Antigen Processing and Presentation

Chapter 7

WHAT'S IN THIS CHAPTER?

Real generosity toward the future lies in giving all to the present.

Albert Camus

A. Overview of Antigen Processing and Presentation

Antigen processing is the complex process by which antigens are produced from macromolecules. Most often, antigen processing refers to the generation of antigenic peptides from proteins. **Antigen presentation** refers to the binding of these peptides to MHC molecules and the positioning of the resulting pMHC complexes on a host cell surface so that they can be inspected by T cells. The processing and presentation of antigenic peptides, as well as some non-peptidic antigens, are discussed in this chapter, whereas the recognition of pMHCs by the TCRs of T cells is discussed in Chapter 8.

Antigen processing provides the host with a means of scanning the molecules constantly being produced and turned over in the body. At any one time, almost every cell in the body displays several hundred thousand pMHCs on its surface. This population represents hundreds of distinct peptides, the vast majority of which are "self" in origin and elicit no T cell response in a healthy individual. In the case of an infection, a substantial proportion (up to 10%) of the peptides may be pathogen-derived, a number more than sufficient to trigger the activation of T cells specific for these pMHCs.

With respect to protein antigens, there are four major pathways of antigen processing, two of which are well defined and two of which remain to be completely elucidated (**Fig. 7-1**). The **exogenous processing pathway** acquires proteins from *outside* the host cell (extracellular proteins) and degrades them to peptides within endocytic compartments. In contrast, the **endogenous processing pathway** acquires proteins that are synthesized inside the host cell (intracellular proteins) and degrades them to peptides in the cytoplasm; these peptides are then delivered into the ER. The two more recently discovered pathways allow a protein antigen to be transferred from one of the preceding pathways into

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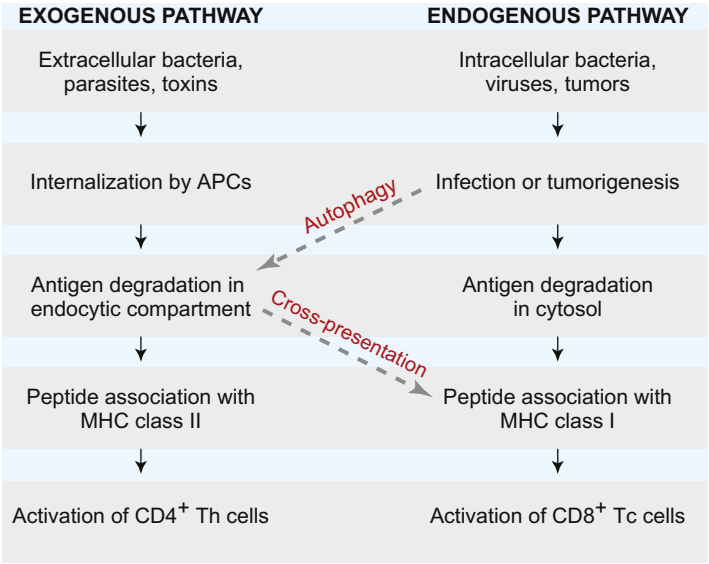
Fig. 7-1
Overview of Antigen Processing Pathways

Antigens processed via the exogenous pathway are presented on MHC class II and activate $CD4^+$ Th cells. Antigens processed via the endogenous pathway are presented on MHC class I and activate $CD8^+$ Tc cells. Peptides escaping from the exogenous pathway may be displayed on MHC class I via cross-presentation. Peptides from intracellular entities that are captured by autophagy can be diverted into the endocytic compartment and presented on MHC class II.



the other. The **cross-presentation** pathway transfers peptides from the exogenous pathway into the endogenous pathway, and the **autophagic** pathway captures cytoplasmic entities and macromolecules and delivers them into the exogenous pathway. These four processing pathways allow antigenic peptides to be presented efficiently to both $CD4^+$ and $CD8^+$ T cells, so that both subsets will respond to extracellular and intracellular entities as required.

The outcome of antigen presentation to T cells is determined in large part by the MHC molecules involved. MHC class I and class II molecules follow different intracellular trafficking routes after synthesis, show critical differences in cell type expression, and bind to different coreceptors on T cells. With respect to MHC class I molecules, after their synthesis in the ER, these proteins remain in this organelle and bind to peptides delivered into the ER by endogenous processing or cross-presentation. The pMHC complexes generated by this binding are then expressed on the host cell surface, where the CD8 coreceptor binding site of the MHC class I protein ensures that $CD8^+$ CTL effectors recognize the host cell as a target. Because MHC class I molecules are expressed on all nucleated cells, $CD8^+$ CTLs can effectively target and kill any cell that has fallen prey to the internal afflictions threatening all host cells, namely infection and cancerous transformation. In contrast, only APCs express MHC class II molecules. Within an APC, newly synthesized MHC class II molecules traffic from the ER to specialized endosomal compartments where they receive peptides generated through exogenous processing or autophagy. The resulting pMHC complexes are expressed on the APC surface where the CD4 coreceptor binding site of the MHC class II protein ensures that $CD4^+$ Th cells are able to survey the proffered peptides. This limitation of MHC class II expression to APCs ensures that the attention of Th cells is efficiently focused on the only cells capable of Th cell activation. Thus, the various antigen presentation systems at work in different host cell types ensure that any peptide associated with "danger," whether from an extracellular or intracellular source, will activate the Th cells that are the lynchpin of adaptive immunity. The products of these Th cells then support the activation of Tc cells that may be needed to combat intracellular threats, B cells that may be required to produce antibody against extracellular threats, and other Th subsets that may be required to fine-tune and regulate the immune response.

B. Nature of Cells That Can Activate T Cells

To become activated, naïve $CD4^+$ and $CD8^+$ T cells must interact with host cells that display pMHCs on their cell surfaces. With respect to $CD4^+$Th cells, activation is carried out by so-called professional APCs, which are those few cell types that constitutively

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or inducibly express high levels of MHC class II and costimulatory molecules. Professional APCs include mature DCs (which can activate naïve Th cells as well as effector and memory Th cells) and macrophages and B cells (which can activate effector and memory Th cells but not naïve T cells). "Non-professional" APCs are cell types (such as fibroblasts and epithelial cells) that can transiently express low levels of MHC class II if exposed to IFNγ during an inflammatory response. With respect to $CD8^+$ Tc cells, although all nucleated host cells express the MHC class I required to present peptides to this lymphocyte subset, again, only mature DCs are able to activate naïve Tc cells. In contrast, $CD8^+$ CTL effectors and memory $CD8^+$ Tc cells can be activated by any cell type expressing the specific peptide-MHC class I complex recognized by the TCR of that effector or memory $CD8^+$ T cell. Thus, any cell that has become aberrant due to cancer or intracellular infection becomes a target cell for CTL-mediated cytolysis.

Mechanisms of T cell activation are discussed in Chapter 9.

NOTE: Intracellular proteins that are released into the extracellular milieu by infected or transformed cells can be internalized by an APC and directed into its exogenous processing pathway. Then, thanks to cross-presentation, the antigen or its fragments can be transferred into the endogenous processing pathway, allowing the APC to display the resulting peptides on MHC class I. Thus, although not under internal attack itself, the APC can activate the Tc cells required to respond to an intracellular threat.

I. Dendritic Cells as APCs

As described in Chapter 2, multiple types of DCs in mammals can be distinguished by their locations in the body as well as by the surface markers and PRRs they express. By virtue of these differences, these various types of DCs play a key role in inducing the T cell response appropriate for dealing with the specific pathogenic threat encountered. We also introduced in Chapter 2 the concept that microenvironmental conditions can directly influence DC differentiation, in that the rare group of DCs called *inflammatory DCs* appears to be derived from monocytes that extravasated into inflamed tissue and subsequently became activated. However, under the steady-state conditions prevailing in healthy individuals, the main types of DCs present in the blood and tissues are either the comparatively rare *plasmacytoid DCs (pDCs)* or the much more numerous *conventional DCs* (which we refer to simply as DCs). In the absence of a threat, most of these pDCs and DCs remain in the immature state; they are induced to undergo maturation only when the healthy host becomes infected or sustains tissue damage. The pDCs specialize in sensing viral RNA and DNA through endosomal PRRs and respond with vigorous production of IFNα and IFNβ, cytokines that have direct antiviral effects. Thus, pDCs make their most important contribution to the innate, rather than the adaptive, immune response. In contrast, conventional DCs are the major drivers of T cell activation and are therefore the subset we focus on in this chapter when discussing the processing and presentation of antigen to T cells.

See Table 2-3 in Chapter 2 for a comparison of the properties of pDCs and DCs.

i) Migratory vs. Lymphoid-Resident DCs

The conventional DCs involved in naïve T cell activation can be further categorized as *migratory* DCs or *lymphoid-resident* DCs (often shortened to "resident DCs"). After their release from the bone marrow into the blood, migratory DCs first access a peripheral tissue rather than a lymphoid site, and collect self and foreign antigens in this location. Antigen-laden migratory DCs can then enter a lymphatic vessel and travel to the nearest secondary lymphoid tissue (most often a lymph node), where they either interact directly with T cells in the node, or act as an antigen source for resident DCs in that node. In contrast, when first released from the bone marrow into the blood, resident DCs move directly into one lymphoid site and do not travel the body in the lymphatic system. These cells remain in their lymphoid tissue of residence, collecting and presenting self and foreign antigens that are either dumped into the tissue via a lymphatic vessel, or are conveyed there by migratory DCs traveling in the lymphatic

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TABLE 7-1 Migratory vs. Lymphoid-Resident DCs

DC Subset	Function	Examples
Migratory	Front-line defense in peripheral tissues Acquire antigens in peripheral tissues and migrate through lymphatics to lymph nodes Deliver antigens to lymphoid-resident DCs or directly initiate T cell responses in local lymph nodes	Langerhans cells in epidermis Dermal DCs in dermis Mucosal DCs in mucosae of body tracts Interstitial DCs in non-lymphoid tissues
Lymphoid-resident	Front-line defense in lymphoid tissues Do not migrate but acquire antigens from migratory DCs or antigens that have accumulated in lymphoid tissues Initiate T cell responses in lymph nodes Initiate T cell responses to blood-borne antigens in the spleen Participate in central tolerance	Resident DCs in lymph nodes Splenic DCs in spleen Thymic DCs in thymus

system. The properties of migratory and lymphoid-resident DCs at steady-state are summarized in **Table 7-1**.

Migratory DCs are abundant at junctures between the body and the outside world, including just under the skin and the mucosae lining the respiratory and gastrointestinal tracts. Several subsets of migratory DCs have been identified that differ slightly in their surface markers, PRR repertoire, tissue distribution and cytokines secreted upon maturation. Consequently, each DC subset may have a different effect on T cell activation, allowing a tailored response to the specific threat encountered. Examples of migratory DCs are **Langerhans cells (LCs)**, which are relatively long-lived DCs present in the epidermis of the skin; *dermal DCs* present in the dermis of the skin; *mucosal DCs* in the mucosae lining the gastrointestinal, respiratory and urogenital tracts; and *interstitial DCs* present in almost all other non-lymphoid peripheral tissues.

Lymphoid-resident DCs include those present in the thymus and spleen and about half of those present in lymph nodes. *Thymic DCs* remain in this organ throughout their short life span and most likely participate in the establishment of central tolerance, presenting peptides from self antigens to T cells developing within the thymus. Immature T cells that strongly recognize these pMHCs (and thus are self-reactive T cells) are then eliminated. *Splenic DCs* reside in the spleen and monitor blood-borne antigens. At least three subtly different subsets of splenic DCs have been identified in mouse spleen based on differential surface marker expression.

Mechanisms of central T cell tolerance induction in the thymus are discussed in Chapter 9. Mechanisms of peripheral tolerance are discussed in Chapter 10.

ii) Immature vs. Mature DCs

In Chapter 2, we introduced the terms "immature DC" and "mature DC." The distinction is an important one because DCs become capable of activating naïve T cells only after they have undergone the maturation process. Until they receive specific maturation signals associated with infection or tissue damage, migratory and resident DCs remain in the immature state, which is specialized for rapid sampling of the surrounding microenvironment and the monitoring of tissue health. Immature DCs continually form and retract their long finger-like processes to capture entities in the surrounding tissue, and can also extend these processes harmlessly through the "tight junctions" that hold epithelial cells together to sample macromolecules in the external environment. Such sampling is mediated by the general engulfment processes of macropinocytosis, clathrin-mediated endocytosis, phagocytosis and autophagy described in Chapter 3, and the receptors mediating this uptake include scavenger receptors (like CD91 and CD36), complement receptors (like CR1 and CR3), and CLRs (like the mannose receptors, DEC-205 and DC-SIGN). Immature DCs also express high levels of FcγRII, which is a low affinity IgG receptor that can facilitate the uptake of protein antigens complexed to IgG (through opsonization) (**Fig. 7-2**).

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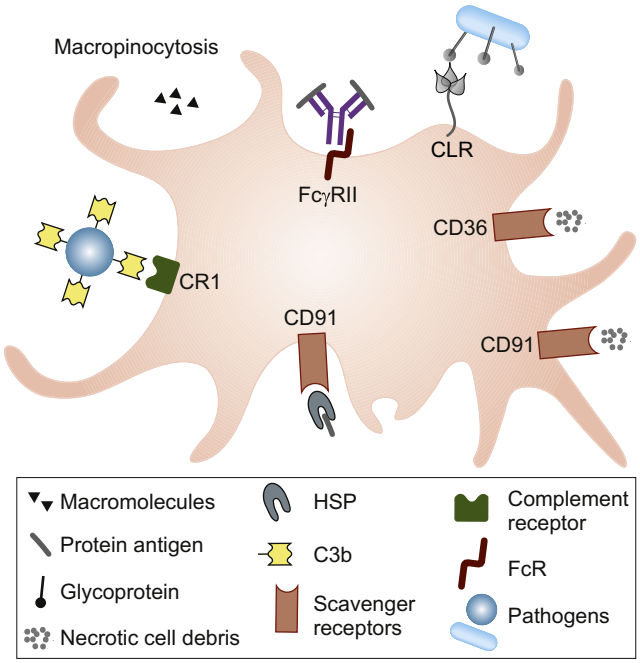

Fig. 7-2
Examples of Antigen Capture by an Immature DC

Immature DCs sample entities in the surrounding tissue microenvironment using macropinocytosis, receptor-mediated endocytosis and phagocytosis. Major antigen uptake receptors on these cells include scavenger receptors, complement receptors, CLRs and FcRs.

NOTE: Immunologists often call the engulfment of macromolecules from the external milieu "antigen uptake," and the receptors on DCs and other APCs that facilitate this process "antigen uptake receptors." These terms are widely used despite the fact that both foreign and host-derived entities are internalized in the same way, and not all captured entities will act as "antigens" (or, more properly, immunogens) with respect to lymphocyte activation.

Antigen uptake constantly supplies immature DCs with myriad proteins from which they derive peptides for display on MHC class I and II. The regular turnover of these pMHCs permits DCs to carry out ongoing surveillance of the protein environment of the host. Immature DCs do not initiate T cell responses because: (1) the supply of MHC class II on an immature DC is low and the pMHCs created by sampling are turned over very quickly, limiting antigen display and thus the DC's capacity to interact with and activate T cells; (2) under steady-state conditions, the pMHCs displayed do not contain foreign or aberrant antigen and so are not recognized by TCRs; and (3) no DAMPS/PAMPs are present to engage the DC's PRRs in such a way that the cell becomes activated.

In a host experiencing inflammation or infection, phagocytes (including immature DCs) efficiently capture whole pathogens by phagocytosis and use their antigen uptake receptors to capture macromolecular carbohydrate and protein antigens released by necrotic host cells. Large quantities of antigenic peptides are displayed on the immature DC's MHC molecules. Because the situation involves infection or trauma, DAMPs/PAMPs are present and engage the TLRs, NLRs, RAGE, and/or RLRs of immature migratory and resident DCs. The maturation of these cells is thus triggered and inflammatory cytokines are released, as described in Chapter 3. A mature migratory DC uses its large array of cytokine and chemokine receptors (particularly CCR1 and CCR7) as well as adhesion molecules to migrate rapidly and efficiently through the lymphatic system to the nearest lymph node (**Fig. 7-3**). Within the node, resident DCs that acquire antigen from either the afferent lymph or migratory DCs are also triggered to mature. Thus, the node becomes filled with mature migratory and resident DCs that are laden with foreign antigen and present pMHCs derived from the antigen directly to naïve Th and Tc cells also present in the node. Any naïve Th and Tc cells that are activated by these mature DCs proliferate and differentiate into resting Th effectors or CTLs that then leave the node and use their homing receptors to find the tissue under

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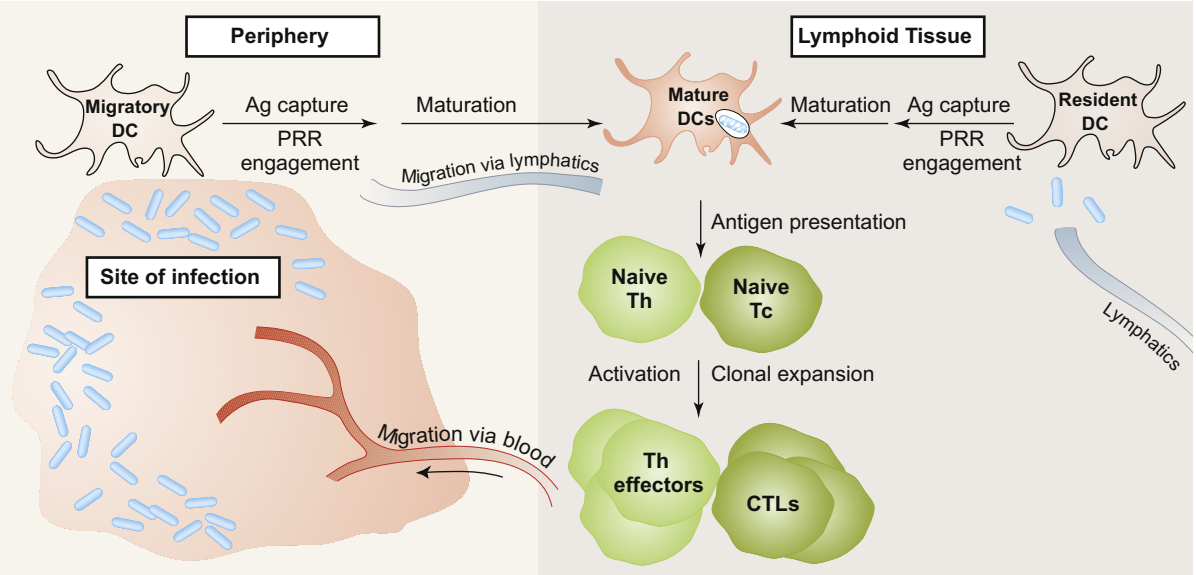

Fig. 7-3
Antigen Presentation by DCs to Naïve T Cells

Immature migratory DCs in peripheral tissues and immature resident DCs in lymphoid tissues continuously capture proteins from their surrounding environments. The maturation of these DCs is triggered only if they acquire antigen and have their PRRs engaged by DAMPs/PAMPs. Migratory DCs that have captured antigen directly from a site of infection initiate maturation as they move via the lymphatics to a lymphoid tissue such as a lymph node. Resident DCs that have either captured antigen conveyed into the lymphoid tissue via a lymphatic, or acquired antigen from an incoming migratory DC, are also triggered to mature. Within the lymphoid tissue, mature DCs of both types present pMHC complexes to naïve Th and Tc cells. Activated antigen-specific Th and Tc cells proliferate and generate Th effectors or CTLs, respectively, that migrate via the circulation to inflamed peripheral tissues. Immature and mature DCs are shown in light and dark brown, respectively.

TABLE 7-2 Immature vs. Mature DCs

	Immature DCs	Mature DCs
Location	Peripheral tissues Secondary lymphoid tissues	Secondary lymphoid tissues
Surface MHC class II	Low	High
Antigen internalization capacity	High	Low
Costimulatory molecules	Low	High
Antigen presentation to T cells	Inefficient	Very efficient
Chemokine receptors	High CCR1, low CCR7	Low CCR1, high CCR7
Arrays of actin filaments	Present	Absent

attack. Once in this tissue, the effector Th cells are activated by pMHCs presented by any APC (including mature DCs, macrophages and activated B cells) that have congregated in response to inflammatory signals, and the CTLs are activated by any host cell (including APCs) presenting antigenic peptides on MHC class I. At the conclusion of the primary response, the mature DCs do not "de-differentiate" and resume immature status but instead die by apoptosis, helping to dampen the T cell response after it is no longer needed. The properties of immature and mature DCs are summarized in Table 7-2.

iii) Mechanism of DC Maturation

The precise mechanism underlying DC maturation has yet to be completely elucidated. Complex changes to gene transcription programs are initiated by PRR engagement and the engagement of a DC's cytokine receptors by cytokines produced by other

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TABLE 7-3 Comparison of Professional APCs

	Mature DCs	Macrophages	B Cells
Level of MHC class II	Very high	High	High
Level of constitutive costimulatory molecule expression	High	Moderate	Low
Capable of cross-presentation	+++	++	+/−
Activates naïve T cells	Yes	No	No
Activates effector and memory T cells	Yes	Yes	Yes

activated innate leukocytes (such as macrophages) in the immediate area. The maturing DC's actin cytoskeleton is reorganized, and the efficiency of its endocytic compartment is increased. The receptors used by immature DCs to internalize antigen are downregulated, and the turnover of pMHCs on the DC surface is dramatically slowed, freezing the antigens displayed into a "peptide snapshot" of the tissue under attack. Surface expression of MHC class II increases by 5- to 20-fold, allowing a mature DC to rapidly present many copies of different antigenic pMHCs to Th cells. If the TCR expressed by a naïve Th cell recognizes one of the pMHCs displayed by the mature DC, costimulatory molecules such as CD28 and CD40L are upregulated on the Th cell surface (see Ch. 9). The binding of CD40L on the Th cell to CD40 on the DC greatly upregulates DC expression of the B7 costimulatory molecules (also called CD80/86) that bind to CD28. The high levels of these molecules, which greatly exceed those expressed by other types of APCs, are necessary to supplement the signal delivered by TCR binding to pMHC. The DC is able to push the naïve Th cell over the activation threshold in a way that other APCs cannot. The properties of mature DCs and other professional APCs are compared in **Table 7-3**.

As well as activating naïve Th cells by presenting peptide on MHC class II, mature DCs can present peptides on MHC class I to activate naïve $CD8^+$ Tc cells. Such presentation readily occurs if the DC is infected with a pathogen that replicates intracellularly. In addition, when a DC phagocytoses a whole pathogen or its components, some of the captured proteins may be diverted from the exogenous antigen processing pathway into the endogenous pathway via cross-presentation (see later). As a result, antigenic peptides from an intracellular pathogen may appear on MHC class I even if the pathogen has not infected the DC. Upon TCR engagement by these pMHCs, the same sequence of costimulatory events is triggered such that the naïve Tc cell is pushed over the activation threshold by the mature DC.

Excellent images of T cells interacting with immature and mature DCs can be seen at http://www.springerimages.com/Images/MedicineAndPublicHealth/1-10.1007_s00277-006-0117-1-1.

iv) Influence of DCs on T Cell Response Type

DC subsets are a key means by which the immune system tailors its response to a particular pathogen. Different DC subsets are present in various sites throughout the body. These subsets express different collections of PRRs, and different pathogens engage different subsets of these PRRs. Depending on which PRRs are engaged, a mature DC not only participates in innate responses by producing the cytokines, chemokines and other molecules best suited to eliminating the attacking pathogen directly, but also develops the properties needed to drive the differentiation of any naïve Th cell it activates down one of several parallel paths. For example, a DC subset that preferentially produces IL-12 and the IFNs in response to engagement of its PRRs causes the naïve Th cell it has activated to differentiate into **Th1 effector cells.** Th1 cells secrete cytokines that assist in adaptive responses against intracellular threats. In contrast, a DC subset that preferentially produces IL-13 helps to influence the naïve Th cell it has activated to differentiate into **Th2 effector cells.** Th2 cells secrete cytokines that assist in adaptive responses against extracellular threats. Finally, a DC subset that preferentially produces IL-6 helps to influence the naïve Th cell it has activated to differentiate into **Th17 effector cells.** Th17 cells both participate in adaptive responses against extracellular threats and play a role in autoimmunity.

Th subsets are discussed in more detail in Chapter 9.

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II. Macrophages as APCs

Macrophages excel at ingesting whole bacteria or parasites or other large native antigens present in peripheral tissues. These phagocytes quickly digest these bulky entities, producing a spectrum of antigenic peptides that are combined with MHC class II and can be presented to Th cells. However, unlike mature DCs, macrophages express only moderate levels of costimulatory molecules and so cannot activate naïve Th cells. Instead, macrophages make their contribution as APCs by activating memory or effector T cells that have homed to an inflamed tissue. The IFNγ secreted by a Th effector interacting with a macrophage hyperactivates the macrophage, increasing antigen presentation and thus pathogen clearance. Activated macrophages also produce cytokines that promote Th effector cell differentiation and upregulate MHC class II on other APCs (including DCs) in the immediate vicinity. Macrophages are thus important amplifiers of the adaptive response.

The differing activation requirements of naïve, effector and memory T cells are discussed in Chapter 9.

III. B Cells as APCs

B cells are considered professional APCs because they constitutively express MHC class II. B cells use their aggregated BCRs to internalize protein antigens by receptor-mediated endocytosis. The internalized antigen enters the exogenous processing pathway of the B cell such that peptide–MHC class II complexes appear on its surface. A B cell acting as an APC is one of the most efficient antigen presenters in the body because the BCR binds specific antigen with high affinity and can thus capture antigens present at very low concentrations. However, B cells do not generally serve as APCs in the primary response to a Td antigen because antigen-specific B cells and the antigen-specific Th cells required to help them are very rare in an unimmunized individual. Thus, the chance that a naïve B cell recognizing antigen X and able to act as an APC is in close proximity to an equally rare naïve anti-X Th cell is exceedingly small. In addition, resting mature naïve B cells express only low levels of the costimulatory molecules required for full Th activation. However, once activated, B cells quickly upregulate B7 expression and become effective APCs. Moreover, in a secondary response, anti-X memory B and Th cells are present in significantly greater numbers. Memory B cells thus frequently serve as APCs in the secondary response and become increasingly prominent in this function upon each subsequent encounter with antigen X.

C. Major Antigen Processing and Presentation Pathways

As introduced previously, there are four major pathways of protein antigen processing and presentation, which we now discuss in detail. We conclude this chapter with a discussion of other antigen processing and presentation pathways that are less well defined but still important for immune defense.

NOTE: Several medically important viruses, including HIV, cytomegalovirus (CMV) and human papillomavirus (HPV), escape immune system destruction by interfering with an infected host cell's antigen processing and presentation pathways. These mechanisms are discussed in Chapter 13.

I. Exogenous Antigen Processing Pathway

i) Generation of Peptides via the Exogenous Pathway

An APC that has internalized a whole microbe or macromolecule encloses the entity in a membrane-bound transport vesicle. As introduced in Chapter 3, the transport vesicle enters the endocytic processing system and is successively fused to a series of protease-containing endosomes of increasingly acidic pH. Within the endolysosome, the proteins of the microbe or macromolecule are degraded into peptides of 10–30 amino acids. The peptides are then conveyed in a transport vesicle to specialized late

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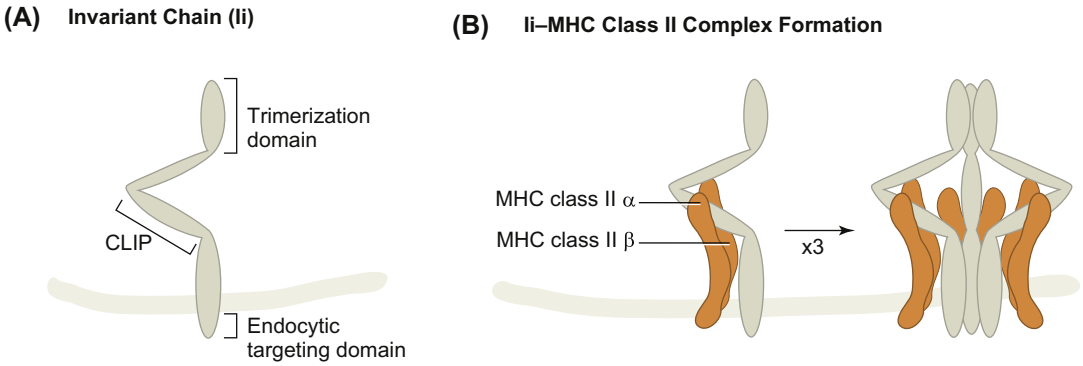

Fig. 7-4
Interaction of MHC Class II and Invariant Chain

(A) Diagram of an Ii monomer showing the trimerization, CLIP, and endocytic targeting domains. **(B)** Within the ER, an MHC class II αβ heterodimer interacts with an Ii monomer such that the CLIP domain is positioned in the peptide-binding groove of the MHC class II protein. Three MHC class II molecules bind to three Ii chains to form a nonameric complex in which each MHC class II binding groove is blocked by a CLIP. *[Adapted from Pieters J. (1997). MHC class II restricted presentation.* Current Opinion in Immunology 9, *89–96.]*

endosomal compartments known as **MIICs** (MHC class II compartments; see later). It is in the MIICs where the peptides bind to newly synthesized MHC class II molecules. Peptides and MHC class II molecules must arrive in the MIICs in a synchronized fashion: if a peptide is not immediately bound by an MHC class II molecule, the peptide is rapidly degraded by lysosomal enzymes contained within the MIIC.

ii) MHC Class II Molecules in the ER and Endosomes

During their synthesis on membrane-bound ribosomes, the α and β chains of the MHC class II molecule are cotranslationally inserted into the ER membrane. A third protein called the **invariant chain** (Ii) is coordinately expressed with the MHC class II chains and is also cotranslationally inserted into the ER membrane. The function of Ii is to bind to newly assembled MHC class II heterodimers and protect the binding groove from being occupied by endogenous peptides present in the ER (see later). The MHC class II molecules are thus "preserved" for the exogenous peptides generated in the endocytic compartment. The part of the Ii molecule that sits in the MHC class II binding groove is called **CLIP** (class II associated invariant chain peptide) (**Fig. 7-4A**). Ii also contains a trimerization domain that allows the formation of a nonameric complex consisting of three MHC class II heterodimers (three αβ pairs) and one Ii trimer (three Ii polypeptides) (**Fig. 7-4B**). Once complexed to Ii, the MHC class II molecules enter the Golgi complex but are then deflected away from the cell's secretory pathway and into its endocytic system by localization sequences in the Ii protein (**Fig. 7-5**). In an endolysosome, most of the Ii protein is degraded by the sequential action of a family of cathepsin enzymes, leaving CLIP stuck in the grooves of the now monomeric MHC class II molecules. The MHC-CLIP complexes then enter the MIICs.

iii) Peptide Loading onto MHC Class II

The next step in exogenous antigen processing is the exchange of the CLIP peptide in the MHC class II binding groove for an exogenous peptide. A non-classical MHC molecule called HLA-DM in humans is essential for this process, but the mechanism remains unclear. (The equivalent molecule in mice is called H-2DM.) Although HLA-DM closely resembles a conventional MHC class II molecule, it does not bind peptides. Instead, the association of HLA-DM with MHC-CLIP likely induces a conformational change that promotes the release of CLIP. HLA-DM then stabilizes the empty MHC class II heterodimer until the exogenous peptide is loaded (refer to **Fig. 7-5**). The actual mechanism of peptide loading has yet to be completely defined, but some studies have indicated that HLA-DM plays an active role in determining which peptides can bind in a given MHC class II antigen-binding site. In any case, once an exogenous peptide lodges stably in the MHC class II groove, the conformation of the MHC

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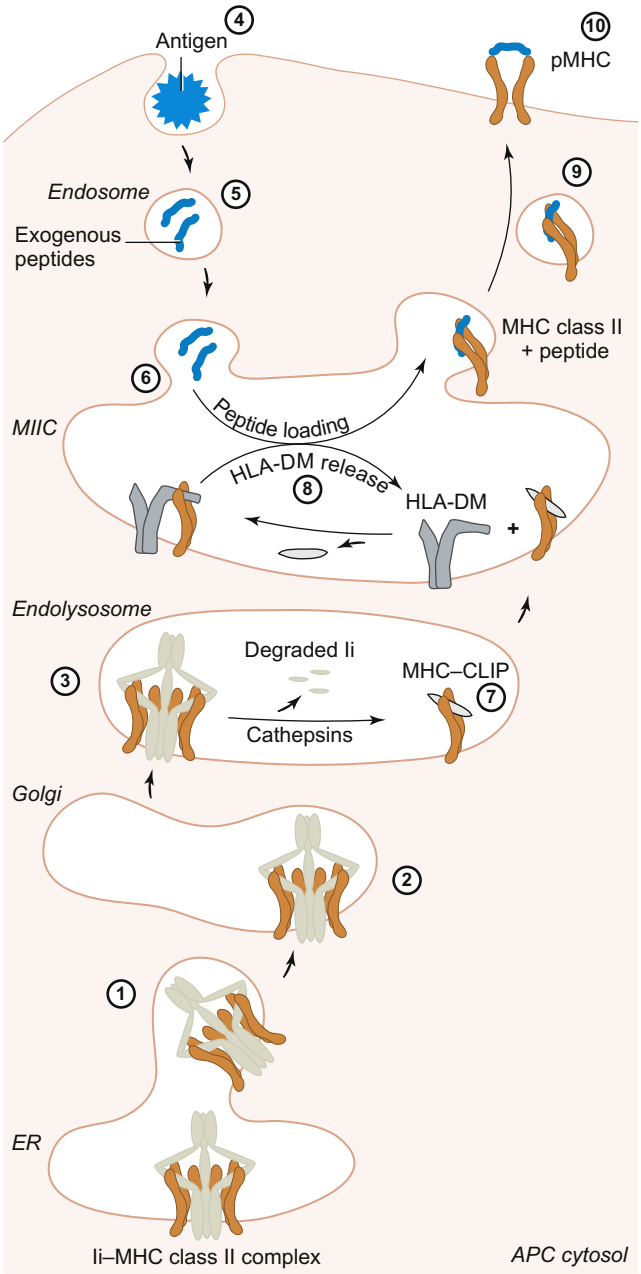
Fig. 7-5
MHC Class II Antigen Presentation Pathway

Within the ER, nonameric complexes are formed in which the peptide-binding groove of each MHC class II heterodimer is blocked by the CLIP domain of an Ii chain (1). These complexes exit the ER, pass through the Golgi (2), and enter an endolysosome (3). If the APC also internalizes an antigen (4), the antigen is degraded by endolysosomal proteases to generate exogenous peptides (5) that are transferred into an MIIC (6). Meanwhile, back in the original endolysosome, the Ii is partially degraded, leaving the CLIP peptide in the MHC class II binding groove (7). The MHC-CLIP complexes are transferred to the MIIC, where the exchange of CLIP for exogenous peptide is mediated by HLA-DM (8). A transport vesicle (9) takes the peptide-loaded MHC class II molecule to the APC surface where it is inserted in the plasma membrane (10).



class II molecule alters again to force dissociation of HLA-DM. The pMHC is then transported out of the MIICs in a vesicle, inserted into the APC membrane by reverse vesicle fusion, and displayed to $CD4^+$ Th cells (refer to **Fig. 7-5**).

II. Endogenous Antigen Processing Pathway

Endogenous antigen processing differs from exogenous processing in three ways: (1) Unlike the limited number of cell types that express MHC class II and so can function as professional APCs, almost any nucleated body cell expresses MHC class I and so can present peptides derived from intracellular antigens to $CD8^+$ CTL effectors (but not naïve Tc cells). Thus, almost any body cell that has become aberrant due to cancer or intracellular infection becomes a target cell for CTL-mediated cytolysis. (2) The processing of the protein antigen takes place in the cytosol rather than in the endocytic system. (3) Peptides generated in the cytosol meet newly synthesized MHC class I molecules in the ER rather than in the MIICs.

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i) Generation of Peptides via the Endogenous Pathway

Viruses or intracellular bacteria that have taken over a host cell force it to use its protein synthesis machinery to make viral or bacterial proteins. Antigenic proteins are thus derived from the translation of viral or bacterial mRNA on host cytosolic ribosomes. Similarly, abnormal proteins synthesized in the cytoplasm of tumor cells can give rise to peptides that appear to be "non-self" to the host's immune system and thus warrant an immune response. The process used to generate peptides from such foreign proteins is basically the same as that used to deal with misfolded or damaged host proteins. In the cytosol of every host cell are large numbers of huge, multi-subunit protease complexes called **proteasomes**. The function of proteasomes is to degrade proteins into peptides. There are two major types of proteasomes: the *standard proteasome* and the *immunoproteasome* **(Fig.7-6)**. Both types of proteasome contain a basic structure called the 20S core proteasome, a hollow cylinder of four stacked polypeptide rings made up of α and β subunits. The α subunits maintain the conformation of the proteasome core while the β subunits are its catalytically active components. The standard proteasome and immunoproteasome differ slightly in the catalytic β subunits included in their 20S cores. In addition to the 20S core, the standard proteasome contains two copies of a structure called the 19S regulatory complex. In addition to its slightly modified 20S core, the immunoproteasome contains two copies of the 19S regulatory complex, and two copies of a proteasome activator (PA) regulatory complex called PA28.

Standard proteasomes are present in all host cells and carry out a housekeeping function by routinely degrading spent and unwanted self proteins. The peptides produced by a standard proteasome are usually 8–18 residues in length. About 20% are the size (8–10 amino acids) that fits neatly into an MHC class I binding groove. Additional trimming of peptides that are initially too large can be carried out by peptidases in the cytosol. The degradation of host proteins in this way and subsequent loading of these peptides onto MHC class I allow constant scanning of self components and the monitoring of the cell's internal health. In contrast to standard proteasomes, immunoproteasomes are not present in most resting host cells, the exceptions being a narrow range of cell types that includes DCs. However, immunoproteasome formation is induced in most host cells by exposure to pro-inflammatory cytokines (such as IFNγ and TNF) that are present at high concentrations during a pathogen attack. Accordingly, immunoproteasomes most often produce peptides from foreign rather than self proteins and are responsible for the majority of the antigen processing associated with immune responses. Most peptides produced by the immunoproteasome are the optimal 8–10 amino acids in length.

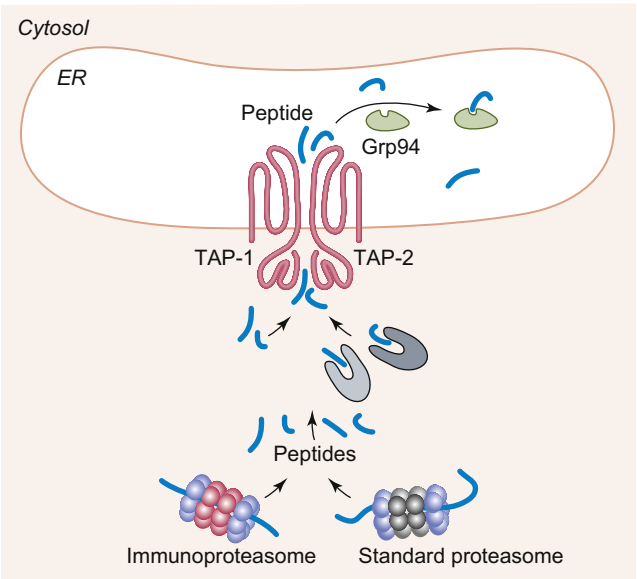

Fig. 7-6
Proteasomes and the TAP Transporter

The TAP transporter is composed of the TAP-1 and TAP-2 subunits and is positioned in the ER membrane. Peptides produced by the immunoproteasome or the standard proteasome in the vicinity of the TAP can bind directly to its peptide-binding site. Alternatively, peptides bound to chaperone proteins (such as the HSP molecules depicted here) are conveyed to the TAP, where they are released. The TAP then transports the peptides into the ER interior, where they bind to ER-resident chaperones (such as the Grp94 molecule depicted here). Peptides of the correct size are then loaded onto MHC class I molecules (not shown).

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ii) Transport of Peptides into the Endoplasmic Reticulum

To induce an immune response, the peptides generated by the proteasomes in a host cell's cytosol must access the binding site of an MHC class I molecule. However, the peptide and the peptide-binding site of the MHC class I molecule are on topologically opposite sides of the cell's membrane system. Unlike the case for MHC class II molecules and their peptides, no vesicle fusion event brings MHC class I molecules and their peptides together. Instead, peptides generated in the cytosol are transported directly into the ER where MHC class I molecules are synthesized.

In the membrane of the ER are positioned transporter structures known as **TAP** (transporter associated with antigen processing) (refer to **Fig. 7-6**). TAP is a heterodimeric molecule composed of two subunits, TAP-1 and TAP-2, which are encoded by genes in the MHC. Structurally, TAP-1 and TAP-2 contain domains that project into the ER lumen, hydrophobic domains that span the ER membrane, and domains that extend into the cytosol and combine to form a single peptide-binding site. Peptides produced by the action of proteasomes are normally subject to very rapid degradation in the cytoplasm but can be rescued from this outcome by binding directly to TAP. Alternatively, the peptides can be bound by "chaperone" proteins, including HSPs and other stress molecules (**see Box 7-1**) that protect the peptides from degradation and escort them to TAP for transfer into the ER. It has been estimated that TAP molecules can translocate 20,000 peptides/min/cell, more than enough to ensure a steady supply for loading onto nascent MHC class I molecules generated in the ER at a rate of 10–100/min. TAP preferentially imports peptides of 8–12 residues in length, although longer peptides can be transported with lower efficiency. Once in the ER lumen, the peptides meet one of four fates. Some peptides are bound immediately to MHC class I molecules, whereas others are temporarily taken up by chaperone proteins resident in the ER (such as Grp94; glucose-regulated protein 94) and protected from further degradation prior to loading onto MHC class I. Still other peptides are trimmed by ER-resident peptidases to achieve the correct length and C-terminus necessary for fitting into the MHC class I groove. Lastly, some peptides are rapidly re-translocated back

Box 7-1 Heat Shock/Stress Proteins as Peptide Chaperones for Antigen Processing

Heat shock proteins (HSPs) are highly conserved members of a larger group of proteins called *stress proteins*. Stress protein expression is sharply increased in cells subjected to environmental assaults such as a sudden temperature increase, cancerous transformation or inflammation. Stress proteins are important for immunity because they bind to proteins and peptides and facilitate constant immune system surveillance of both the intracellular and extracellular protein environment.

Intracellular Surveillance

Some stress proteins act as quality control monitors in the ER, binding to misfolded proteins and preventing them from leaving the ER. Other stress proteins are cytosolic and have a "chaperone" function in that they facilitate polypeptide folding and protect newly synthesized proteins from intracellular degradation. Several HSPs act as intracellular disposal tags, binding to an unwanted protein and conveying it to the proteasome for destruction. HSPs and other stress proteins can also function as chaperones protecting the peptide products of proteasomal degradation in the cytosol. These chaperones facilitate the transfer of endogenous peptides into the ER and the loading of these peptides onto MHC class I. Conversely, certain stress molecules may participate in chaperone-mediated autophagy, diverting intracellular proteins into endocytic compartments for degradation such that the resulting peptides are presented on MHC class II.

Extracellular Surveillance

When a cell dies of necrosis, complexes of peptides or proteins that were bound to stress proteins intracellularly are released to the extracellular environment. Activated macrophages and immature DCs express receptors that recognize HSPs and mediate the uptake of HSP–peptide complexes by these APCs. The HSP–peptide complexes then enter the exogenous antigen processing system, and the peptides emerge displayed on MHC class II. HSPs can also promote the cross-presentation of extracellular peptides on MHC class I if these peptides happen to access the cytosol. A cytosolic HSP can bind to such peptides and convey them to the ER for loading on MHC class I.

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into the cytosol where they are either degraded or re-imported back into the ER via TAP and subjected to further trimming.

iii) MHC Class I Molecules in the ER

The α chain of an MHC class I molecule is synthesized on a membrane-bound ribosome and is cotranslationally inserted into the membrane of the ER. As it enters the ER membrane, the MHC class I α chain associates with a transmembrane chaperone protein called calnexin and a non-transmembrane enzyme called ERp57. Calnexin facilitates proper polypeptide folding and association of the α chain with the coordinately expressed β2m chain. In humans, calnexin is then replaced by a soluble chaperone protein called calreticulin. (In mice, either the original calnexin molecule or an incoming calreticulin molecule associates with the heterodimer after association with β2m.) ERp57 binds to both calnexin and calreticulin and works with these molecules to catalyze the formation of disulfide bonds in MHC class I α chains. ERp57 also promotes the loading of peptide into the MHC class I binding groove.

iv) Peptide Loading onto MHC Class I

To bring a newly synthesized MHC class I heterodimer (with its chaperones) into the vicinity of TAP and the peptides, the MHC class I molecule transiently interacts with a protein called **tapasin** that binds to ERp57, MHC class I and TAP (**Fig. 7-7**). Tapasin helps to stabilize the empty MHC class I heterodimer in a conformation suitable for peptide loading. Exactly how a peptide, either free or bound to a chaperone, accesses the MHC class I binding groove has yet to be determined, but tapasin is important for this process. Tapasin also works with calreticulin to prevent improperly loaded peptide–MHC class I complexes from leaving the ER. Peptide loading is a crucial step in antigen presentation because an MHC class I heterodimer that is transported to the cell surface without a peptide in its groove is unstable and rapidly lost. With the insertion of a pMHC into the membrane of a host cell, it is ready for inspection by $CD8^+$ T cells.

Focus on Relevant Research

"Immunogenetics of *Toxoplasma gondii* Informs Vaccine Design" by Henriquez, F.L., Woods, S., Cong, H., McLeod, R., and Roberts, C.W. (2010) *Trends in Parasitology 26, 550–555.*

The parasite *Toxoplasma gondii* is an important pathogen worldwide and among the leading causes of death in AIDS patients. Antiparasitic drugs are only minimally effective against this scourge, making the development of a prophylactic *T. gondii* vaccine a key objective for researchers in this field. In this article, Henriquez *et al.* review cutting-edge research that (1) points to particular MHC class I alleles as being critical for effective protection against *T. gondii* and (2) tentatively identifies important parasite proteins and short peptides whose processing and presentation may promote $CD8^+$ T cell-mediated immunity against the parasite. Techniques reviewed by the authors include the generation and analysis of mutant mice missing key MHC class I alleles, "caged" MHC tetramer technology, and the use of predictive bioinformatics algorithms. Figures in the article illustrate (1) the natural mechanisms by which *T. gondii* infects a cell and presumably elicits an immune response and (2) the means by which a vaccination strategy might "mimic" the natural infection such that the *T. gondii* proteins are properly processed and displayed. Epitopes for both $CD4^+$ and $CD8^+$ T cells must be provided by the vaccine immunogen, and a PAMP or "TLR ligand" must be present that can engage the appropriate PRRs of immature DCs and induce their maturation. Ideally, these mature DCs would then display the vaccine epitopes on their surfaces in a suitable context for activating pathogen-specific naïve T cells. In vaccine formulations, components that play the role of TLR ligands in the absence of actual infection are known as "adjuvants." Adjuvants are described further in Chapter 14.

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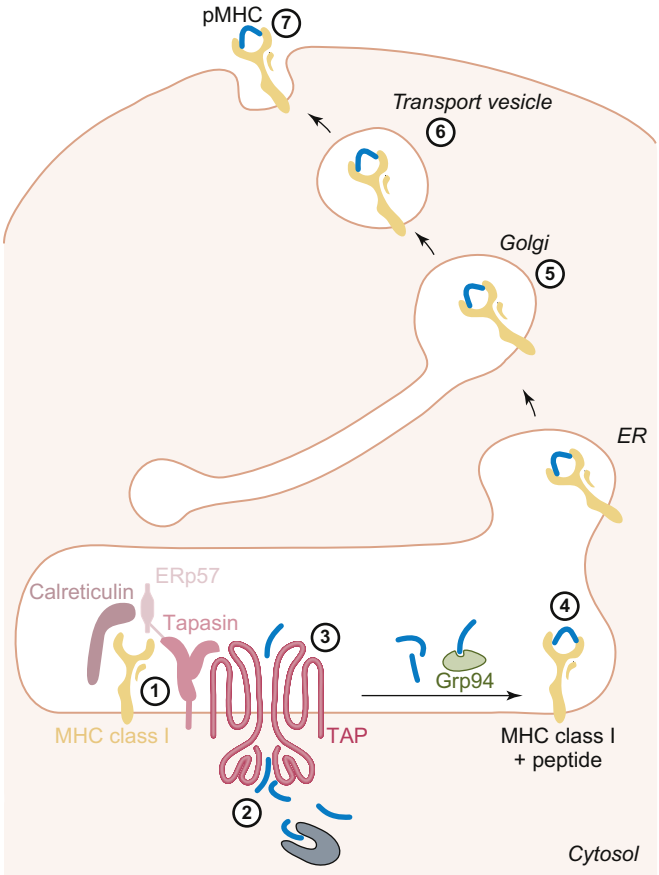
Fig. 7-7
MHC Class I Antigen Presentation Pathway

An MHC class I protein assembled in the ER and bound to its chaperones calreticulin and ERp57 first interacts with tapasin to stabilize the empty molecule and bring it close to the TAP transporter (1). Meanwhile, antigenic peptides generated by proteasomal degradation, which may be bound to HSP chaperones, arrive on the cytosolic side of the TAP (2). The TAP imports the peptides into the ER (3), where they are bound by ER-resident chaperones. The peptides are then transferred into the peptide-binding groove of the MHC class I molecule (4), and the loaded MHC molecule passes through the Golgi (5) and into a transport vesicle (6) prior to insertion in the host cell membrane (7).



III. Cross-Presentation on MHC Class I

Cross-presentation refers to the display on MHC class I of peptides from *extracellularly acquired* antigens. Although this process is thought to be a major means by which DCs can activate naïve Tc cells, the underlying mechanisms are still not well understood. Cross-presentation was discovered when researchers found that $CD8^+$ CTL responses could be mounted to certain antigens that were known to be extracellular, and that peptides from viral antigens could be presented on MHC class I even when the endogenous processing pathway was blocked. The phenomenon was called "cross-presentation" because the viral antigen appeared to physically "cross over" from an infected host cell to an uninfected APC that presented peptides from the antigen on MHC class I as if the antigen had originated in the interior of the APC. Although various APCs appear to be capable of cross-presentation *in vitro*, DCs are the main cell type responsible for antigen cross-presentation *in vivo*. DC subsets vary in their ability to carry out cross-presentation, with resident DCs located in the lungs and blood vessel walls being particularly proficient. In contrast, migratory DCs are less able to cross-present and concentrate on displaying peptides on MHC class II.

How can exogenous peptides be loaded onto MHC class I molecules? APCs (particularly DCs) most often acquire viral antigens by internalizing debris from infected cells that have undergone necrotic or apoptotic death. In rare cases, DCs may also acquire portions of the membranes of live infected cells (by an ill-defined process sometimes called "nibbling"). In all these situations, because the viral protein has entered the APC from the extracellular environment, it is initially directed to the endocytic system in the usual way. Thus, viral peptides appear on the APC surface associated with MHC class II, and a Th response to the antigen can be mounted. However, during the initial processing of the viral proteins in the early endosomes, a fraction of the resulting polypeptides may be actively transported from the endosomes into the cytosol by endosomal membrane

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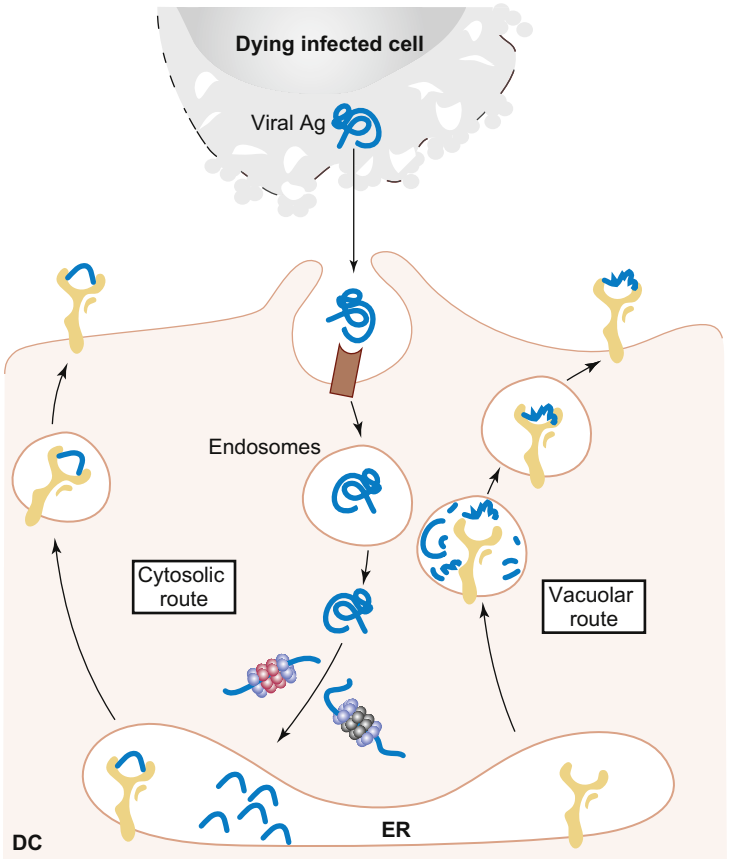

Fig. 7-8
Models of Cross-Presentation on MHC Class I

When an APC takes up antigen from its extracellular environment, such as the viral protein illustrated here, the antigen enters the cell by the usual exogenous processing pathway. However, the antigen can then cross into the endogenous processing pathway by either a cytosolic route involving proteasomes (left), or a vacuolar route involving diversion of MHC class I molecules into endosomes (right). In both cases, peptides derived from the antigen are combined with MHC class I molecules and are presented on the APC surface.

proteins whose role in this translocation process is not yet clear. In the cytosol, the polypeptides are taken up by proteasomes and degraded to peptides (Fig. 7-8). The viral peptides are then transported via TAP into the ER and loaded onto MHC class I just as if the viral protein had originated within the APC itself. This pathway has been dubbed the "cytosolic" route of cross-presentation. Alternatively, there is some evidence for cross-presentation via a "vacuolar" route that does not involve proteasomes. According to this model, some MHC class I molecules may be diverted from the ER into an early endosome where an extracellularly acquired viral antigen is undergoing degradation by lysosomal enzymes. The combination of these diverted MHC class I molecules with the viral peptides within the endosome creates pMHC complexes that subsequently can be transported to the cell surface for antigen presentation. Lastly, a very few extracellular proteins appear to be able to cross the plasma membrane directly, bypass the endocytic system entirely and enter a proteasome. Peptides generated via this latter mechanism could presumably associate with MHC class I via the cytosolic route. Regardless of their pathway of cross-presentation, the end result is the display of viral peptide-MHC class I complexes on the APC surface that can be inspected by antiviral $CD8^+$ T cells.

Antigens from intracellular bacteria and parasites can also be processed and their peptides displayed on MHC class I via cross-presentation.

IV. Autophagic Presentation on MHC Class II

Just as cross-presentation enables the display of exogenous peptides on MHC class I molecules, autophagy allows the display of endogenous peptides on MHC class II. Recent analyses of peptides bound to MHC class II molecules of mouse and human APCs have shown that 20–30% of these ligands originate from cytosolic or nuclear proteins. As described in Chapter 3, unwanted cytosolic entities are routinely dealt with by a form of autophagy known as macroautophagy. Macroautophagy is generally reserved for intracellular entities that are too large for direct proteasomal degradation and/or cannot be kept soluble by chaperone proteins. The reader will recall that, during macroautophagy, a double-layered isolation membrane circularizes around the

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entity to form an autophagosome (refer to Fig. 3-12). The autophagosome can then participate in innate responses by fusing with a lysosome to generate an autophagolysome in which the entire structure is degraded. PAMPs generated by this degradation can then be shunted into endosomal vesicles expressing TLRs in their membranes. To participate in adaptive responses, an autophagosome can fuse instead with an MIIC containing MHC class II molecules. Lysosomal enzymes within the MIIC degrade the autophagosome and its contents, releasing peptides that can be loaded onto MHC class II via HLA-DM. These endogenous peptide-MHC class II complexes are then displayed on the APC surface for inspection by $CD4^+$ T cells.

D. Other Methods of Antigen Presentation

I. Antigen Presentation by MHC Class Ib Molecules

The glycoproteins encoded by the MHC class Ib genes (refer to **Box 6-1**) are closely related in structure to the classical MHC class I molecules. However, MHC class Ib molecules are less polymorphic, are expressed at lower levels and have a more limited pattern of tissue distribution. Some MHC class Ib molecules occur in a secreted form and do not bind to antigenic peptides. Other MHC class Ib molecules are transmembrane proteins that can bind to certain subsets of foreign peptides and present them to subsets of αβ and γδ T cells in a TAP-dependent manner. However, the peptide-binding groove in these MHC class Ib molecules is partially occluded such that a narrower range of shorter peptides is presented.

Antigen presentation to γδ T cells and NKT cells is discussed in depth in Chapter 11.

II. Non-Peptide Antigen Presentation by CD1 Molecules

In Chapter 6, we described "MHC-like" molecules that are encoded outside the MHC but feature an MHC-like fold in their structures. Five MHC-like **CD1 molecules** have been identified: CD1a, CD1b and CD1c (which make up "Group 1"); CD1d (the sole "Group 2" member); and CD1e (which shows only low homology to the other CD1 molecules and so is not considered a member of either group). The CD1 proteins are of particular interest with respect to antigen presentation because these non-polymorphic proteins can present lipid-based (rather than peptide) antigens to certain T cell subsets. The function of CD1 molecules is therefore thought to be the monitoring of the health of the host's cellular lipids, as well as the detection of pathogen lipids. Human APCs can express all five CD1 isoforms, whereas mouse APCs express only CD1d. CD1d is constitutively expressed on APCs of both species, whereas the expression of the Group 1 CD1 molecules on human APCs is upregulated by TLR signaling. Indeed, the expression of a particular CD1 isoform by human APCs may be dictated by cytokines or other signals present in the immediate inflammatory microenvironment.

The antigen-binding groove in CD1 molecules is much more hydrophobic than that of classical MHC molecules, suiting it to lipid binding. In humans, CD1a, CD1b and CD1c molecules present lipid antigens derived from diacylglycerol, phospholipid, lipopeptide, polyketide, glycolipid, and glycosphingolipid molecules to subsets of αβ Th and Tc cells. CD1e has a large lipid-binding pocket but is not expressed on the APC surface and so does not participate directly in antigen presentation. In both mice and humans, CD1d molecules present a very restricted collection of ceramide-based antigens to NKT cells and some T cell subsets. It is thought that the TCRs of these cells "see" a combined epitope composed of amino acids of the CD1 molecule plus a small portion of the carbohydrate head group of the lipid. NKT and T cells can be very discriminating in their recognition of CD1-presented antigens, failing to respond if the orientation of even a single hydroxyl group of the antigen is changed. The consequences of CD1-mediated antigen presentation are virtually identical to those of

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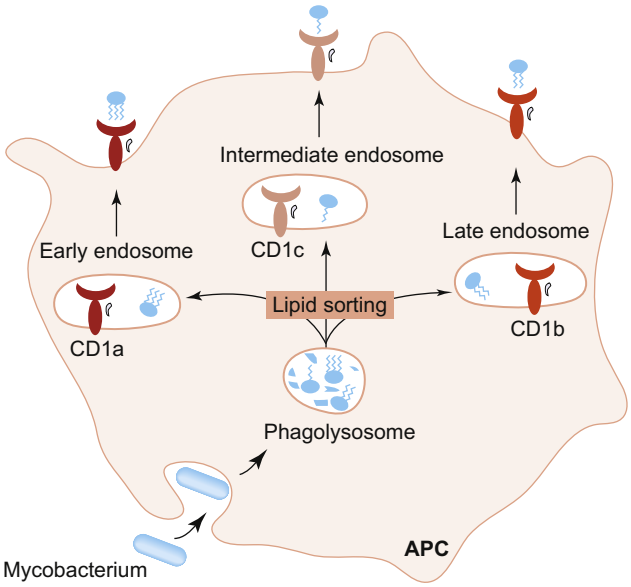

Fig. 7-9
Presentation of Mycobacterial Lipid Antigens by Human CD1 Molecules

A mycobacterium that has been phagocytosed by an APC is digested in a phagolysosome. The bacterial lipids are sorted by structure into the indicated endosome where they are loaded onto the indicated CD1 family members. The CD1-lipid complexes are then displayed on the APC surface for presentation to particular subsets of αβ and γδ T cells and NKT cells.

MHC-mediated peptide presentation; that is, activated NKT cells and Th effectors secrete cytokines, while Tc cells generate CTLs that kill target cells by cytolysis. The activities of these CD1-restricted effectors have been shown to influence the outcome of infections with various viruses and species of bacteria, fungi and protozoa.

Antigen processing and presentation by CD1 molecules appears to utilize elements of both the exogenous and endogenous pathways. Like MHC class I, CD1 chains must be associated with β2m to be transported to the cell surface, but, unlike MHC class I, antigen loading of CD1 molecules does not take place in the ER. Instead, like MHC class II, CD1 molecules are targeted to the endocytic system where they are loaded with antigen, but no association with either Ii or HLA-DM is required. Because of the presence or absence of particular amino acid motifs, different human CD1 molecules accumulate in different endosomal compartments. For example, CD1a molecules are found in early endosomes, while CD1c molecules tend to collect in intermediate endosomes, and CD1b molecules are directed to late endosomes. CD1e appears to translocate from the Golgi to the lysosomes in response to infection and then facilitates the selection of exogenous and endogenous lipids to be displayed by other CD1 family members. In addition to this endosomal sorting of CD1 molecules, different lipid antigens also tend to accumulate in different endosomal compartments. Depending on the structure of the lipid, it is sorted and confined to either an early endosome, an intermediate endosome or a late endosome. At the molecular level, this sorting seems to be determined at least partially by the length of the lipid's alkyl chains, with the earliest endosomes containing lipids with the longest alkyl chains. These measures allow the APC to ensure that the right lipid is loaded onto the right CD1 molecule. Once loaded with lipid, a CD1 molecule is then transported to the plasma membrane for display on the APC surface. In addition, extracellular lipids with short alkyl chains can be loaded directly into the binding grooves of CD1 molecules that have already made it to the APC surface. The actual kinetics and mechanisms involved in the antigen loading of CD1 molecules and their subsequent presentation on the APC surface remain unclear. A schematic representation of CD1-mediated antigen presentation appears in **Figure 7-9**.

MHC molecules presenting foreign peptides are the body's signposts to the immune system that a T cell–mediated adaptive response is required. The next chapter discusses the genes and proteins of TCRs, the antigen receptor molecules that carry out pMHC recognition.

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Chapter 7 Take-Home Message

- The pMHCs recognized by the TCRs of T cells are assembled by four major antigen processing and presentation pathways: exogenous, endogenous, cross-presentation and autophagy.
- Professional APCs include mature DCs, macrophages and B cells. These cell types take up antigen efficiently and express MHC class II and costimulatory molecules inducibly or constitutively. However, only mature DCs can activate naïve Tc and Th cells.
- Immature DCs may be migratory or lymphoid-resident. DC maturation is triggered by PRR engagement by DAMPs/PAMPs plus pro-inflammatory cytokine signaling. Macrophages and B cells are efficient APCs for effector and memory T cells.
- In the exogenous pathway, extracellular antigens are internalized by APCs and degraded within endosomal compartments. The resulting peptides bind to MHC class II molecules to form pMHCs that are transported from the endosomes to the APC surface for recognition by $CD4^+$ Th cells.
- In the endogenous pathway, antigens that are produced intracellularly as a result of host cell infection or transformation are degraded by cytoplasmic proteasomes. The resulting peptides are actively transported into the ER where they bind to MHC class I molecules to form pMHC complexes that are transported to the cell surface for recognition by $CD8^+$ CTLs.
- Cross-presentation refers to the display on MHC class I of peptides from extracellularly acquired antigens. DCs can use cross-presentation to activate naïve Tc cells.
- Autophagic presentation refers to the display on MHC class II of peptides from intracellularly acquired antigens. DCs can use autophagic presentation to activate naïve Th cells.
- Non-classical MHC class Ib molecules present very short peptide antigens to subsets of αβ and γδ T cells.
- The CD1 proteins are MHC-like molecules that present lipid-based antigens to subsets of αβ and γδ T cells and NKT cells.

Did You Get it? a Self-Test Quiz

Section A

1) Distinguish between antigen processing and antigen presentation.
2) Distinguish between the exogenous and endogenous antigen processing and presentation pathways.
3) How did cross-presentation get its name?
4) Which major antigen processing pathway would be used to initiate an immune response against a liver cancer and why?
5) Why is it $CD4^+$ rather than $CD8^+$ T cells that respond to extracellular pathogens?

Section B

1) Can you define these terms? Langerhans cell, inflammatory DC, interstitial DC
2) What cell types can function as professional APCs and why?
3) Distinguish between the two main types of DCs present in a host at steady state.
4) Distinguish between migratory and lymphoid-resident DCs.
5) What is the main function of pDCs? thymic DCs? splenic DCs?
6) Why can't immature DCs activate T cells?
7) Does apoptotic cell death induce DC maturation? If not, why not?
8) Give three ways in which immature DCs differ from mature DCs.
9) Briefly outline how DCs influence Th cell differentiation.
10) Why are macrophages considered amplifiers of the adaptive response?
11) Why are B cells efficient APCs for the secondary response?

Section C

1) Can you define these terms? MIICs, CLIP, Ii
2) Where in the cell does the final degradation of extracellularly derived proteins most often occur?
3) Describe the structure and function of the invariant chain.
4) Where in the cell do exogenous peptides and MHC class II meet?
5) What is the role of HLA-DM during the loading of peptides onto MHC class II?
6) How can stress molecules contribute to antigen presentation?
7) Distinguish between standard proteasomes and immunoproteasomes in terms of structure and function.
8) Why are endogenous peptides transported into the ER?

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Did You Get it? a Self-Test Quiz—Continued

9) Describe the structure and function of TAP.

10) How does tapasin facilitate pMHC formation?

11) What is a chaperone protein? Give three examples of such proteins and their functions.

12) How is cross-presentation thought to allow DCs to activate naïve Tc cells?

13) Describe two ways in which an uninfected APC might acquire antigens from intracellular pathogens.

14) How do the cytosolic and vacuolar routes of cross-presentation differ?

15) How does autophagy promote the display of intracellular peptides on MHC class II?

Section D

1) How does antigen presentation by MHC class I and Ib molecules differ?

2) What types of antigens are presented by CD1 molecules?

3) Describe how elements of both the standard exogenous and endogenous antigen processing pathways are used in antigen processing and presentation by CD1 molecules.

Can You Extrapolate? Some Conceptual Questions

1) Why might poor lymphatic drainage result in less effective immune responses?

2) In the case of the following mutations, each of which renders the affected component non-functional, would you expect the greatest effect to be on the activation of $CD4^+$ T cells, $CD8^+$ T cells, or both?

Functional mutation of

a) HLA-DM

b) TAP

c) Standard proteasome

d) Immunoproteasome

e) CLIP

f) β2m

3) The HA (hemagglutinin) molecule of influenza virus is a membrane-bound glycoprotein found on the surfaces of both virions and influenza-infected cells. The amino acid sequence of HA includes a leader (L) sequence that directs the protein into transport vesicles after it is translated in the ER. The HA protein is then displayed on the surface of the infected host cell after the transport vesicle fuses with the host cell's plasma membrane. If a new strain of influenza virus ($HA\text{-}L^-$) is engineered in which the HA gene has no leader sequence, how might the CTL-mediated response to the $HA\text{-}L^-$ virus compare with the CTL-mediated response to the wild type virus? You may assume that the wild type and $HA\text{-}L^-$ viruses infect cells equally well.

Would You Like To Read More?

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