Trends in Immunology

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Series: The Biology of Antigen Presentation

Feature Review

Present Yourself! By MHC Class I and MHC Class II Molecules

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Since the discovery of MHC molecules, it has taken 40 years to arrive at a coherent picture of how MHC class I and MHC class II molecules really work. This is a story of the proteases and MHC-like chaperones that support the MHC class I and II molecules in presenting peptides to the immune system. We now understand that the MHC system shapes both the repertoire of presented peptides and the subsequent T cell response, with important implications ranging from transplant rejection to tumor immunotherapies. Here we present an illustrated review of the ins and outs of MHC class I and MHC class II antigen presentation.

Why Do We Need to Present Antigens?

T cells help eliminate pathogens present in infected cells and also help B cells make better and different kinds of antibodies to protect against extracellular microbes and toxic molecules. To accomplish these important functions, T cells have to interact intimately with other cells and then find and instruct or eliminate the ones that are harboring or have been exposed to these pathogenic threats. However, T cells are unable to peek beneath the surface of cells to identify ones that have ingested bacteria or are synthesizing viral or mutant proteins. Instead, antigen presentation systems have evolved that display on the cell surface information about the various antigens that cells are synthesizing or have ingested. These antigen presentation pathways monitor the major subcellular compartments where pathogens could be lurking and report their findings to the appropriate kinds of T cells. Endogenously synthesized antigens in the cytosol of all cells are presented to $CD8^+$ T cells as peptides bound to MHC I molecules, thereby allowing the $CD8^+$ lymphocytes to identify and eliminate virally infected cells or cancers. Antigens ingested into endocytic compartments of macrophages, dendritic cells, or B cells are presented to $CD4^+$ T cells as peptides bound to MHC II molecules. T cells have antigen receptors that recognize antigenic peptide, but only in the context of MHC I or MHC II molecules that are displaying the antigen on the cell surface. Consequently, T cells are directed to work with cells while not being 'distracted' by free antigen, against which they would be unable to do anything. Moreover, the pattern of expression of MHC I and II molecules directs T cells to interact with exactly the right kind of cells. Here we review the current understanding of the mechanisms of antigen presentation as well as their implications in health and disease. The studies discussed here have paved the way for the increasingly refined analyses of the biology of antigen presentation – in various physiological and clinical contexts, cells, and organs – that are the focus of this special issue.

The MHC Scaffold, or How Did MHC I and MHC II Molecules Evolve?

MHC I and II molecules present protein fragments to $CD8^+$ and $CD4^+$ T cells, respectively. These molecules are essential for cell-mediated immunity and therefore appeared at the inception of the adaptive immune system 500 million years ago [1]. For their construction they used two

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MHC molecules are critical in transplantation, autoimmunity, infections, and tumor immunotherapy.

The biology of antigen presentation by MHC I and MHC II molecules provides targets for manipulation of these diseases.

This biology also explains the fragments presented to the immune system and the cellular evolutionary race to escape immune control of infected and transformed cells.

Many of the players determining antigen degradation and subsequent peptide loading on MHC molecules are defined and are helping to improve the accuracy of predicting presented fragments.

The combined understanding of antigen presentation by MHC molecules allows exploitation to improve the responses of the cellular arm of the immune system to vaccinations and immunotherapies.

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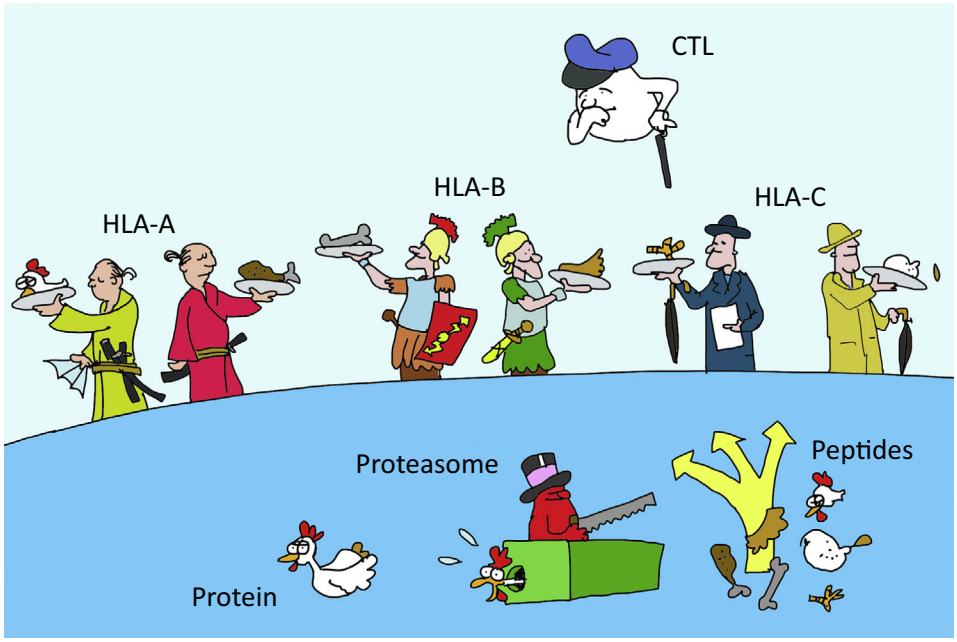

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Figure 1. MHC Locus and Allelic Products with Polymorphism. Most mammalian species express three different MHC I and three different MHC II molecules (shown here for the human MHC I HLA-A, HLA-B, and HLA-C locus products). Since these are polymorphic and genetically encoded, between three and six (depending on differences between the inherited genes) alleles will be expressed on cells. These are polymorphic in the peptide-binding groove region of MHC molecules to present different peptides of a defined antigen to a $CD8^+$ T cell (CTL).

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immunoglobulin domains topped by two parallel alpha helixes resting on a platform of beta-pleated sheets. This capital structure generated a peptide-binding groove between the alpha helices [2,3] that is, 'evolutionarily speaking', likely to have been borrowed from earlier chaperone structures. There are arguments for this: (i) chaperones bind unfolded stretches of proteins and the prototypic unfolded structure is a peptide; (ii) various phylogenetically older chaperones have a somewhat similar structure [4]; and (iii) there are various MHC molecule 'look alikes' that act as specific chaperones in the process of MHC-restricted antigen presentation. These include tapasin [5], tapasin-binding protein related (TAPBPR) [6,7], DM [8,9], and DO [10], as we discuss below. However, MHC I and MHC II molecules are unique in the proteome because of their extreme polymorphism (>10 000 different alleles of MHC I molecules have been identified thus far). This has interesting consequences. Polymorphic residues on the top alpha helices interact with the T cell receptor (TCR) and are the basis for the specificity of TCRs for an antigen peptide plus a particular allelic form of an MHC molecule (a phenomenon called MHC restriction). Polymorphic residues in the MHC peptide-binding groove change the nature and location of so-called pockets. These variable pockets are filled by complementary variable amino acid side chains of peptides (so-called anchor residues), with the effect that different fragments from a defined antigen are presented by different polymorphic MHC molecules [11,12] (Figure 1). Next to the anchor residues, most other amino acids in a peptide fill a free space and can be (almost) any of the 20 amino acids [13,14]. By having pockets with specificity for only a few side chains and allowing the remaining six to ten amino acids to vary between all possibilities, each kind of MHC molecule can present a very large repertoire of peptides. Moreover, by having three to six different MHC I as well as three to 12 different MHC II molecules (the exact number depending on how many different MHC alleles were inherited from one's parents and how the MHC II subunits are paired), cells can present a large fraction of the universe of peptides, although not all sequences. In theory, then, MHC I molecules can present a peptidome of around $6 \times 20^{6-7}$

different peptides and MHC II can display up to 12×20^{10}. In actuality, such a large array of peptides cannot all be presented because there are only around 200 000 MHC I and 20 000 MHC II molecules on cells such as B and T cells [15]. Moreover, since some peptides are present in high numbers (from highly expressed proteins), the real number of different peptides presented by one cell is likely to be less than 10 000. Importantly, when a pathogen alters a critical anchor residue in one of its antigenic epitopes, it may prevent presentation of this antigen in one individual but not in another with different MHC molecules that will simply select different peptides from the same pathogen [16]. Therefore, MHC polymorphism is good for the survival of the population and not necessarily the individual. How is this extensive polymorphism maintained in the species? One possibility is based on evidence that females can distinguish by smell MHC allele differences in males and prefer as mates individuals with whom they do not share MHC alleles. The consequence of such a preference would be to promote maximal expression of polymorphic MHC alleles in offspring and the maintenance of diverse alleles in the population [17]. In the human species, perfumes may mask the natural scent of this basis of partner choice, with unknown effects. The obvious modern disadvantage of MHC polymorphism is transplant rejection, but even this may serve a useful function in nature by preventing the seeding of cancer cells between individuals. This is illustrated by the fact that MHC-deficient oral cancers are currently being transferred between Tasmanian devils through bites and decimating the population of these animals in the wild [18,19].

How to Present Your Inner Self? MHC Class I Molecules

MHC I molecules present peptides from the proteins that are synthesized by cells. In healthy cells, all of these proteins are autologous ones to which $CD8^+$ T cells are tolerant. However, when cells are expressing mutant sequences (e.g., in cancers), microbial genes (e.g., from viral infections), or foreign polymorphic genes (e.g., from transplants), these 'non-self' antigenic peptides are included in the presented peptidome, allowing $CD8^+$ T cells to detect and destroy these abnormal cells. How MHC I manages to present a blueprint of the intracellular proteome has been established over the past 25 years [20] (Figure 2). Briefly, normal and abnormal proteins are degraded by the proteasome into peptide fragments [21,22]. These fragments are further trimmed and to a large extent destroyed by cytosolic peptidases [23] but some survive by escaping into the endoplasmic reticulum (ER) through a peptide transporter called transporter associated with antigen processing (TAP) that is embedded in the ER membrane [20]. In the ER, TAP forms the center of a peptide-loading complex that includes a dedicated chaperone, tapasin, empty MHC I molecules awaiting peptides, and two common chaperones, calreticulin and the protein disulfide isomerase ERp57 [24]. TAP translocates peptides that then are considered for binding by MHC I molecules. These empty MHC I molecules are held in a peptide-receptive state by the chaperone tapasin and tapasin also promotes MHC I binding of peptides with a slow off rate, thereby helping to shape the repertoire of presented peptides [25]. In this reaction, MHC I molecules test the binding of many peptides and subsequently release most of these until a proper (low-off-rate) peptide is bound [26]. These are usually peptides of a very specific length of eight to ten amino acids with appropriate anchor residues. Peptides that are too long can be trimmed by an ER-resident aminopeptidase, ERAP1 (and ERAP 2 in some species) [27–29], before consideration by MHC I molecules that are either in the peptide-loading complex or associating with another tapasin look-alike chaperone in the ER called TAPBPR. Like tapasin, TAPBPR also shapes the peptide repertoire on MHC I molecules [30,31]. Interestingly, binding of peptides longer than eight or nine residues, but not shorter ones, triggers a conformational change in ERAP1 that activates its hydrolysis [32–34]. Through this mechanism, ERAP1 trims most peptides down to eight or nine residues, corresponding to the size needed for optimal binding to MHC I molecules. Ultimately, the peptides that are available to be presented are the ones that have been cleaved to the right size and have somehow escaped further hydrolysis to a size that is too small to stably bind to MHC I molecules (Figure 3). Peptides that are unable to bind an MHC I molecule are ultimately translocated back into the cytosol for

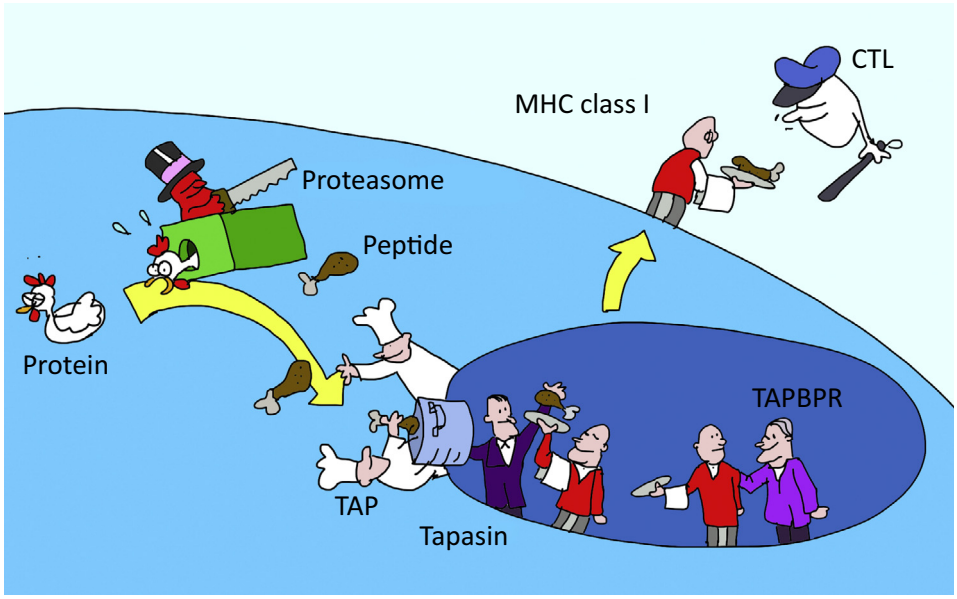

Figure 2. A Simple Illustration of MHC I Antigen Presentation. Antigens are degraded by the proteasome to yield peptide fragments. These peptides are then translocated from the cytosol into the endoplasmic reticulum (ER) lumen where MHC I waiting for peptides is retained by a series of chaperones including the dedicated chaperone tapasin in the peptide-loading complex. A second dedicated chaperone [tapasin-binding protein related (TAPBPR)] can further optimize the peptides in MHC I. Only MHC I with optimal peptides is allowed to leave the ER to present the peptide fragments at the cell surface to $CD8^+$ T cells (CTL).

degradation [35]. Whether there are mechanisms that help to protect some of these peptides from destruction or release from the ER during the time before they bind MHC I molecules is not entirely clear.

Peptides should be considered the third subunit of MHC I molecules as they are required to stabilize these complexes when the MHC I molecules are not bound to chaperones in the ER [36]. Peptides also allow the MHC I molecules to be released from the ER quality control system (the various chaperones) for transport to the cell surface for presentation to $CD8^+$ T cells [37,38]. This system of low-off-rate peptide selection, exporting only peptide-loaded MHC I complexes, may help prevent healthy cells from easily replacing their bound endogenous peptides for exogenous antigenic peptides, an event that would lead to the presentation of peptides that do not reflect the status of a given cell and possible execution by $CD8^+$ T cells.

Complexity in the MHC Class I Antigen Presentation Pathway

The general scheme outlined above is somewhat more complex when taking into consideration the diversity of the MHC I family. The different loci expressed (in humans, HLA-A, -B, and -C) and the many polymorphic allelic forms behave somewhat differently with respect to expression, peptide binding, and stability [39,40]. Peptides may also be generated from multiple sources. This is because the proteasome not only degrades proteins as part of normal protein turnover but also degrades abnormal ones that arise from errors in translation, folding, and/or pairing. The degradation of these disabled proteins prevents their aggregation and also potentially more directly couples protein translation to antigen presentation [41–43]. Such antigens are called defective ribosomal products (DRiPs) and may be produced in greater amounts under high-protein-synthesis-conditions, such as occur during viral infection. The degradation of DRiPs

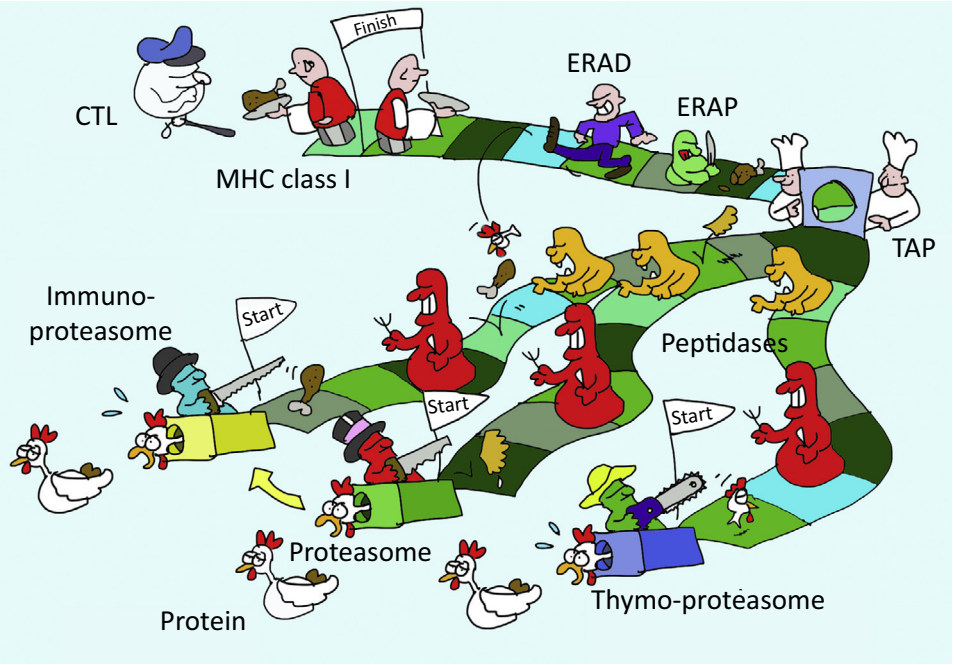

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Figure 3. Survival of the Fittest for MHC I Presentation and the Many Proteasomes. There are three types of proteasome with unique tissue expression. These proteasomes have altered cleavage specificities yielding (in part) different degradation fragments. These fragments are released in the hostile environment of the cytosol where most of the peptides will be destroyed by peptidases. A few peptides survive this massacre through translocation in the ER by transporter associated with antigen processing (TAP). Here they can be further trimmed by ER-resident aminopeptidase (ERAP) or translocated back into the cytosol by the ER-associated degradation (ERAD) system. Only a few peptides survive chaperone-mediated survival selection for low-off-rate peptides for a defined MHC I allele and these are ultimately presented to a $CD8^+$ T cell (CTL).

would quickly generate peptides after initial translation of the antigen and this may allow rapid detection of infected cells [44,45].

While the source of antigens can vary, so can the cleavage of these antigens by different proteasomes. Many presented peptides can be generated through the phylogenetically older 'conventional' proteasome. However, the development of the immune system coincided with the evolution of alternative forms of active-site subunits for this particle, leading to the assembly of an immunoproteasome. A set of these subunits (β1i, β2i, and β5i) [46] is constitutively expressed in dendritic cells and lymphocytes and can be induced in all other cells by interferons; for example, during viral infections [47,48]. When these subunits are expressed, they preferentially incorporate into newly assembling particles to form immunoproteasomes that generate a distinct set of peptides during protein degradation[49]. This shift from constitutive to immunoproteasome in cells often enhances the generation of peptides presented by MHC I molecules, including many unique ones [50]. Generation of new peptides will be at the cost of other peptide fragments that are cleaved to make the new fragment [51]. Another set of alternative active-site subunits (β1i, β2i, and β5t) is expressed uniquely only in cortical thymic epithelial cells (cTECs), where they are incorporated into thymoproteasome particles [52]. Among the peptides generated by thymoproteasomes, several are unique and these play a critical role in the auditioning of developing $CD8^+$ T cells during positive selection and in allowing many of these cells to avoid subsequent negative selection [50]. Proteasomes, but also the cytosolic and ER-associated peptidases, are variable in content and number. From this stew of proteolytic activities an

estimated 0.02% of the peptides generated by the proteasome survive for presentation to the immune system [53] (Figure 3).

Not How to Present, But What to Present

MHC I molecules present peptides from a cell's expressed genes and thereby allow the immune system to monitor the proteins synthesized in a cell. However, cells may also alter signaling in response to transformation or infection, resulting in an altered phosphoproteome [54], acetylome, or glycome [55] or other (small) post-translational modifications [56–58]. The peptide transporter TAP allows peptides with these small modifications to enter the ER and some of these can bind to MHC I molecules for presentation to $CD8^+$ T cells. These modified peptides are not genetically encoded but are neoepitopes to which the immune system may not be tolerant [59,60]. This can allow the immune system to detect cells in abnormal states (e.g., transformed ones) for elimination. Other non-genetically encoded antigenic peptides arise by peptide splicing by the proteasome, where the proteasome performs the opposite reaction linking two peptide fragments into a new one [61]. Whether this is just a consequence of the reverse proteolysis reaction of the proteasome or is influenced by particular cellular states is unclear, but these peptides can be presented and stimulate $CD8^+$ T cell responses. Through these various mechanisms the repertoire of MHC I-presented peptide (the 'presentome') is expanded beyond the genetically encoded sequences and additional options for the detection of abnormal cells are available, but there is also a risk of autoimmune reactions.

How to Hide One's Inner Self? Pathogen and Tumor Escape from Antigen Presentation

While the MHC class I pathway evolved to allow detection and elimination of the nidi of viruses in an infected host, some viruses have coevolved cloaking mechanisms to avoid such detection (Figure 4). For example, a large majority of the human species is chronically infected with cytomegalovirus (CMV) and herpes simplex virus (HSV). It is clear that these viruses have evolved

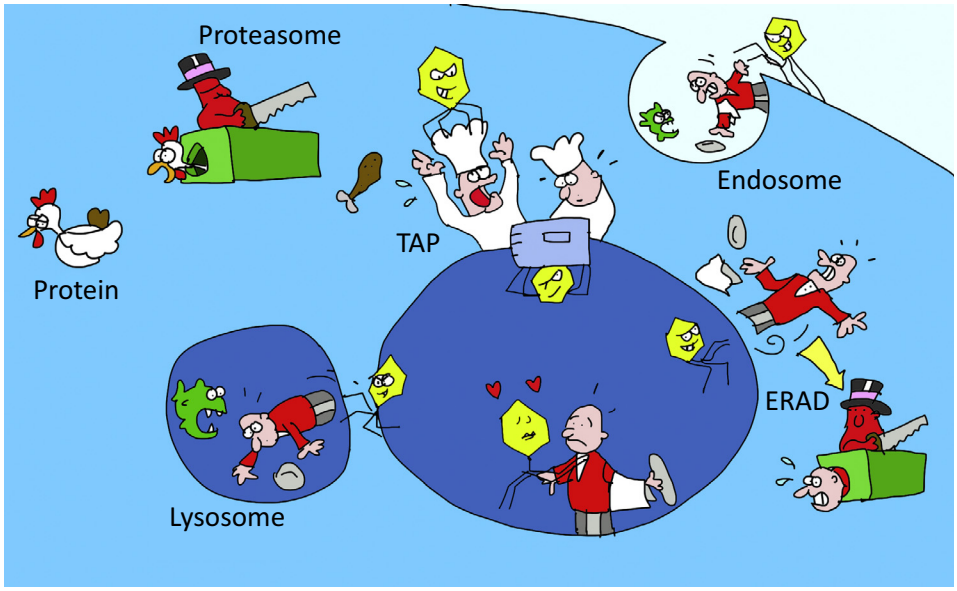

Figure 4. Various Viral Immune Evasion Strategies for MHC I Antigen Presentation. Pathogens have evolved different ways to obstruct processes selective in the antigen presentation pathway. Examples include viral proteins inhibiting peptide transport by transporter associated with antigen processing (TAP), retaining MHC I in the endoplasmic reticulum (ER), and recognizing MHC I in the ER to target these back into the cytosol for degradation by the proteasome [the ER-associated degradation (ERAD) system]. Other viral proteins recognize MHC I at the cell surface for internalization and destruction in lysosomes.

ways to tamper with the process of antigen presentation [62]. CMV encodes proteins that inhibit the peptide transporter TAP or that induce the degradation of MHC I molecules in the ER or plasma membrane [63,64]. Other viruses shut down genomic MHC class I expression[65], produce a peptide mimic that blocks TAP, or block the transport of MHC I molecules to the cell surface [66]. Any step in the MHC class I antigen presentation pathway not interfering with cell viability can be expected to be manipulated by viruses to prevent their presentation.

Since most of the steps in the MHC class I pathway are not essential for viability and because cancer cells are often genetically unstable, tumors can, under the selection pressure imposed by $CD8^+$ T cells, generate variants that have lost key components of the MHC class I pathway and escape control by $CD8^+$ T cells [67]. Reduction of MHC I molecule expression in human tumors is often detected by pathologists [68]. This is one of the reasons for assuming an important role of the immune system in the control of particular tumors, especially those with more neoantigens (from mutated proteins) such as melanoma and lung cancer [69,70]. Understanding how a given tumor can evade detection by $CD8^+$ T cells could help in determining the immunotherapies that are most likely to succeed against that tumor.

Some Exceptions in Self-Presentation by MHC Class I Molecules

The MHC class I antigen presentation system is constructed in such a way that most cells exclusively present their own antigens. Because of this, it was thought that $CD8^+$ T cells selectively eliminate infected cells without destroying neighboring 'innocent bystanders'. However, this concept is challenged by the fact that the cytosols of many healthy cells (unlike cancer cells) are connected by so-called gap junctions. These gap junctions allow peptide fragments to pass into directly connected neighboring cells for entry in their antigen presentation pathway and presentation through the neighbor's MHC I molecules to $CD8^+$ T cells [71,72]. It is likely that cytosolic peptidases will limit the spread of such peptides beyond the most proximal neighboring cells. However, since the proximal cells are at high risk of viral invasion, gap junctions may allow their elimination even before real entry of the pathogen.

A more intensely studied system where MHC I presents antigens that differ from those made by the cell itself is cross-presentation [73,74] (Figure 5). This pathway operates in dendritic cells and other phagocytes and is important because it plays a central role in immune surveillance [74–76]. It allows dendritic cells to acquire antigens from other infected cells and cancers in the periphery and then report their presence to naïve $CD8^+$ T cells in lymphoid organs in ways that initiate an immune response. Phagocytes acquire these antigens when they ingest them by phagocytosis (e.g., by eating cell debris [77–79] or possibly even taking a 'bite' of living cells [80]) or via receptor-mediated endocytosis (e.g., of glycan-modified proteins through lectin receptors or antibody-bound antigens through Fc receptors [81,82]). There is more than one mechanism by which ingested antigens can be cross-presented, but one is likely to involve cytosolic transfer of antigen from the phagosome into the cytosol for degradation by the proteasome [83,84]. The fragments may then be loaded on MHC I molecules in the ER or translocated back into the phagosome for local MHC class I peptide loading [78]. In another mechanism, some antigens are degraded by lysosomal proteases and loaded onto recycling MHC class I molecules in a pathway similar to MHC class II molecules [85–87]. Toll-like receptor (TLR) signaling can induce the accumulation of MHC I in recycling endosomes to promote cross-presentation [88]. A potential limitation of this latter mechanism is that it could result in priming $CD8^+$ T cells to peptides differing from the ones that the T cells will encounter in infected cells (as the priming peptides are generated by proteases different from the proteasome). However, this may also be a problem in the phagosome-to-cytosol mechanism of cross-presentation because, in the absence of inflammation, immunoproteasomes in dendritic cells may generate different fragments compared with the constitutive proteasome in most peripheral (and, notably, tumor) cells [89]. Thus, whether and how cross-presentation allows activation of $CD8^+$ T cells recognizing

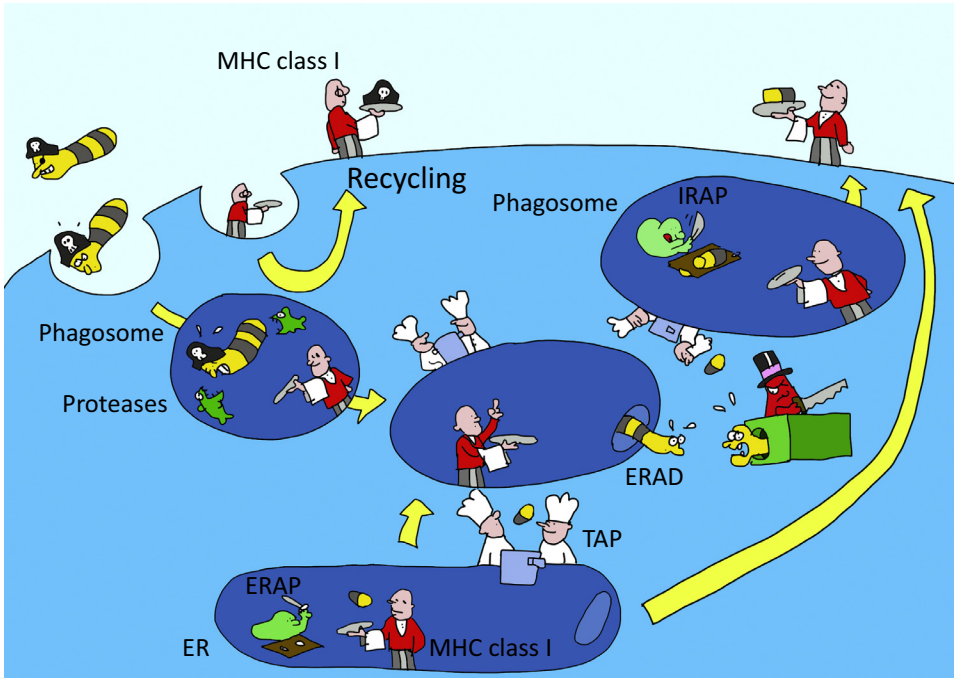

Figure 5. Crossing Boundaries: Various Models of Cross-Presentation by MHC I. MHC I molecules can present exogenous antigens and antigens delivered in apoptotic bodies and other types of cell debris. For dendritic cells, this process can result in cross-priming of $CD8^+$ T cells. MHC I can recycle through the endosomal pathway to acquire antigen fragments made by proteases such as insulin-regulated aminopeptidase (IRAP) [129] for cross-presentation. Endosomes may also acquire transporter associated with antigen processing (TAP) and other endoplasmic reticulum (ER) molecules that may help export antigens into the cytosol for proteasomal hydrolysis; the resulting peptides may be re-imported into the endosomes for MHC I loading and recycling and/or be delivered in the normal antigen presentation pathway as shown in Figure 2.

the full range of antigenic peptides presented by peripheral cells is unclear. Regardless of the exact mechanism, cross-presentation allows MHC I molecules to present peptides from antigens that usually are handled by MHC II molecules. The system employed by MHC II molecules for antigen presentation is understood in considerably more molecular detail and may share elements used for cross-presentation by MHC I molecules.

MHC Class II Molecules

MHC II molecules are both similar and different from MHC I molecules, as are their mechanisms of presentation. MHC II molecules are expressed on immune cells such as B cells, monocytes, macrophages, and dendritic cells and on epithelial cells following inflammatory signals, while MHC I molecules are expressed more ubiquitously. MHC II molecules on dendritic cells present antigen to naïve $CD4^+$ T cells to activate them; later MHC II molecules participate in the interaction of B cells and macrophages with these specific $CD4^+$ effector T cells [90]. This is a critical function, as exemplified by patients with deficits in MHC class II expression (bare lymphocyte syndrome), which results in extreme susceptibility to infections with a variety of microorganisms and death at a young age [91]. The structure of MHC class II resembles that of MHC class I and they are both polymorphic proteins (and thus transplantation antigens) [90,92]. Interestingly, *Gadus morhua* fish (Atlantic cod) lack MHC II but express an MHC I molecule containing endocytosis signals that effectively takes over MHC II function, illustrating the strong relationship and conserved function of these two MHC classes [93]. However, the natures of the

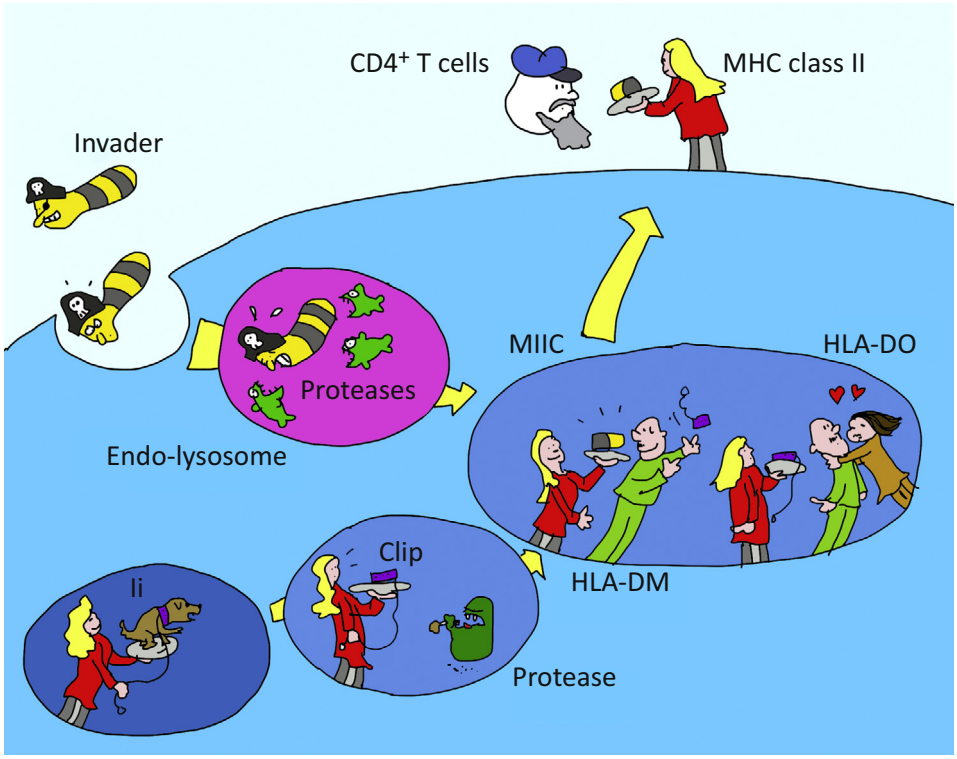

Figure 6. A Simple Illustration of MHC II Antigen Presentation. MHC II proteins are made in the endoplasmic reticulum (ER) where they pair with a third chain, the invariant chain (Ii). Ii fills (through a peptide sequence called CLIP) the MHC II peptide-binding groove and allows efficient exit of MHC II from the ER. Ii also guides MHC II through the cell to a late endosomal compartment, MIIC. Ii is degraded by endosomal proteases, as are antigens taken up by endocytosis or phagocytosis. The CLIP segment is protected from destruction and exchanged for an antigenic peptide with the help of a dedicated chaperone called DM (HLA-DM in humans). Another chaperone expressed in a few immune cell types (immature B cells, some DC forms), called DO (HLA-DO), can compete for DM binding to MHC II and thereby affect the peptide repertoire on MHC II that is ultimately presented at the cell surface to $CD4^+$ T cells.

presented peptides usually differ, as does the underlying biology of MHC class II antigen presentation.

How to Present the Outside World?

MHC class II presents peptide fragments that are generally larger than those presented by MHC class I, because the peptide-binding groove of MHC class II is open, allowing peptides to extend out of this site [94]. The MHC class II-associated peptides are derived from extracellular proteins and from self-proteins that are degraded in the endosomal pathway (Figure 6) [95]. MHC II molecules associate during their assembly in the ER with the invariant chain Ii, which acts as a pseudopeptide by filling the MHC class II peptide-binding groove and targets MHC II molecules into the endosomal pathway through its cytosolic dileucine motif [20,96]. In a compartment commonly named MIIC [97], MHC II molecules then meet antigenic fragments generated by resident proteases. For these peptides to bind MHC II molecules, the invariant chain has to be degraded by the same mix of proteases, especially cathepsin L and S [90]. This leaves an invariant chain fragment (called CLIP) inaccessible for proteases and remaining in the peptide-binding groove of MHC II molecules [98]. This CLIP fragment has to be exchanged for higher-affinity peptides with the help of a dedicated MHC class II-like chaperone called DM (in humans, HLA-DM) [99]. The structure of HLA-DM in association with MHC II (HLA-DR1) reveals that DM locally opens the groove to release low-affinity peptides such as CLIP. DM release from MHC

class II then locks the proper peptide fragments in the MHC class II peptide-binding groove [100]. After some period of residence in MIIC, MHC II molecules move to the plasma membrane either via vesicular transport or in the form of tubules [101–103]. Since the targeting information in the invariant chain has been removed after its degradation in MIIC, MHC II molecules can stably reside on the plasma membrane.

Variations in MHC Class II Presentation

As for MHC I molecules, there are various MHC II loci in most species (in humans there are three, named HLA-DR, HLA-DQ, and HLA-DP). Also, MHC II molecules are polymorphic (>3000 alleles known) [90] and their polymorphic amino acids similarly cluster in and around the peptide-binding groove, shaping the peptide-binding pockets. Consequently, different MHC II alleles bind different peptides by virtue of their different anchor residues [104]. This is likely to explain why certain MHC II alleles are strongly linked with defined autoimmune diseases [105] and immune responses against external environmental and food antigens [90]. This is well illustrated for the link between gluten sensitivity (celiac disease) and the MHC class II allele HLA-DQ2. HLA-DQ2 is able to present a peptide from gluten after deamination by tissue transglutaminase to activate $CD4^+$ T cells and drives the disease [106]. The dominant antigens for autoimmune diseases are often unclear but there are some suggestions. For example, insulin has been suspected to mediate HLA-DR3/4-associated type 1 diabetes and myelin basic protein to participate in HLA-DR1 related multiple sclerosis [107,108]. One additional mechanism involves the presentation of an atypical conformation of a peptide [109] as the result of peptide loading of MHC II molecules in compartments that lack DM molecules, such as may occur in recycling endocytic compartments [110] or in the ER [111,112]. In the absence of DM function, the non-optimal peptide conformation bound to MHC II is not corrected. A different conformation of a self-peptide can be recognized as non-self by $CD4^+$ T cells, which may drive the induction of autoantibodies [109]. However, the fact that many people with these MHC class II alleles never develop autoimmune diseases, and that for most of these conditions much less than half of identical twins are concordant for disease, indicates that epigenetic and other factors must also be involved and these are as yet unclear.

Besides genetic variation resulting in polymorphism of MHC II molecules, another variation in the life of MHC class II lies with the associated invariant chain, which is actually not so invariant. Indeed, there are multiple splice variants, including one (p44) that contains an additional protease inhibitor (cystatin) domain [90]. This variant can be expected to modify the protease activities involved in antigen preparation for MHC II molecules. The proteases (cathepsins in the endosomal pathway) also vary in the different MHC class II-expressing immune cells, as do natural inhibitors for these cathepsins (called cystatins) [90,96]. It is likely that antigens are degraded after denaturation (which involves reduction of their disulfide bonds by the enzyme GILT [113], and acidic pH [114]) by a swarm of proteases that will generate and destroy potential peptides for a defined MHC II allele.

Peptides are selected for presentation by MHC II with the help of the chaperone DM. DM can be further controlled by a dedicated co-chaperone called DO (HLA-DO in humans) that strongly pairs with DM [115]. DO associates with DM at the same interface as that occupied by MHC II molecules [100] and thus inhibits DM-assisted peptide loading of MHC II molecules unless the DM–DO complex enters very acidic endosomes along with MHC II molecules [116,117]. DO thus shapes the peptide repertoire by preventing peptide binding in earlier vesicular compartments. Deleting DO induces type 1 diabetes in mouse models and possibly other autoimmune diseases [118].

Other variations entail the transport and surface half-life of MHC II molecules. MHC II transport from MIIC to the plasma membrane is not constitutive but controlled in dendritic cells,

monocytes, and B cells. Activation of dendritic cells promotes MHC II transport to the cell surface and strongly enhances the half-life of these molecules on the plasma membrane [119,120]. As a result, activated dendritic cells have high numbers (around 2 million per cell) of MHC II molecules on the cell surface that continue to present antigens for long periods. However, there are other ways to control MHC II expression. For example, in human monocytes IL-10 increases the expression of a ubiquitin ligase membrane-associated RING-CH1 (MARCH-1) that ubiquitinates the tail of cell surface MHC II molecules initiating their rapid internalization and destruction [121]. Genome-wide analyses have identified many factors that control the complex process of control of MHC II expression and transport in dendritic cells [122]. Many pathogens, especially those residing in MHC II-containing phagosomes, also inhibit MHC II expression or peptide loading [123,124]. Such immune evasion by pathogens may be caused by manipulation of DM interactions with MHC II molecules, manipulation of pH levels, alteration of protein networks, or induction of MHC ubiquitination by MARCH homologs [125] or other processes required for optimal antigen presentation by MHC II molecules.

Concluding Remarks and Future Perspectives

The work of many laboratories over past decades has informed our understanding of the function of MHC class I and MHC class II molecules at the immunological, cell biological, genetic, and atomic level. Many of the major mechanisms of both the MHC I and the MHC II pathways are understood and we can look forward to an even more comprehensive understanding of antigen presentation mechanisms in the coming years. With such knowledge, we can look forward to better understanding how these processes help to maintain health and/or contribute to disease pathogenesis. We anticipate that there will be a strong potential to translate this knowledge into clinical medicine. One important area is in cancer immunotherapy. While the role of MHC class I and II antigen presentation in tumor immunology was initially restricted to curing cancer in mouse models, the recent development of checkpoint inhibitor antibodies has translated this into clinical responses in human melanoma, lung cancer, and other tumors [126]. Characterizing the defects in antigen presentation in such tumors might in theory help identify patients who can or cannot respond to such interventions. Moreover, if methods can be developed to reverse such defects, they might be able to improve the efficacy of these immunotherapies. Similarly, manipulating antigen presentation pathways might overcome the inhibition of antigen presentation induced by certain microbes and then help eliminate these chronic infections. The rules for antigen presentation by MHC I molecules can be used to predict neoepitopes detected by $CD8^+$ T cells [127] and this information might be exploited to actively immunize cancer patients in conjunction with removal of checkpoint control and to monitor patient responses. Targeting antigens to dendritic cells and in particular into specific presentation pathways has the potential to generate more robust and effective kinds of responses to vaccines and immunotherapies. Antigen presentation might also be manipulated in the opposite way to dampen autoimmune diseases; for example, with tolerogenic peptides or tolerogenic antigen-presenting cells. Despite the remarkable advances in our knowledge about antigen presentation, the picture remains incomplete but should continue to improve. For example, forward genetics screens are uncovering unsuspected new components in the pathways [122,128]. Filling these gaps (see Outstanding Questions) should provide a higher-resolution understanding of the pathways and their contribution to disease pathogenesis as well as increase the opportunities to exploit these pathways to develop better immunotherapies to prevent and/or treat disease.

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Supplemental Information

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Outstanding Questions

While antigen presentation by MHC molecules has been intensively studied for 40 years, many aspects remain unclear. The following is only a partial list.

Why can peptides presented by MHC molecules not be more accurately predicted? What are we missing?

What are the many proteases involved in generating and destroying presented peptides and what are their relative contributions and specificities?

How do the antigen presentation pathways so successfully present a broad peptidome from very large numbers of different proteins of very different abundances and in the face of robust peptide destruction?

Are there mechanisms that protect peptides from destruction before they bind to MHC molecules?

The components of the MHC antigen presentation pathway have remarkable heterogeneity in expression and activity in different tissues. Does that lead to different presentation of self-antigens and contribute to autoimmune responses?

Why do we have three different proteasomes?

Why do we express only three MHC I and three MHC II locus products and not more to cover all possible peptides for presentation?

What is the major mechanism that generates DRIPS–inaccurate transcription, translation, folding, or assembly?

Is antigen cross-presentation the result of many different systems or are there dominant systems?

For cross-presentation, how are antigens transported from endosomes into the cytosol?

For cross-presentation, how are MHC I molecules kept stable during transport to and after arrival in endosomes?

Are there mechanisms that promote the loading of peptides in endosomes for cross-presentation?

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Do dendritic cells or specific subsets of dendritic cells have unique components that promote cross-presentation?

How are hydrophobic peptides delivered in the peptide-binding groove of MHC I and MHC II molecules? Are there unique chaperones for such peptides?

Is there an MHC class II peptide-loading complex?

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