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Generation of MHC class II:peptide ligands for CD4 T cell allorecognition of MHC Class II molecules

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Abstract

Purpose of review—The molecular and cellular mechanisms that underlie allorecognition of MHC class II molecules has been the subject much debate and experimentation in recent decades. In this review, we discuss several aspects of MHC class II structure, peptide acquisition and TcR-MHC:peptide interactions that have particular relevance to recognition of cells bearing allogeneic class II molecules.

Recent findings—First, MHC polymorphism is heavily biased toward those amino acids that influence stable peptide binding by MHC class II. Second, the peptide repertoire presented by class II molecules is highly diverse and can be edited substantially by the molecular catalyst HLA-DM and by tissue-specific expression of HLA-DO, stress and cytokines. Third, T cell receptor docking onto MHC peptide typically involves substantial contacts with the bound peptide in the MHC class II molecule. Finally, there is increasing evidence that T cell recognition of MHC is in part germline-encoded through T cell receptor V region contacts with MHC class II alpha helices.

Summary—Together, these conclusions support the view that allorecognition of MHC class II molecules is likely to parallel key aspects of conventional CD4 T cell recognition, with allele-dependent variation in peptide representation accounting in large part for the high precursor frequency of alloreactive CD4 T cells

Keywords

MHC class II; allorecognition; T cell receptor; MHC:peptide

Introduction

MHC class II molecules (HLA-DR, DQ and DP in the human) play a critical role in allorecognition, by recruiting large numbers of CD4 T cells that specifically recognize the “foreign” MHC class II molecules expressed by the engrafted cells or tissues. Activation of CD4 T cells not only leads to expansion of CD4 effector cells, but also can promote expansion and differentiation of antigen-specific CD8 T cells and B cells. This review will focus on several issues, with particular detail on new advances in the past year. First, where are allelic polymorphisms localized in MHC class II protein relative to what we now know about peptide selection by MHC molecules or T cell receptor binding to MHC molecules?

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Second, if the peptide repertoire presented by class II molecules influences recognition by alloreactive CD4 T cells, what do we know about the source, diversity and intracellular mechanisms that control the array of peptides presented by MHC class II proteins? A final question that is related to the first two is the whether there is any new insight into the mechanisms that explain the apparently high precursor frequency of cells that are reactive with allogeneic MHC molecules. In this review, we summarize recent advances that help form a conceptual framework to understand CD4 T cell recognition of alloantigens.

MHC class II structure and peptide acquisition

MHC class II proteins are heterodimers, denoted α and β chains, with each chain consisting of two extracellular domains and short cytoplasmic and transmembrane domains. The membrane-distal domains contact the T cell receptor and membrane-proximal domains of class II interact with the CD4 co-receptor. MHC class II α and β chains assemble with a third non-MHC encoded glycoprotein chain, the invariant chain, early during biosynthesis in the endoplasmic reticulum. Later in biosynthesis, invariant chain enhances localization of class II molecules in endosomes/lysosomal compartments, the site of peptide acquisition, where it is eventually degraded by endosomal proteases, leaving a small remnant, termed CLIP, within the antigen-binding groove of class II. Also in endosomal compartments, peptide fragments of either foreign or self antigens are generated from proteolysis and become available for MHC class II binding. The most important concept to emerge in recent years regarding peptide loading by MHC class II molecules is that it is a catalytic exchange reaction. In endosomes, CLIP is replaced by antigenic peptides, a reaction that is catalyzed by an MHC-encoded protein "DM" (HLA-DM or H-2M in the mouse). DM, a class II-like heterodimeric molecule, binds to class II molecules in the low pH of the endosomes and promotes both peptide release and peptide binding (Figure 1). In acidic endosomes, DM and class II interactions are initiated, leading to catalytic release of CLIP from class II molecules and subsequent loading of antigenic peptide. Current models for DM suggest that binding to class II molecules stabilizes an "open" conformation of the peptide-binding pocket of class II that allows the exchange reaction to rapidly occur.

Although in early studies, DM was observed to enhance antigen presentation [1–6], consistent with its ability to release CLIP and make more class II molecules available for peptide acquisition [7,8], later studies led to a more complex view. Most striking was that a high proportion of epitopes recognized by alloreactive or self-peptide specific CD4 T cells were extinguished by DM expression [9,10]. These studies and similar early work [11,12] indicated that DM functions as a "peptide editor" that could either increase or decrease expression of peptide:MHC class II complexes. That DM editing has important immunological implications was made clear by antigen presentation studies indicating that for both protective responses to pathogens and self tolerance induction, the process of "DM editing" dramatically diminishes the expression of particular MHC class II:peptide complexes on the surface of an antigen presenting cell (APC) as it concurrently promotes expression of others (reviewed in [13,14]). Accordingly, class II molecules that ultimately are exported to the cell surface of APC and available for CD4 T cell recognition typically display only a fraction of the foreign or self antigen-derived peptides that have the potential to bind to those class II molecules.

There has been significant research towards identifying characteristics of the class II:peptide complex that determine the susceptibility to DM editing. Many studies with purified class II and DM proteins and more recent functional studies, suggest that the most salient feature that predicts susceptibility to DM editing is the kinetic stability of the class II:peptide complex, where stable peptides are resistant and unstable peptides are susceptible to removal by DM during endosomal loading (reviewed in [13,15]). Thus, selective DM editing shapes

the repertoire of peptides presented to enrich for a subset that has a characteristically stable expression with the presenting class II molecule.

For consideration of the issues important to transplantation, it is perhaps most relevant to consider the possibility first, that allorecognition by CD4 T cells depends the particular peptide(s) presented by class II molecules (discussed below), and second, that DM-mediated editing of the peptide repertoire presented by class II molecules is likely to be uneven in different cell types, both those within the hematopoietic system and beyond it. The potency of DM editing in selecting the peptide repertoire presented by class II will be determined by at least three factors: the levels of DM expressed within the class II positive cells, the kinetics of co-localization of DM with class II molecules in endosomes and the expression of HLA-DO, another MHC encoded protein that is thought to antagonize or “mute” the potency of DM (see Figure 1). This class II-like protein is expressed in a large proportion of B cells, and its expression is regulated during B cell activation and differentiation [16,17]. Several groups have shown that DO can modulate the function of DM by decreasing its ability to exchange peptides, particularly at early endosomal pH (reviewed in [18]). It is speculated that the expression of DO in B cells prevents class II peptide loading in early endosomal compartments and that it may dissociate from DM in later endosomal compartments where the BCR-conjugated antigen becomes available. DO has also been found to be expressed in some subsets of dendritic cells [19], but not in macrophages or other types of class II/DM positive antigen presenting cells. DO has a stable interaction with the DM heterodimer and both molecules have endosomal sorting signals in their cytoplasmic tails. Therefore, quantitative or tissue-specific patterns in the expression of the DO heterodimer may change the location of DM in endosomal compartments and thus the consequences of its peptide editing function. These differences in the expression of HLA-DM and HLA-DO can thus change the array of peptides that are displayed with class II molecules at the cell surface of different cell types and therefore can control tissue specific recognition of alloantigens.

Peptides constitutively presented by the MHC class II molecule

A major advance in our understanding of peptide acquisition and presentation by MHC class II molecules has been through the development of highly sophisticated and sensitive biochemical methods to characterize the complex mixture of peptides that occupy the peptide binding pocket of MHC class II molecules. The earliest and most surprising results from the analyses of peptides eluted from class II MHC molecules is that the diversity of distinct peptides presented by class II can number in the thousands, of which a significant fraction is derived from internally synthesized proteins [20,21]. Depending on the MHC class II allele, peptides derived from internally synthesized antigens constitute from 70–90% of the peptides sequenced. These results point out that although class II molecules are indeed specialized to present antigens from an exogenous source, in the absence of pathogenic challenge, most of the peptides displayed on the cell surface of an APC likely represent the array of proteins synthesized within that APC. The quantity of peptide recovered from class II molecules is close to equimolar to the MHC class II molecule itself, suggesting that the vast majority of MHC molecules are bound by autologous peptides rather than being “empty”. This finding is in agreement other studies that have analyzed the fate of class II molecules that are devoid of peptide [22] and through the use of antibodies that selectively react with unoccupied class II molecules [23].

Perhaps one of the most intriguing findings derived from sequencing of MHC class II-bound peptides is the finding an extraordinarily high percentage of source proteins are MHC molecules themselves [20,21]. Depending on the allele, MHC-related peptides can constitute from 10–60% of the total. The prevalence of genetically polymorphic peptides within the

peptide-binding site therefore increases the diversity of the expressed MHC molecules. Thus, a given MHC molecule that is expressed on cells of divergent MHC backgrounds is essentially unique, due to the presence of divergent MHC-derived peptides within its peptide-binding site. This model has particular relevance when one considers the roles of MHC polymorphism and of peptide in allorecognition. Also noteworthy is that a significant fraction of peptides of the peptides presented by class II is derived from proteins that localize to the cytosol. Recent data suggest that the process of autophagy delivers most cytosolic antigens to the MHC class II loading compartment [24, 25–33] can mediate uptake of cytosolic materials into the class II loading compartment by several distinct intracellular pathways, and may be upregulated during virus infection [25], cellular stress [34] or under the influence of cytokines [35–37]. Thus, under inflammatory conditions, the repertoire of peptides presented by class II can change.

Degree and localization of polymorphic residues in the MHC class II molecule

The most striking feature of MHC molecules is their allelic diversity [38,39]. The HLA-DRB1 chain locus alone bears over 1000 alleles. While the population and molecular forces driving MHC to become so polymorphic are not completely resolved, it is clear that allelic variation has a profound influence on the repertoire of peptides presented by the MHC molecules. Successful crystallographic studies of MHC molecules with bound peptides, as well as increasing numbers of sequenced allelic variants of class II have shown that the vast majority polymorphism is localized to those amino acid residues that line the peptide binding groove (Figures 2 and 3). Thus, allelic polymorphism shapes the repertoire of peptides presented by class II. At a population level, having diverse peptides presented from circulating pathogenic organisms will help to ensure that at least a fraction of individuals generate a durable and ultimately protective immune response.

In the context of allorecognition, these structural and genetic studies make it reasonable to conclude that much of alloreactivity toward MHC class II molecules may be secondary to allelic polymorphisms that influence the peptide repertoire displayed by MHC on the surface of cells. Also following from our increasing awareness that most allelic polymorphisms are sequestered in the peptide binding groove of class II is the concept that many class II molecules may offer very similar molecular landscapes in the alpha helical regions of the peptide binding pocket that are available for T cell receptor (TcR) binding (Figure 3).

The molecular features of MHC class II protein-T cell receptor interactions

Central to understanding both the molecular mechanisms and consequences of allorecognition is knowledge of the structural elements within the MHC and T cell receptor proteins that control binding, signaling and activation of alloreactive T cells. In the simplest sense, one can ask the question: what explains the high frequency of interactions between host T cells and cells bearing allogeneic MHC molecules? Is the recognition an extension of the molecular constraints involved in T cell receptor recognition of foreign peptides presented by host MHC molecules or is fundamentally distinct from this recognition event, perhaps involving unique contact residues on both the MHC and T cell receptor proteins? In the first case, one would expect the sequence of the bound peptide to play an important role in recognition, as would the contact residues on the MHC that influence T cell receptor and peptide binding. In the second case, peptide might be largely ignored, with recognition by the TcR instead conveyed by the MHC molecule itself, perhaps in a way analogous to superantigen binding to MHC proteins. This issue has been debated for decades (reviewed in [40]), but some clarification has come from the steadily accumulating data and insight

derived from co-crystallization of T cell receptors and MHC:peptide ligands (reviewed in [41]).

It is now clear for both allorecognition and recognition of foreign peptides presented by host MHC class II molecules that the T cell receptor docks onto the MHC protein in a relatively fixed orientation (Figure 4). In general, the docking mode of the TcR is diagonally oriented along the long axis of MHC-peptide, with the variable region of the TcR α chain oriented at the amino terminus of the peptide and MHC class II β chain and the TcR β chain oriented over the carboxyterminal segment of the peptide and the MHC class II α chain. The six complementarity determining regions (CDR) of the TcR have rather predictable contacts, with the CDR1 and CDR2 from the alpha chain primarily contacting the MHC class II β chain and the CDR1 and CDR2 from the TcR β chain making their major contacts with the MHC class II alpha chain helical regions. The most highly variable CDR3 regions of the beta and alpha chains of the TcR are oriented largely over the bound peptide. Although there is considerable flexibility and adjustments possible to allow the docked TcR to bind with sufficient affinity to peptide MHC for binding and signaling, and a certain amount of “induced” fit that allows the TcR to adjust its own structure to make optimal contacts with its peptide MHC ligand ([42**, 43**, 45–46], the basic docking orientation has been surprisingly consistent among many co-crystals studied thus far.

The strikingly similar docking mode of the T cell receptor onto MHC molecules has answered one question related to allorecognition, which is whether the peptide bound to the MHC molecule influences allorecognition. Many published studies have suggested that this is so, but the issue has remained controversial. With the accumulating evidence that T cell receptors, both alloreactive and host-MHC restricted, dock in a fairly fixed orientation with extensive contacts with the peptide made through sequences in the CDR3 regions of the TcR, coupled with the data suggesting that very few MHC class II molecules exist in a peptide-free form at the cell surface, it is now clear that peptide cannot be ignored by alloreactive T cells. Peptides bound to MHC class II molecules that are the target of CD4 T cell alloreactivity (and class I molecules for alloreactive CD8 T cells) undoubtedly will be influenced by the solvent exposed residues in the bound peptide. Therefore, peptide selection during endosomal loading, and peptide diversity presented by MHC class II will influence alloreactivity. The degree of specificity of alloreactive T cells for the particular peptide bound to the MHC is still controversial, but on balance, there is no reason to suspect that in general, allorecognition will be any less peptide specific than that of conventional T cells. Polymorphisms in MHC (See Figures 2 and 3) are largely clustered within the regions of the MHC class II molecule that can influence stable peptide acquisition. Therefore, different MHC alleles will present different subsets of self-peptides. Importantly, this new set of self peptides will have not been recognized as “self” during central tolerance induction in the thymus and the host CD4 T cells will consider them “foreign”.

Discovery of a consistent orientation between the T cell receptor and MHC proteins has brought to the surface an issue originally suggested by Jerne, in considering the nature of self-non-self discrimination by the immune system [47], that has been the subject of much thought, experimentation and debate once MHC-restricted recognition of antigen by T cells was discovered. The question is this: Is MHC reactivity encoded in the germline of T cell receptors or is MHC restricted recognition a product of random receptor structures stringently selected to be self MHC reactive. If the first case is true, then allorecognition simply follows from a T cell receptor repertoire genetically biased to MHC that has been pruned to eliminate a “too high” affinity for self, whether through MHC contacts, conferred mostly by the CDR1 and CDR2 regions of the T cell receptors or through peptide contacts, largely determined by CDR3 regions. Many investigators have used very creative approaches to address the question of a germ-line MHC bias within the T cell receptor V

regions. This subject has been thoughtfully and comprehensively reviewed recently [40,48,49].

Several recent studies have used approaches that eliminate confounding issues that have made assessment of germline encoded MHC biases in the T cell receptor repertoire. The first confounding issue is the toll that negative selection takes on TcR specificity. Because T cell specific with strong affinity for self will be deleted during central tolerance, the mature T cell repertoire may have already lost many characteristics of the germline-encoded repertoire. Studies where the impact of negative selection during T cell development have been diminished through imaginative experimental designs have revealed a strikingly high frequency of alloreactivity and reactivity to self MHC [50–52], estimated to be 100-fold to 1000-fold higher than reactivity to any one antigenic peptide:MHC complex [40]. The second issue that has hindered attempts to discern an MHC bias within the germline of the T cell receptor V regions is that co-crystallization of TcR with MHC:peptide ligands is technically very challenging and accordingly the number of co-crystals are limited and typically involve distinct TcR:MHC pairs, with preference for those that may be rather unique in their interactions, such as those belonging to autoreactive T cells (reviewed in [53]). Therefore, until recently, there have been few comparisons available among TcR with the same V regions, to evaluate if they engage MHC with similar or identical contact segments. However, in a recent study, Garcia and colleagues [54**] compared structures of four distinct V β 8.2 containing co-crystals, three of which recognized I-A^u and one of which recognized an alternate allele of murine class II molecules (I-A^k). The most striking observation made was that in all the complexes, the V β CDR1 and CDR2 interacted with a fixed constellation of class II alpha chain residues. It was thus suggested that this interaction represents one example of a germline-derived TcR-MHC “codon”. Impressive data has since been accumulating [55–57**] that supports the view that a significant proportion of the MHC bias in recognition by the T cell receptor repertoire is due to germline-encoded TCR contacts with the MHC molecules. As more structures are solved, it is quite likely that additional cohorts of TcR V region segments will be discovered to have predictable contacts with discreet regions of the MHC proteins, thus explaining the collective bias of the TcR repertoire reactive with MHC. Because most of the MHC residues that are solvent exposed and available to form these “codons” of recognition are genetically conserved or have only conservative amino acid substitutions (see Figure 2), it is likely that polymorphism in MHC does not provide a barrier to recognition of host MHC and thus does not require any special accommodations to be recognized by host, MHC-restricted T cell receptors.

Conclusions

The structural and cellular elements that underlie allorecognition of MHC class II molecules by CD4 T cells have remained controversial for decades. In this review, we focus on recent advances in MHC class II genetics and structure, peptide acquisition and TcR-MHC:peptide interactions. Together, these advances suggest the possibility that allogeneic MHC class II molecules present a similar molecular landscape as syngeneic MHC that promotes recognition by host CD4 T cells, but an alternate panel of “self” peptides, that can be regarded as “foreign” by the host. Therefore, CD4 T cell alloreactivity may be due to interactions with allogeneic MHC have many parallels with MHC-restricted recognition of syngeneic MHC and pathogen or tumor-derived antigenic peptides.

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Figure 1. MHC peptide represents the end product of an exchange reaction and editing function mediated by HLA-DM

Shown is a representation of late endosomal compartments (in turquoise) where antigen fragments, MHC class II:CLIP and HLA-DM and/or HLA-DO localize. CLIP is the peptide remnant of invariant chain that typically remains within the peptide-binding pocket of class II after proteolytic degradation of the invariant chain protein. HLA-DM binds to class II at endosomal pH at the peptides amino terminus and is thought to promote a conformational change that allows CLIP release and replacement with peptide fragments of self or foreign antigens. During this process, there is selection of peptides that bind to class II with high kinetic stability. HLA-DO can bind to HLA-DM and modulate its catalytic and editing activity in endosomal compartments.

Figure 2. TcR contact residues and frequency of amino acid substitutions within the HLA HLA-DR1

Shown here as a representative structure is HLA-DR1 (PDB ID: 1FYT)^d with polymorphic residues illustrated in varying colors based on the frequency of substitutions between alleles. The alpha chain (residues 2–81) is shown in light blue, the beta chain (residues 7–92) in dark blue, and bound peptide in magenta with the amino and carboxytermini labeled. Residues that are observed in crystal structures to contact TcR CDR1 and CDR2 are solid or hatched white. Substitution frequencies of 3–10% are shown in green, 11–40% in yellow, and >40% in red. Blue (or solid white for TcR contacts) denotes conserved residues. Note that HLA-DRA1 is highly conserved; none of the TcR contact residues are polymorphic. The TcR contact residues of the HLA-DRB have substitution rates of >40% at residue 70, >25% at residues 60, 73, 77, and 85; all other residues are conserved. HLA-DR1 structure was obtained from PDB [58,59] (<http://www.pdb.org/>) and drawn with Swiss-Pdb Viewer (DeepView v4.0) (<http://www.expasy.org/spdbv/>). The HLA-DR1 structure was obtained from PDB [58,59] (<http://www.pdb.org/>) and drawn with Swiss-Pdb Viewer [60] (DeepView v4.0) (<http://www.expasy.org/spdbv/>).

Figure 3. Frequencies and localization of allelic amino acid substitutions within HLA-DQ
 The HLA-DQ alpha chain (residues 2–81) and beta chain (residues 5–94) are colored light and dark blue respectively, and bound peptide in magenta with the amino and carboxytermini labeled. Shown as a representative structure is HLA-DQ8 (PDB ID: 2NNA)^G with polymorphic residues illustrated in varying colors based on the frequency of amino acid substitution between the alleles: frequencies 3–10% green, 11–40% yellow, >40% red, and blue denotes conserved residues. The HLA-DQ8 structure was obtained from PDB [58,59] (<http://www.pdb.org/>) and drawn with Swiss-Pdb Viewer [60] (DeepView v4.0) (<http://www.expasy.org/spdbv/>).

Figure 4. TcR “footprint” on MHC class II

A representative structure of MHC class II, HLA-DR1 (PDB ID:1FYT) [61], is shown to illustrate the area of the MHC class II surface that interacts with the CDRs of the T cell receptor (TcR) predicted by co-crystallization studies (reviewed in [41]). The peptides amino and carboxytermini are indicated. The area of HLA-DR1 that is predicted to be in contact with TcR is represented by the shaded area within the oval, with the approximate site of TcR CDR loops labeled. Adjustments in binding orientation of the TcR (as depicted by the yellow arrows) have been seen in alternative co-crystals of MHC and TcR.