responded vigorously to a HEL peptide in which position 59 was substituted by aspartic acid. Deamidation of this residue is dependent on its position within the three-dimensional structure since deamidation was not observed in lysozyme, but was observed when the peptide was placed in a surface-exposed loop of a recombinant antibody [41•]. In celiac disease, the three-dimensional

Figure 4



Structural modifications of peptides and proteins. Deamidation of **(a)** glutamine or **(b)** asparagine adds a negative charge, whereas **(c)** deimidation of arginine to citrulline results in loss of a positive charge. Deamidation of gliadin is critical in celiac disease and increases binding of peptides from gliadin to the disease-associated DQ2 and DQ8 molecules [32,33]. Antibodies to citrullinated proteins (filaggrin, and fibrin α and β) are found in patients with RA and antibodies to citrulline stain cells and extracellular protein deposits in sections from inflamed joints [46,47•]. PAD is also present in the myelin and can modify up to 6 of the 19 arginine residues in MBP, one of the candidate antigens for T cells in MS [48–51].

structure of gliadin may also be responsible for the preferential deamidation of certain glutamine residues, such as glutamine 65, by transglutaminase.

Modifications of autoantigens implicated in rheumatoid arthritis and multiple sclerosis

The charge properties of a protein or peptide can also be drastically altered by deimidation of arginine to citrulline, which eliminates the positive charge of the arginine headgroup (Figure 4c). Recent work in RA indicates that autoantibodies in this autoimmune disease are directed against citrullinated peptides. Antibodies to citrullinated peptides appear to be quite specific for RA and are not found in patients with other rheumatic diseases [46]. In contrast, other autoantibodies identified in RA patients, such as rheumatoid factor, are not specific for this disease. The antibodies were first identified using filaggrin, a protein produced during the late stages of differentiation of epithelial cells. Filaggrin is released from profilaggrin by proteolytic cleavage and at this stage the protein is dephosphorylated and ~20% of arginine residues are converted to citrulline by peptidylarginine deiminase (PAD). The antibodies recognize the acidic/neutral isoforms of filaggrin, which suggested that citrullination could be critical for autoantibody binding. Antibody-binding to peptides from the carboxy-terminal segment of filaggrin is observed when one or multiple arginine residue(s) is (are) substituted by citrulline [46].

Since filaggrin is present in squamous epithelia, but apparently not in joints, efforts have been made to identify joint proteins modified in this fashion. Histological analysis of rheumatoid synovial membranes with a monoclonal antibody to citrulline showed strong labeling of intracellular proteins and extracellular protein deposits. Amino-terminal sequencing identified deimidated forms of the α and β chains of fibrin as abundant deimidated proteins deposited in joints [47•]. Histological identification of intracellular deimidated proteins suggests that other deimidated proteins may also be present at this inflammatory site. Susceptibility to RA is strongly associated with DR4 and DR1 [4–6], but it is not yet known whether these MHC molecules present deimidated peptides to relevant T cell populations.

A PAD enzyme is also found in the central nervous system and subcellular fractionation has demonstrated that it is localized in the myelin fraction [48]. The enzyme can modify selected arginine residues in myelin basic protein (MBP), an abundant myelin antigen that represents one of the candidate target antigens in MS. Up to six arginine residues of MBP can be selectively citrullinated, resulting in a loss of six positive charges in the MBP isomer that is least cationic (termed MBP-C8). MBP-C8 induces EAE (a model for MS) in Lewis rats, like the most cationic isomer (termed MBP-C1). Citrullination modified the T cell epitope specificity since a T cell line from Lewis rats immunized with MBP-C8 showed only a minimal response to the peptide that is immunodominant for T cells from MBP-C1-immunized rats [49]. T cells specific for MBP-C8 have been identified in patients with MS [50] and a higher frequency of T cells reactive with MBP-C8 has been detected in patients with MS than in control subjects [51].

Implications of peptide modifications for T cell tolerance

Certain splice variants of the Golli-MBP and proteolipid protein (PLP) genes are expressed in the thymus and other lymphoid structures, which may result in T cell tolerance

to certain epitopes of these antigens [52–54]. T cells are not tolerant to segments of myelin proteins that are not expressed in the thymus due to alternative splicing, such as the immunodominant PLP (139–151) peptide in SJL mice [53,54], and may also not be tolerant to modifications of myelin proteins that are introduced only in the nervous system. Similarly, modifications that are preferentially produced at a site of chronic inflammation could create structural variants of peptides to which T cells are not tolerant or only partially tolerant.

Further investigation of these and other post-translational modifications (i.e. oxidation of cysteine or methionine) will require suitable experimental approaches. For example, modifications introduced by specific enzymes could be investigated by treatment of native or recombinant proteins with the relevant enzyme, such as PAD. Mapping of epitopes with synthetic peptides could be accomplished by use of modified residues in the synthesis (i.e. citrulline) or by enzymatic modification of peptides (i.e. with PAD) following synthesis.

Conclusions

The crystal structures of HLA-DR and -DQ molecules, as well as their murine homologs, provide a structural framework for analyzing the functional properties of these MHC molecules in autoimmune processes. DQ8, which confers susceptibility to type 1 diabetes and celiac disease, can accommodate negatively charged peptide side-chains in the basic P9 pocket, as well as in the P1 pocket. In celiac disease, negatively charged anchor residues can be created in gliadin by deamidation of critical glutamine residues. Such post-translational modifications can alter the charge properties of peptides and may therefore be relevant for other antigens. A structural understanding of the relevant MHC molecules may also be useful for the development of therapeutic strategies that inhibit antigen presentation in autoimmune diseases.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Todd JA, Bell JI, McDevitt HO: **HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus.** *Nature* 1987, **329**:599-604.

2. Nepom GT, Erlich H: **MHC class-II molecules and autoimmunity.** *Annu Rev Immunol* 1991, **9**:493-525.

3. Wucherpfennig KW, Strominger JL: **Selective binding of self peptides to disease-associated major histocompatibility complex (MHC) molecules: a mechanism for MHC-linked susceptibility to human autoimmune diseases.** *J Exp Med* 1995, **181**:1597-1601.

4. Gregersen PK, Silver J, Winchester RJ: **The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis.** *Arthritis Rheum* 1987, **30**:1205-1213.

5. Dessen A, Lawrence CM, Cupo S, Zaller DM, Wiley DC: **X-ray crystal structure of HLA-DR4 (DRA*0101, DRB1*0401) complexed with a peptide from human collagen II.** *Immunity* 1997, **7**:473-481.

6. Hammer J, Gallazzi F, Bono E, Karr RW, Guenot J, Valsasnini P, Nagy ZA, Sinigaglia F: **Peptide binding specificity of HLA-DR4 molecules: correlation with rheumatoid arthritis association.** *J Exp Med* 1995, **181**:1847-1855.

7. Wucherpfennig KW, Yu B, Bhol K, Monos DS, Argyris E, Karr RW, Ahmed AR, Strominger JL: **Structural basis for major histocompatibility complex (MHC)-linked susceptibility to autoimmunity: charged residues of a single MHC binding pocket confer selective presentation of self-peptides in pemphigus vulgaris.** *Proc Natl Acad Sci USA* 1995, **92**:11935-11939.

8. Stern LJ, Brown JH, Jardetzky TS, Gorga JC, Urban RG, Strominger JL, Wiley DC: **Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide.** *Nature* 1994, **368**:215-221.

9. Jardetzky TS, Gorga JC, Busch R, Rothbard J, Strominger JL, Wiley DC: **Peptide binding to HLA-DR1: a peptide with most residues substituted to alanine retains MHC binding.** *EMBO J* 1990, **9**:1797-1803.

10. Horn GT, Bugawan TL, Long CM, Erlich HA: **Allelic sequence variation of the HLA-DQ loci: relationship to serology and to insulin-dependent diabetes susceptibility.** *Proc Natl Acad Sci USA* 1988, **85**:6012-6016.

11. Acha-Orbea H, McDevitt HO: **The first external domain of the nonobese diabetic mouse class II I-A β chain is unique.** *Proc Natl Acad Sci USA* 1987, **84**:2435-2439.

12. • Lee KH, Wucherpfennig KW, Wiley DC: **Structure of a human insulin peptide-HLA-DQ8 complex and susceptibility to type 1 diabetes.** *Nat Immunol* 2001, **2**:501-507.
First crystal structure of a human DQ molecule. Demonstrates structural features of DQ8 that are relevant in the pathogenesis of type 1 diabetes.

13. Wegmann DR, Norbury-Glaser M, Daniel D: **Insulin-specific T cells are a predominant component of islet infiltrates in pre-diabetic NOD mice.** *Eur J Immunol* 1994, **24**:1853-1857.

14. Wong FS, Karttunen J, Dumont C, Wen L, Visintin I, Pilip IM, Shastri N, Pamer EG, Janeway CA Jr: **Identification of an MHC class I-restricted autoantigen in type 1 diabetes by screening an organ-specific cDNA library.** *Nat Med* 1999, **5**:1026-1031.

15. Alleva DG, Crowe PD, Jin L, Kwok WW, Ling N, Gottschalk M, Conlon PJ, Gottlieb PA, Putnam AL, Gaur A: **A disease-associated cellular immune response in type 1 diabetics to an immunodominant epitope of insulin.** *J Clin Invest* 2001, **107**:173-180.

16. Yu B, Gauthier L, Hausmann DH, Wucherpfennig KW: **Binding of conserved islet peptides by human and murine MHC class II molecules associated with susceptibility to type I diabetes.** *Eur J Immunol* 2000, **30**:2497-2506.

17. Kwok WW, Domeier ME, Johnson ML, Nepom GT, Koelle DM: **HLA-DQB1 codon 57 is critical for peptide binding and recognition.** *J Exp Med* 1996, **183**:1253-1258.

18. Kwok WW, Domeier ML, Raymond FC, Byers P, Nepom GT: **Allele-specific motifs characterize HLA-DQ interactions with a diabetes-associated peptide derived from glutamic acid decarboxylase.** *J Immunol* 1996, **156**:2171-2177.

19. Hattori M, Buse JB, Jackson RA, Glimcher L, Dorf ME, Minami M, Makino S, Moriwaki K, Kuzuya H, Imura H *et al.*: **The NOD mouse: recessive diabetogenic gene in the major histocompatibility complex.** *Science* 1986, **231**:733-735.

20. • Corper AL, Stratmann T, Apostolopoulos V, Scott CA, Garcia KC, Kang AS, Wilson IA, Teyton L: **A structural framework for deciphering the link between I-A^{g7} and autoimmune diabetes.** *Science* 2000, **288**:505-511.
See annotation to [21•].

21. Latek RR, Suri A, Petzold SJ, Nelson CA, Kanagawa O, Unanue ER,
• Fremont DH: **Structural basis of peptide binding and presentation by the type I diabetes-associated MHC class II molecule of NOD mice.** *Immunity* 2000, **12**:699-710.
Both papers [20•,21•] describe the crystal structure of the murine MHC class II molecule that confers susceptibility to type 1 diabetes in NOD mice.

22. Reich EP, von Grafenstein H, Barlow A, Swenson KE, Williams K, Janeway CA Jr: **Self peptides isolated from MHC glycoproteins of non-obese diabetic mice.** *J Immunol* 1994, **152**:2279-2288.

23. Hausmann DH, Yu B, Hausmann S, Wucherpfennig KW: **pH-dependent peptide binding properties of the type I diabetes-associated I-A^{g7} molecule: rapid release of CLIP at an endosomal pH.** *J Exp Med* 1999, **189**:1723-1734.

24. Stratmann T, Apostolopoulos V, Mallet-Designe V, Corper AL, Scott CA, Wilson IA, Kang AS, Teyton L: **The I-A^{g7} MHC class II molecule linked to murine diabetes is a promiscuous peptide binder.** *J Immunol* 2000, **165**:3214-3225.

25. Chao CC, McDevitt HO: **Identification of immunogenic epitopes of GAD 65 presented by I-A^{g7} in non-obese diabetic mice.** *Immunogenetics* 1997, **46**:29-34.

26. Zechel MA, Elliott JF, Atkinson MA, Singh B: **Characterization of novel T-cell epitopes on 65 kDa and 67 kDa glutamic acid decarboxylase relevant in autoimmune responses in NOD mice.** *J Autoimmun* 1998, **11**:83-95.

27. Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E: **Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer.** *J Exp Med* 1989, **169**:345-350.

28. Sollid LM: **Molecular basis of celiac disease.** *Annu Rev Immunol* 2000, **18**:53-81.

29. Bao F, Yu L, Babu S, Wang T, Hoffenberg EJ, Rewers M, Eisenbarth GS: **One third of HLA DQ2 homozygous patients with type 1 diabetes express celiac disease-associated trans-glutaminase autoantibodies.** *J Autoimmun* 1999, **13**:143-148.

30. Gillett PM, Gillett HR, Israel DM, Metzger DL, Stewart L, Chanoine JP, Freeman HJ: **High prevalence of celiac disease in patients with type 1 diabetes detected by antibodies to endomysium and tissue transglutaminase.** *Can J Gastroenterol* 2001, **15**:297-301.

31. van de Wal Y, Kooy YM, van Veelen PA, Pena SA, Mearin LM, Molberg O, Lundin KE, Sollid LM, Mutis T, Benckhuijsen WE *et al.*: **Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin.** *Proc Natl Acad Sci USA* 1998, **95**:10050-10054.

32. Molberg O, McAdam SN, Korner R, Quarsten H, Kristiansen C, Madsen L, Fugger L, Scott H, Noren O, Roepstorff P *et al.*: **Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease.** *Nat Med* 1998, **4**:713-717.

33. van de Wal Y, Kooy Y, van Veelen P, Pena S, Mearin L, Papadopoulos G, Koning F: **Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity.** *J Immunol* 1998, **161**:1585-1588.

34. Arentz-Hansen H, Korner R, Molberg O, Quarsten H, Vader W,
• Kooy YM, Lundin KE, Koning F, Roepstorff P, Sollid LM *et al.*: **The intestinal T cell response to alpha-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase.** *J Exp Med* 2000, **191**:603-612.
Demonstrates that the majority of DQ2-restricted T cells from patients with celiac disease recognize a segment of gliadin with a single deamidated glutamine. This modification greatly increases the affinity of two overlapping gliadin peptides to the disease-associated DQ2 molecule.

35. Moustakas AK, van de Wal Y, Routsias J, Kooy YM, van Veelen P, Drijfhout JW, Koning F, Papadopoulos GK: **Structure of celiac disease-associated HLA-DQ8 and non-associated HLA-DQ9 alleles in complex with two disease-specific epitopes.** *Int Immunol* 2000, **12**:1157-1166.

36. Anderson RP, Degano P, Godkin AJ, Jewell DP, Hill AV: ***In vivo***
• **antigen challenge in celiac disease identifies a single trans-glutaminase-modified peptide as the dominant A-gliadin T-cell epitope.** *Nat Med* 2000, **6**:337-342.
Demonstrates that short-term challenge of patients who have celiac disease with the antigen – they ate bread for several days – results in the expansion of DQ2 restricted T cells.

37. Vartdal F, Johansen BH, Friede T, Thorpe CJ, Stevanovic S, Eriksen JE, Sletten K, Thorsby E, Rammensee HG, Sollid LM: **The peptide binding motif of the disease associated HLA-DQ (alpha 1* 0501, beta 1* 0201) molecule.** *Eur J Immunol* 1996, **26**:2764-2772.

38. van de Wal Y, Kooy YM, Drijfhout JW, Amons R, Papadopoulos GK, Koning F: **Unique peptide binding characteristics of the disease-associated DQ(α1*0501, β1*0201) vs the non-disease-associated DQ(α1*0201, β1*0202) molecule.** *Immunogenetics* 1997, **46**:484-492.

39. Quarsten H, Molberg O, Fugger L, McAdam SN, Sollid LM: **HLA binding and T cell recognition of a tissue transglutaminase-modified gliadin epitope.** *Eur J Immunol* 1999, **29**:2506-2514.

40. Quarsten H, Paulsen G, Johansen BH, Thorpe CJ, Holm A, Buus S, Sollid LM: **The P9 pocket of HLA-DQ2 (non-Aspβ57) has no particular preference for negatively charged anchor residues found in other type 1 diabetes-predisposing non-Aspβ57 MHC class II molecules.** *Int Immunol* 1998, **10**:1229-1236.

41. McAdam SN, Fleckenstein B, Rasmussen IB, Schmid DG, Sandlie I,
• Bogen B, Viner NJ, Sollid LM: **T cell recognition of the dominant I-A^k-restricted hen egg lysozyme epitope. Critical role for asparagine deamidation.** *J Exp Med* 2001, **193**:1239-1246.
Demonstrates that spontaneous deamidation of a peptide residue can affect TCR recognition.

42. Viner NJ, Nelson CA, Deck B, Unanue ER: **Complexes generated by the binding of free peptides to class II MHC molecules are antigenically diverse compared with those generated by intracellular processing.** *J Immunol* 1996, **156**:2365-2368.

43. Peterson DA, DiPaolo RJ, Kanagawa O, Unanue ER: **Quantitative analysis of the T cell repertoire that escapes negative selection.** *Immunity* 1999, **11**:453-462.

44. Fremont DH, Monnaie D, Nelson CA, Hendrickson WA, Unanue ER: **Crystal structure of I-A^k in complex with a dominant epitope of lysozyme.** *Immunity* 1998, **8**:305-317.

45. Wright HT: **Sequence and structure determinants of the non-enzymatic deamidation of asparagine and glutamine residues in proteins**. *Protein Eng* 1991, **4**:283-294.

46. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ: **Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies.** *J Clin Invest* 1998, **101**:273-281.

47. Masson-Bessiere C, Sebbag M, Girbal-Neuhauser E, Nogueira L,
• Vincent C, Senshu T, Serre G: **The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin.** *J Immunol* 2001, **166**:4177-4184.
Demonstrates that deimidated proteins, such as fibrin α and β, are abundant in the synovial membrane in RA.

48. Pritzker LB, Nguyen TA, Moscarello MA: **The developmental expression and activity of peptidylarginine deiminase in the mouse.** *Neurosci Lett* 1999, **266**:161-164.

49. Cao L, Sun D, Whitaker JN: **Citrullinated myelin basic protein induces experimental autoimmune encephalomyelitis in Lewis rats through a diverse T cell repertoire.** *J Neuroimmunol* 1998, **88**:21-29.

50. Martin R, Whitaker JN, Rhame L, Goodin RR, McFarland HF: **Citrulline-containing myelin basic protein is recognized by T-cell lines derived from multiple sclerosis patients and healthy individuals.** *Neurology* 1994, **44**:123-129.

51. Tranquill LR, Cao L, Ling NC, Kalbacher H, Martin RM, Whitaker JN: **Enhanced T cell responsiveness to citrulline-containing myelin basic protein in multiple sclerosis patients.** *Mult Scler* 2000, **6**:220-225.

52. Pribyl TM, Campagnoni CW, Kampf K, Kashima T, Handley VW, McMahon J, Campagnoni AT: **The human myelin basic protein gene is included within a 179-kilobase transcription unit: expression in the immune and central nervous systems.** *Proc Natl Acad Sci USA* 1993, **90**:10695-10699.

53. Anderson AC, Nicholson LB, Legge KL, Turchin V, Zaghouani H, Kuchroo VK: **High frequency of autoreactive myelin proteolipid protein-specific T cells in the periphery of naive mice: mechanisms of selection of the self-reactive repertoire.** *J Exp Med* 2000, **191**:761-770.

54. Klein L, Klugmann M, Nave KA, Tuohy VK, Kyewski B: **Shaping of the autoreactive T-cell repertoire by a splice variant of self protein expressed in thymic epithelial cells.** *Nat Med* 2000, **6**:56-61.