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Jorge Oksenberg and David Brassat

Immunogenetics of Autoimmune Disease

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Chapter 1

PREFACE

Autoimmunity is the downstream outcome of a rather extensive and coordinated series of events that include loss of self-tolerance, peripheral lymphocyte activation, disruption of the blood-systems barriers, cellular infiltration into the target organs and local inflammation. Cytokines, adhesion molecules, growth factors, antibodies, and other molecules induce and regulate critical cell functions that perpetuate inflammation, leading to tissue injury and clinical phenotype. The nature and intensity of this response as well as the physiological ability to restore homeostasis are to a large extent conditioned by the unique amino acid sequences that define allelic variants on each of the numerous participating molecules. Therefore, the coding genes in their germline configuration play a primary role in determining who is at risk for developing such disorders, how the disease progresses, and how someone responds to therapy.

Although genetic components in these diseases are clearly present, the lack of obvious and homogeneous modes of transmission has slowed progress by preventing the full exploitation of classical genetic epidemiologic techniques. Furthermore, autoimmune diseases are characterized by modest disease risk heritability and multifaceted interactions with environmental influences. Yet, several recent discoveries have dramatically changed our ability to examine genetic variation as it relates to human disease. In addition to the development of large-scale laboratory methods and tools to efficiently recognize and catalog DNA diversity, over the past few years there has been real progress in the application of new analytical and data-management approaches. Further, improvements in data mining are leading to the identification of co-regulated genes and to the characterization of genetic networks underlying specific cellular processes. These advances together with increasing societal costs of autoimmune diseases provide an important impetus to study the role of genomics and genetics in the pathogenic disregulation of immune homeostasis. In this book, we hope to provide a broad overview of current knowledge on how allelic diversity influences susceptibility in a wide variety of autoimmune diseases. Understanding the genetic roots of these disorders has the potential to uncover the basic mechanisms of the pathology, and this knowledge undoubtedly will lead to new and more effective ways to treat, and perhaps to prevent and cure.

There are approximately 30 recognized autoimmune diseases, affecting 10% of the population. With the aid of novel analytical algorithms, the combined study of genomic and phenotypic information in well-controlled and adequately powered datasets will refine conceptual models of pathogenesis, and a framework for understanding the mechanisms of action of existing therapies for each disorder, as well as the rationale for novel curative strategies.

Jorge Oksenberg, Ph.D.
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CHAPTER 1

HLA and Autoimmunity:
Structural Basis of Immune Recognition

Kai W. Wucherpfennig

Abstract

The MHC region on human chromosome 6p21 is a critical susceptibility locus for many human autoimmune diseases. Susceptibility to a number of these diseases, including rheumatoid arthritis, multiple sclerosis and type 1 diabetes, is associated with particular alleles of HLA-DR or HLA-DQ genes. Crystal structures of HLA-DR and HLA-DQ molecules with bound peptides from candidate autoantigens have demonstrated that critical polymorphic residues determine the shape and charge of key pockets of the peptide binding site and thus determine the interaction of these MHC molecules with peptides. These data provide strong support for the hypothesis that these diseases are peptide-antigen driven. In HLA-DR associated autoimmune diseases such as rheumatoid arthritis and pemphigus vulgaris, key polymorphic determinants are primarily localized to the P4 pocket of the binding site and determine whether the pocket has a positive or negative charge. Peptide binding studies have demonstrated that these changes in the P4 pocket have a significant impact on the repertoire of self-peptides that can be presented by these MHC class II molecules. In HLA-DQ associated diseases such as type 1 diabetes and celiac disease, the β57 polymorphism is critical for peptide presentation since it determines the charge of the P9 pocket of the binding site. The crystal structure of HLA-DQ8 demonstrated that the P9 pocket has a positive charge in HLA-DQ molecules associated with type 1 diabetes, due to the absence of a negative charge at β57. Striking structural similarities were identified between the human DQ8 and murine I-A^{g7} molecules that confer susceptibility to type 1 diabetes, indicating that similar antigen presentation events may be relevant in humans and the NOD mouse model. Recent studies in the NOD mouse indicated that I-A^{g7} can promote expansion in the thymus of a CD4 T cell population which recognizes a peptide ligand that stimulates a panel of islet-specific T cell clones. MHC class II molecules that confer susceptibility to an autoimmune disease may thus promote positive selection of potentially pathogenic T cell population in the thymus and later induce the differentiation of these cells into effector populations by presentation of peptides derived from the target organ.

General Structural Features of MHC Class II Molecules

The peptide binding site of MHC class II molecules is formed by the N-terminal domains of the α and β chains, with each chain contributing approximately half of the floor as well as one of the two long α helices that form the peptide binding site (Fig. 1).[1,2] The binding site is open at both ends so that peptides of different length can be bound, explaining why nested sets of peptides have been identified for a given epitope in peptide elution studies.[1,3,4] Peptides are typically bound with a high affinity and a long half-life ($t_{1/2}$ of several days or even weeks) and mass spectrometry experiments have demonstrated that at least several hundred different

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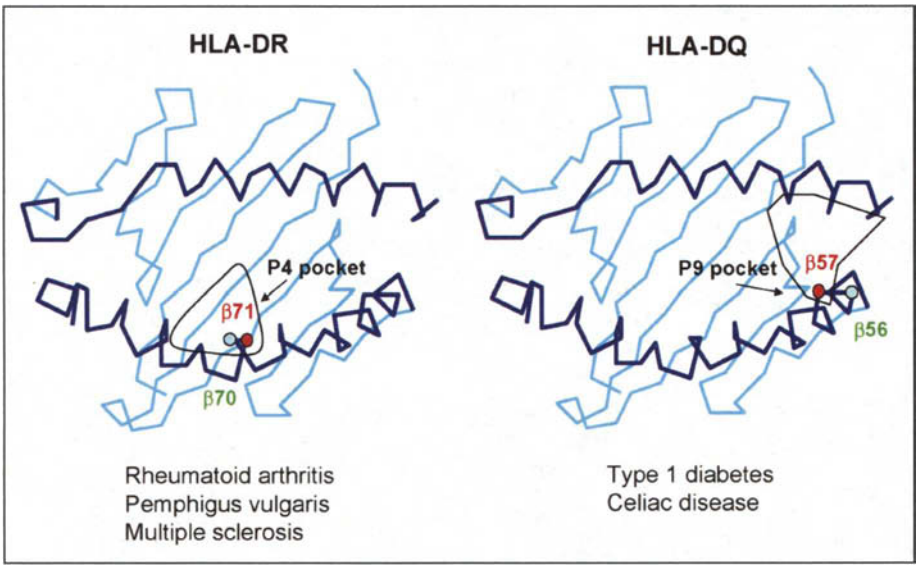

Figure 1. Key polymorphic MHC class II residues in DR and DQ associated human autoimmune diseases. The polymorphic DR β70 and β71 residues are important in DR associated autoimmune diseases and determine the shape and charge of the P4 pocket of the binding site. In the rheumatoid arthritis associated DR alleles (DRB1*0401, DRB1*0404 and DRB1*0101), β71 carries a positive charge (lysine or arginine). In contrast, both β70 and β71 are negatively charged in the pemphigus vulgaris (PV) associated DR allele (DRB1*0402). PV is an antibody-mediated autoimmune disease of the skin and the PV-associated DR4 subtype differs from a rheumatoid arthritis-associated DR4 subtype at only three positions in the binding site (DR β67, β70 and β71). In the multiple sclerosis associated DRB1*1501 molecule, β71 is a small, uncharged amino acid (alanine), resulting in a P4 pocket that is large and hydrophobic. The β57 polymorphism is critical in DQ associated autoimmune diseases. Susceptibility to type 1 diabetes is most closely associated with the DQB gene, and position β57 is not charged (an alanine) in the disease associated DQ8 and DQ2 molecules. In contrast, an aspartic acid residue is present at position β57 in the DQ molecules that either confer dominant protection from type 1 diabetes or are not associated with susceptibility to the disease. DQ2 and DQ8 also confer susceptibility to celiac disease, an inflammatory disease of the small intestine caused by dietary proteins, in particular wheat gliadins.

peptides are bound by a given MHC class II molecule.[3-6] Two modes of interaction permit high affinity binding of peptides: a sequence-independent mode based on formation of hydrogen bonds between the backbone of the peptide and conserved residues of the MHC class II binding site, and sequence-dependent interactions in which peptide side chains occupy defined pockets of the binding site.[1,2] Since peptides of different length can be bound by MHC molecules, the peptide residue that occupies the first pocket is referred to as the P1 anchor. Peptides are bound to MHC class II molecules in an extended conformation and five peptide side chains (P1, P4, P6, P7 and P9) in the core nine-amino acid segment can occupy pockets of the binding site.[2]

Structural Properties of HLA-DR Molecules Associated with Human Autoimmune Diseases

Structural and functional studies on DR molecules that confer susceptibility to rheumatoid arthritis (RA), pemphigus vulgaris (PV) and multiple sclerosis (MS) have identified features of the peptide binding site that are important for the binding of peptides from self-antigens. Particularly relevant are the polymorphic residues that shape the P4 pocket located in the center of the binding groove.

Susceptibility to rheumatoid arthritis is associated with the 'shared epitope', a segment of the DRβ chain helix (β67-74) that is very similar in sequence among disease-associated DR4 (DRB1*0401 and 0404) and DR1 (DRB1*0101) molecules.[7] In structural terms, this 'shared epitope' primarily defines the shape and charge of the P4 pocket.[8] The P4 pocket has a positive charge in the RA-associated DR1 and DR4 subtypes, due to the presence of a basic residue (lysine or arginine) at position β71 and the absence of an acidic residue at the other polymorphic residues that contribute to this pocket. In contrast, DR4 subtypes that do not confer susceptibility to RA carry a negative charge at positions β70 and β71 (DRB1*0402) or β74 (DRB1*0403, DRB1*0406, DRB1*0407) in the P4 pocket. Peptide binding studies have demonstrated that the RA-associated DR4 subtypes have a preference for negatively charged or small peptide side chains in the P4 pocket and that the β71 polymorphism is particularly important in determining binding specificity.[9]

Interestingly, susceptibility to pemphigus vulgaris is associated with a DR4 subtype (DRB1*0402) in which acidic residues are present at both β70 and β71 of the P4 pocket, resulting in a pocket with a negative charge.[10] PV is an autoimmune disease of the skin induced by autoantibodies against desmoglein-3, a keratinocyte surface protein, and these autoantibodies interfere with the interaction among keratinocytes and thus induce the formation of blisters in the skin and mucous membranes.[11] The PV-associated DR4 subtype is rare in the general population and differs from the RA-associated DRB1*0404 subtype only at three positions of the peptide binding site.[12] Two of these polymorphic residues (β70 and β71) are located in the P4 pocket and determine which peptides from the desmoglein-3 autoantigen can be presented to CD4 T cells. We have identified a peptide from human desmoglein-3 that is presented by the PV-associated DR4 subtype, but not other DR4 subtypes, to T cell clones isolated from patients with the disease. Presentation of this peptide was abrogated by mutation of residues β70 and β71, but not by mutation of β67, indicating that the polymorphic residues of the P4 pocket are critical. A second desmoglein-3 peptide that was also presented by the PV-associated DR4 molecule was identified using the same approach.[10] These data indicate that polymorphic MHC class II residues localized to one particular pocket of the DR binding site represent a key feature of MHC-linked susceptibility in a human autoimmune disease.

Susceptibility to multiple sclerosis (MS) is associated with the DR2 (DRB1*1501) haplotype. This MHC class II haplotype carries two functional DRβ chain genes (DRB1*1501 and DRB5*0101) and two different DR dimers can thus be formed by pairing with the nonpolymorphic DRα chain.[13] The structure of the DRB1*1501 molecule was determined with a bound peptide from human myelin basic protein (MBP) that is recognized by T cell clones isolated from patients with MS and normal donors.[14] Biochemical studies had demonstrated that two hydrophobic anchor residues (valine at P1 and phenylalanine at P4) were critical for high affinity binding.[15] A large, primarily hydrophobic P4 pocket was found to be a prominent feature of the DRB1*1501 peptide binding site. This pocket was occupied by a phenylalanine of the MBP peptide which made an important contribution to the binding of the MBP peptide to this MHC class II molecule. The presence of a small, uncharged residue (alanine) at the polymorphic DRβ71 position created the necessary room for the binding of a large hydrophobic side chain in the P4 pocket. The binding of aromatic side chains by the P4 pocket of DRB1*1501 is also facilitated by two aromatic residues of the P4 pocket (β26 Phe and β78 Tyr, of which β26 is polymorphic).[14] An alanine at β71 is relatively rare among DRB1 alleles since most alleles encode lysine, arginine or glutamic acid at this position.

These structural studies demonstrate that the polymorphic residues that shape the P4 pocket of the peptide binding site can be important determinants in DR associated human autoimmune diseases. Other polymorphic residues also contribute to the peptide binding specificities of these MHC class II molecules, but these key polymorphisms drastically change the repertoire of peptides that can be presented. The P4 pocket is the most polymorphic pocket of the DR binding site and the DR molecules associated with susceptibility to RA, PV and MS differ substantially in the shape and charge of the P4 pocket: the pocket carries a positive charge in the RA-associated DR1 and DR4 subtypes, a negative charge in the PV-associated DR subtype and is large and hydrophobic in the MS-associated DR2 (DRB1*1501) molecule.

Structure and Function of HLA-DQ Molecules That Confer Susceptibility to Type 1 Diabetes and Celiac Disease

Crystal Structure of HLA-DQ8 with a Bound Peptide from Human Insulin

The MHC region is the most important susceptibility locus for type 1 diabetes (*IDDM1*) and accounts for an estimated 42% to the familial clustering of the disease. By comparison, the contribution of other loci to familial clustering is relatively small, with an estimated 10% for *IDDM2* (insulin gene) and an even smaller fraction for other candidate loci.[16] Susceptibility is most closely associated with the DQB gene in the MHC class II region, based on linkage studies in families and association studies in patient and control groups.[17,18] The two alleles of the DQB gene that confer the highest risk for type 1 diabetes – DQB1*0201 and DQB1*0302 – encode the β chains of the DQ2 (DQA1*0501, DQB1*0201) and DQ8 (DQB1*0301, DQB1*0302) heterodimers. The risk for type 1 diabetes is greatly increased in individuals who are homozygous for these DQB genes and therefore express DQ8/DQ8 or DQ2/DQ2, and is even higher in subjects who are heterozygous and coexpress DQ8 and DQ2.[17,18] Analysis of MHC genes in different populations has demonstrated that these alleles of the DQB gene confer susceptibility in different ethnic groups, including Caucasians, Blacks and Chinese, providing further support for the hypothesis that the DQB gene rather than a closely linked gene is critical. A notable exception is Japan where the frequency of type 1 diabetes and these particular DQB alleles is relatively low, and where a different allele of DQB (DQB1*0401) confers susceptibility to the disease.[19,20]

These disease associations are highly specific since DQB alleles that encode proteins which differ at only one or a few polymorphic residues do not confer susceptibility to type 1 diabetes. Susceptibility to type 1 diabetes is strongly associated with the polymorphic DQ β57 residue. DQ molecules associated with susceptibility to type 1 diabetes carry a nonaspartic acid at this position (an alanine in DQ8 and DQ2), while an aspartic acid residue is present at β57 in DQ molecules that confer dominant protection from the disease (such as DQB1*0602) or are not associated with susceptibility to the disease.[17] The same polymorphic position is also critical in the NOD mouse model of the disease since β57 is a serine in I-A^{g7}, rather than an aspartic acid as in most murine I-A molecules.[21]

DQ8 was crystallized with a peptide from human insulin (B chain, res. 9-23) that represents a prominent T cell epitope for islet infiltrating CD4 T cells in NOD mice.[22,23] A T cell response to the insulin B (9-23) peptide has also been documented in patients with recent onset of type 1 diabetes and in prediabetics.[24] 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).[25] The crystal structure demonstrated 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 side chains carry a negative charge (glutamic acid at P1 and P9). A tyrosine residue is bound in the P4 pocket, which is very deep and hydrophobic (Figs. 2 and 3).[23] 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 (Fig. 3). Both DQ8 and DQ2 carry an alanine at β57, rather than an aspartic acid residue which is present in alleles that do not confer susceptibility to type 1 diabetes. In MHC class II molecules with aspartic acid at this position, the P9 pocket is electrostatically neutral since the salt bridge between β57 aspartic acid and α76 arginine neutralizes the basic α76 residue, as shown in Figure 3C for the complex of DR1 and a influenza hemagglutinin peptide.[2] In contrast, the P9 pocket of DQ8 has a positive charge (blue color in Fig. 2), 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 α76 arginine (Fig. 3B).[23] The formation of a salt bridge between the peptide and α76 accounts for the

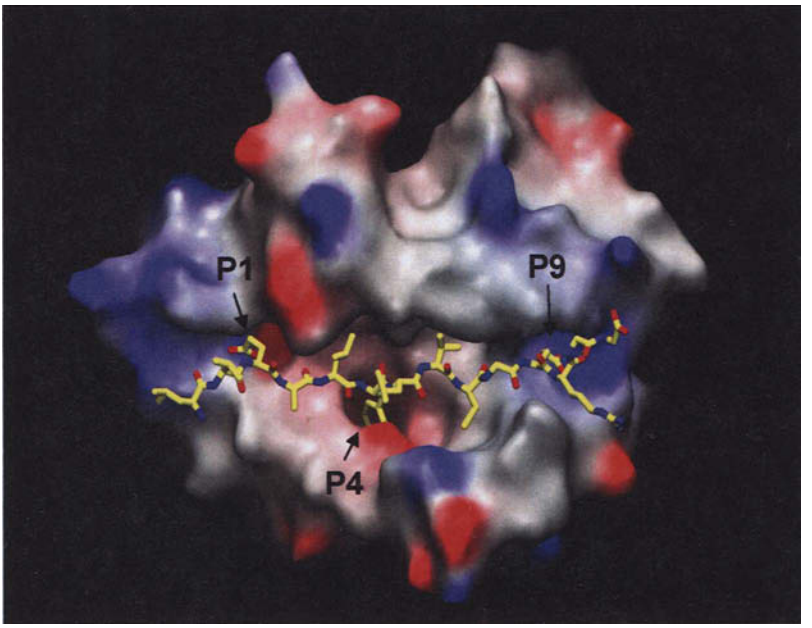

Figure 2. Crystal structure of the type 1 diabetes-associated DQ8 molecule with a bound peptide from human insulin. DQ8 was cocrystallized with the insulin B (9-23) peptide that is recognized by islet infiltrating T cells in NOD mice. An unusual feature of the structure is the presence of two acidic peptide side chains in pockets of the binding site (glutamic acid in both P1 and P9 pockets). The P9 pocket has a positive charge in DQ8 (blue color), due to the absence of a negative charge at β57. The P4 pocket of DQ8 is very deep and occupied by a tyrosine residue of the insulin peptide.

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 for DQ8. However, it is important to note that other residues can also be accommodated in the P9 pocket of DQ8, albeit with a reduced affinity.[25,26] The β57 polymorphism therefore has a drastic impact on the peptide binding specificity of DQ molecules: a preference for acidic peptide side chains is observed when β57 is a nonaspartic acid residue but such acidic side chains 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.[27,28] An important similarity between these structures is that the P9 pocket of both DQ8 and I-A^{g7} is basic. Peptide binding studies demonstrated that the P9 pocket of I-A^{g7} has a preference for negatively charged residues, as observed for DQ8.[29] In the I-A^{g7}/GAD peptide complex, a glutamic acid side chain occupies the P9 pocket and forms hydrogen bonds with α76 arginine and β57 serine (Fig. 3D).[27] Despite these important similarities, most of the polymorphic residues that shape the P9 pocket actually differ between DQ8 and I-A^{g7}, including residues β55-57 (Pro-Pro-Ala in DQ8 and Arg-His-Ser in I-A^{g7}, as shown in Figure 3B and 3D. The difference in the residues that shape the P9 pocket indicates that the alleles of DQB and I-Aβ that confer susceptibility to type 1 diabetes have evolved independently from their DQ and I-A ancestors, respectively, to converge with similar peptide-binding properties that confer some unknown advantage in immune protection that has the unfortunate side-effect of increasing the risk for type 1 diabetes.[23]

Due to the structural similarities, DQ8 and I-A^{g7} can present the same peptides.[25] The majority of peptides that were identified as T cell epitopes of insulin, GAD65 and HSP60 in

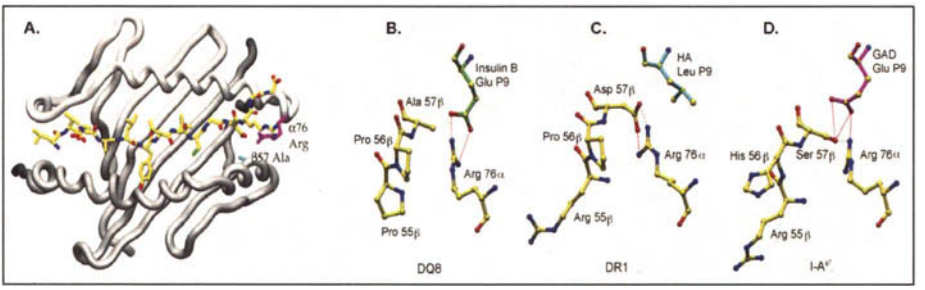

Figure 3. The β57 polymorphism determines the charge of the P9 pocket of the DQ8 peptide binding site. DQ β57 (blue color in Fig. 3A) is located on the helical segment of the DQ β chain and reaches into the P9 pocket of the binding site. Due the absence of a negative charge at this position, the positive charge of arginine 76 of the DQ α chain (α76 Arg, pink color) is not neutralized by formation of a salt bridge. As a result, the P9 pocket of DQ8 has a positive charge and a strong preference for acidic peptide side chains. In the DQ8 structure, a glutamic acid residue from the insulin peptide occupies this pocket and forms a salt bridge with α76 (Fig. 3B). β57 is also a nonaspartic acid residue in the MHC class II molecule (I-A^{g7}) expressed in NOD mice which develop spontaneous type 1 diabetes. Again, the P9 pocket carries a positive charge and has a strong preference for an acidic peptide side chain (glutamic acid in the structure of I-A^{g7} with a bound peptide from GAD65) (Fig. 3D). In contrast, a salt bridge is formed between β57 and α76 when an aspartic acid residue is located at β57. This results in a P9 pocket that is electrostatically neutral, as exemplified here by the structure of DR1 in which a hydrophobic residue of the bound influenza hemagglutinin peptide (leucine) occupies the P9 pocket (Fig. 3C). Reprinted from Nature Immunology with permission from the publisher.[23]

NOD mice also bind to DQ8. 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}.[23,27,28]

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 NOD mice.

Presentation of Deamidated Gliadin Peptides by HLA-DQ8 and HLA-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. 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.[30] Celiac disease is much more prevalent in patients with type 1 diabetes (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 antibody positive patients). 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.[31,32]

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. Patients with celiac

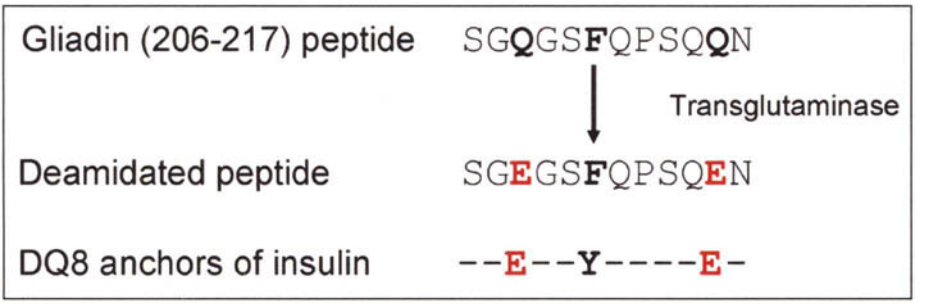

Figure 4. Enzymatic modification of a gliadin peptide creates a DQ8-restricted T cell epitope in celiac disease. Susceptibility to celiac disease, an inflammatory disease of the small intestine, is associated with DQ8 and DQ2. These MHC class II molecules present peptides from dietary proteins (gliadins) to gut-infiltrating T cells, and the T cell epitopes are created by deamidation of glutamine residues of gliadin by transglutaminase. This enzymatic modification converts glutamines to glutamic acid and thus creates the negatively charged anchor residues required for DQ8 and DQ2 binding. Modification of two glutamines in the gliadin (206-217) peptide results in a peptide that has very similar anchor residues to the insulin B (9-23) used for cocrystallization with DQ8: glutamic acid residues at P1 and P9, as well as an aromatic residue (tyrosine versus phenylalanine) at P4. These data thus explain how DQ8 confers susceptibility to two different autoimmune diseases - type 1 diabetes and celiac disease.

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. Gliadins are very rich in glutamine and proline residues, and treatment of gliadin with transglutaminase dramatically increases the stimulatory capacity of the protein for DQ2 and DQ8 restricted T cell clones.[30,33]

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. Mass spec 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 (Fig. 4).[34] 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. 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 (Figs. 2, 4).

Conversion of a single glutamine to glutamic acid (res. 65) is critical for the DQ2 restricted T cell response to gliadin. This gliadin segment (res. 57-75) contains two overlapping T cell epitopes, res. 57-68 and 62-75, centered around residue 65. 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 as well as binding to DQ2. Binding of modified gliadin peptides to DQ8 and DQ2 is thus dependent on enzymatic modifications that create acidic peptide side chain(s).[35]

These studies thus provide a structural explanation for the association of susceptibility to two different autoimmune diseases with DQ8 and DQ2. The β57 polymorphism is critical in disease susceptibility since it permits binding of peptides with acidic side chains in the P9 pocket of the DQ8 binding site. The studies in celiac disease indicate that such epitopes can arise as the result of post-translational modifications. Recent studies have implicated enzymatic modifications of self-antigens in other autoimmune diseases, in particular rheumatoid arthritis. Enzymatic conversion of an arginine to citrulline by peptidyl arginine deiminase removes a positive charge from the arginine head group and thereby drastically alters the electrostatic properties of proteins or peptides. Autoantibodies to citrullinated proteins have