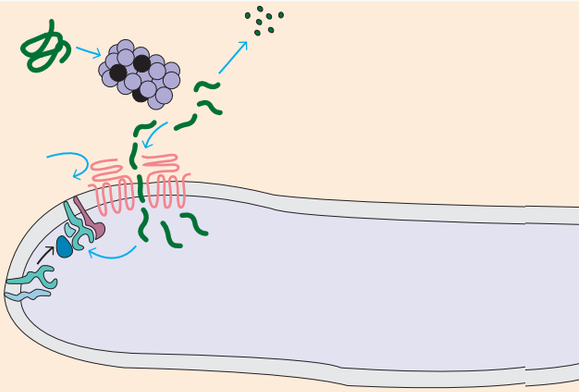
chapter 8

# Antigen Processing and Presentation



**Antigen Processing for Presentation by Class I MHC Molecules**



Recognition of foreign protein antigens by a T cell requires that peptides derived from the antigen be displayed within the cleft of an MHC molecule on the membrane of a cell. The formation of these peptide-MHC complexes requires that a protein antigen be degraded into peptides by a sequence of events called **antigen processing.** The degraded peptides then associate with MHC molecules within the cell interior, and the peptide-MHC complexes are transported to the membrane, where they are displayed (**antigen presentation**).

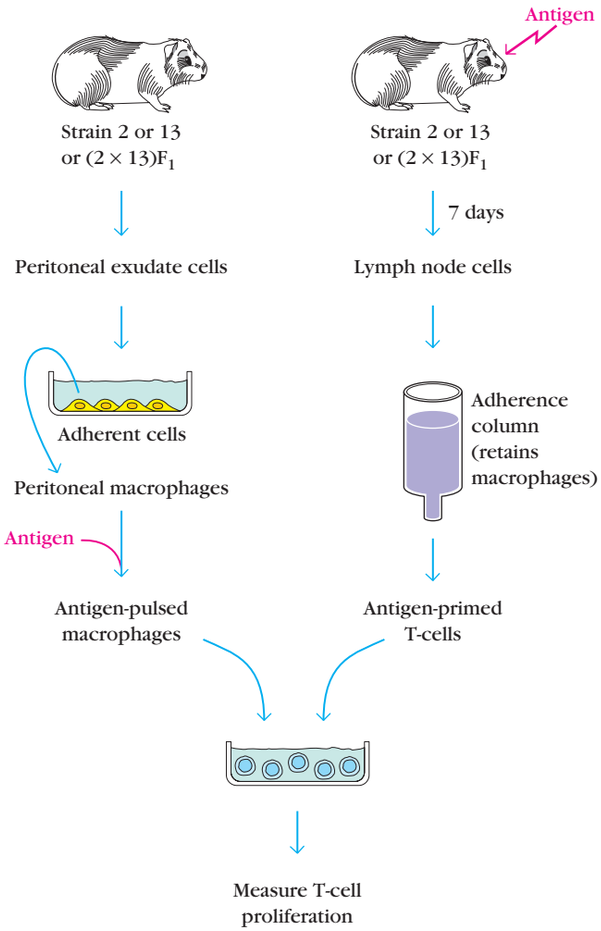
Class I and class II MHC molecules associate with peptides that have been processed in different intracellular compartments. Class I MHC molecules bind peptides derived from **endogenous antigens** that have been processed within the cytoplasm of the cell (e.g., normal cellular proteins, tumor proteins, or viral and bacterial proteins produced within infected cells). Class II MHC molecules bind peptides derived from **exogenous antigens** that are internalized by phagocytosis or endocytosis and processed within the endocytic pathway. This chapter examines in more detail the mechanism of antigen processing and the means by which processed antigen and MHC molecules are combined. In addition, a third pathway for the presentation of nonpeptide antigens derived from bacterial pathogens is described.

## Self-MHC Restriction of T Cells

Both $CD4^+$ and $CD8^+$ T cells can recognize antigen only when it is presented by a self-MHC molecule, an attribute called *self-MHC restriction.* Beginning in the mid-1970s, experiments conducted by a number of researchers demonstrated self-MHC restriction in T-cell recognition. A. Rosenthal and E. Shevach, for example, showed that antigen-specific proliferation of $T_H$ cells occurred only in response to antigen presented by macrophages of the same MHC haplotype as the T cells. In their experimental system, guinea pig macrophages from strain 2 were initially incubated with an antigen. After the "antigen-pulsed" macrophages had processed the antigen and presented it on their surface, they were mixed with T cells from the same strain (strain 2), a different strain (strain 13), or $(2 \times 13)$ $F_1$ animals, and the magnitude of T-cell proliferation in response to the antigen-pulsed macrophages was measured.

The results of these experiments, outlined in Figure 8-1, showed that strain-2 antigen-pulsed macrophages activated strain-2 and $F_1$ T cells but not strain-13 T cells. Similarly, strain-13 antigen-pulsed macrophages activated strain-13 and $F_1$ T cells but not strain-2 T cells. Subsequently, congenic and recombinant congenic strains of mice, which differed from each other only in selected regions of the H-2 complex, were used as the source of macrophages and T cells. These experiments confirmed that the $CD4^+$ $T_H$ cell is activated and proliferates only in the presence of antigen-pulsed macrophages that share class II MHC alleles. Thus, antigen recognition by the $CD4^+$ $T_H$ cell is *class II MHC restricted.*

In 1974 R. Zinkernagel and P. Doherty demonstrated the self-MHC restriction of $CD8^+$ T cells. In their experiments, mice were immunized with lymphocytic choriomeningitis (LCM) virus; several days later, the animals' spleen cells, which included $T_C$ cells specific for the virus, were isolated and incubated with LCM-infected target cells of the same or different haplotype (Figure 8-2). They found that the $T_C$ cells killed only syngeneic virus-infected target cells. Later studies with congenic and recombinant congenic strains showed




| Antigen-primed T cell | Antigen-pulsed macrophages | | |
|---|---|---|---|
| | Strain 2 | Strain 13 | $(2 \times 13)F_1$ |
| Strain 2 | + | − | + |
| Strain 13 | − | + | + |
| $(2 \times 13)F_1$ | + | + | + |

**FIGURE 8-1** Experimental demonstration of self-MHC restriction of $T_H$ cells. Peritoneal exudate cells from strain 2, strain 13, or (2 × 13) $F_1$ guinea pigs were incubated in plastic Petri dishes, allowing enrichment of macrophages, which are adherent cells. The peritoneal macrophages were then incubated with antigen. These "antigen-pulsed" macrophages were incubated in vitro with T cells from strain 2, strain 13, or (2 × 13) $F_1$ guinea pigs, and the degree of T-cell proliferation was assessed. The results indicated that $T_H$ cells could proliferate only in response to antigen presented by macrophages that shared MHC alleles. *[Adapted from A. Rosenthal and E. Shevach, 1974,* J. Exp. Med. ***138:****1194, by copyright permission of the Rockefeller University Press.]*

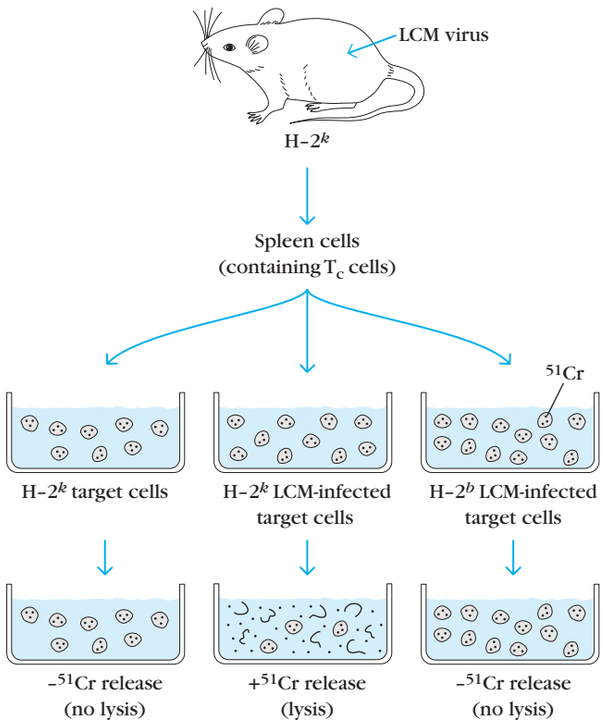



**FIGURE 8-2** Classic experiment of Zinkernagel and Doherty demonstrating that antigen recognition by $T_C$ cells exhibits MHC restriction. H-$2^k$ mice were primed with the lymphocytic choriomeningitis (LCM) virus to induce cytotoxic T lymphocytes (CTLs) specific for the virus. Spleen cells from this LCM-primed mouse were then added to target cells of different H-2 haplotypes that were intracellularly labeled with $^{51}Cr$ (black dots) and either infected or not with the LCM virus. CTL-mediated killing of the target cells, as measured by the release of $^{51}Cr$ into the culture supernatant, occurred only if the target cells were infected with LCM and had the same MHC haplotype as the CTLs. *[Adapted from P. C. Doherty and R. M. Zinkernagel, 1975,* J. Exp. Med. ***141:****502.]*

that the $T_C$ cell and the virus-infected target cell must share class I molecules encoded by the K or D regions of the MHC. Thus, antigen recognition by $CD8^+$ $T_C$ cells is *class I MHC restricted.* In 1996, Doherty and Zinkernagel were awarded the Nobel prize for their major contribution to the understanding of cell-mediated immunity.

## Role of Antigen-Presenting Cells

As early as 1959, immunologists were confronted with data suggesting that T cells and B cells recognized antigen by different mechanisms. The dogma of the time, which persisted until the 1980s, was that cells of the immune system recognize the entire protein in its native conformation. However, experiments by P. G. H. Gell and B. Benacerraf demonstrated that, while a primary antibody response and cell-mediated response were induced by a protein in its native conformation, a secondary antibody response (mediated by B cells) could be induced only by native antigen, whereas a secondary

cell-mediated response could be induced by either the native or the denatured antigen (see Table 3-5). These findings were viewed as an interesting enigma, but implications for antigen presentation were completely overlooked until the early 1980s.

## Processing of Antigen Is Required for Recognition by T Cells

The results obtained by K. Ziegler and E. R. Unanue were among those that contradicted the prevailing dogma that antigen recognition by B and T cells was basically similar. These researchers observed that $T_H$-cell activation by bacterial protein antigens was prevented by treating the antigen-presenting cells with paraformaldehyde prior to antigen exposure. However, if the antigen-presenting cells were first allowed to ingest the antigen and were fixed with paraformaldehyde 1–3 h later, $T_H$-cell activation still occurred (Figure 8-3a,b). During that interval of 1–3 h, the antigen-presenting cells had processed the antigen and had displayed it on the membrane in a form able to activate T cells.

Subsequent experiments by R. P. Shimonkevitz showed that internalization and processing could be bypassed if antigen-presenting cells were exposed to peptide digests of an antigen instead of the native antigen (Figure 8-3c). In these experiments, antigen-presenting cells were treated with glutaraldehyde (this chemical, like paraformaldehyde, fixes the cell, making the membrane impermeable) and then incubated with native ovalbumin or with ovalbumin that had been subjected to partial enzymatic digestion. The digested ovalbumin was able to interact with the glutaraldehyde-fixed antigen-presenting cells, thereby activating ovalbumin-specific $T_H$ cells, whereas the native ovalbumin failed to do so. These results suggest that antigen processing involves the digestion of the protein into peptides that are recognized by the ovalbumin-specific $T_H$ cells.

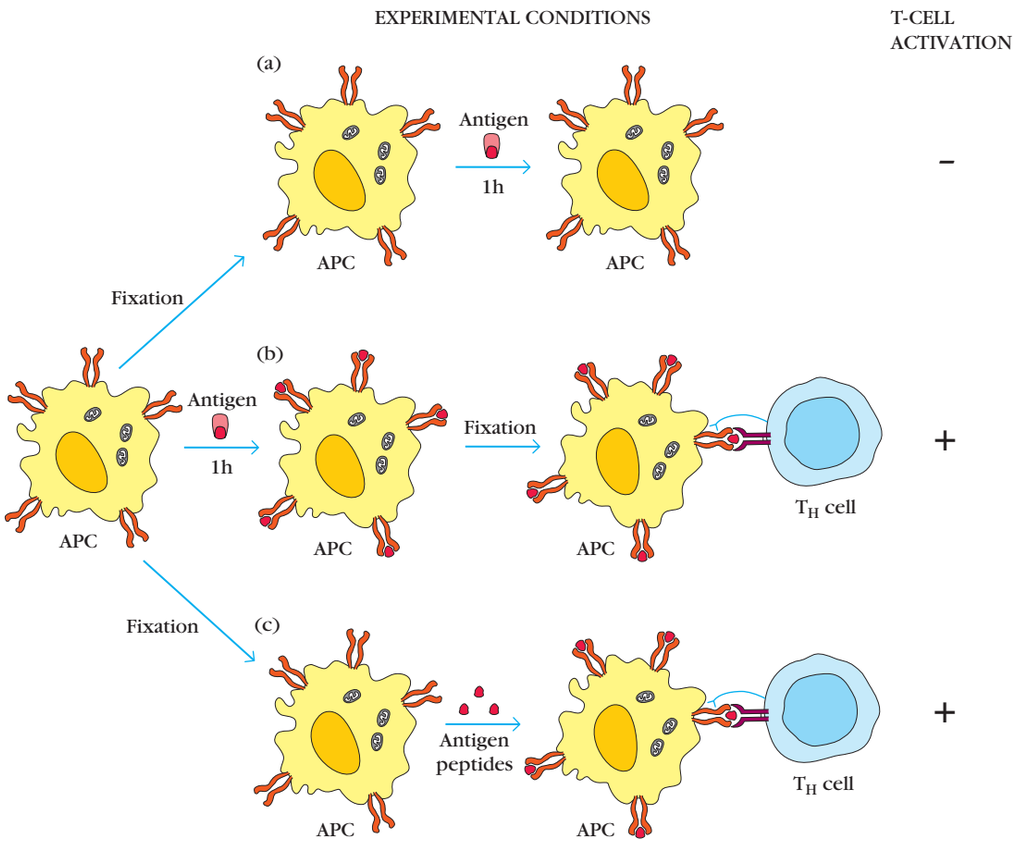



**FIGURE 8-3** Experimental demonstration that antigen processing is necessary for $T_H$-cell activation. (a) When antigen-presenting cells (APCs) are fixed before exposure to antigen, they are unable to activate $T_H$ cells. (b) In contrast, APCs fixed at least 1 h after antigen exposure can activate $T_H$ cells. (c) When APCs are fixed before antigen exposure and incubated with peptide digests of the antigen (rather than native antigen), they also can activate $T_H$ cells. $T_H$-cell activation is determined by measuring a specific $T_H$-cell response (e.g., cytokine secretion).

**TABLE 8-1** Antigen-presenting cells

| Professional antigen-presenting cells | Nonprofessional antigen-presenting cells | |
|---|---|---|
| Dendritic cells (several types) | Fibroblasts (skin) | Thymic epithelial cells |
| Macrophages | Glial cells (brain) | Thyroid epithelial cells |
| B cells | Pancreatic beta cells | Vascular endothelial cells |

At about the same time, A. Townsend and his colleagues began to identify the proteins of influenza virus that were recognized by $T_C$ cells. Contrary to their expectations, they found that internal proteins of the virus, such as matrix and nucleocapsid proteins, were often recognized by $T_C$ cells better than the more exposed envelope proteins. Moreover, Townsend's work revealed that $T_C$ cells recognized short linear peptide sequences of the influenza protein. In fact, when noninfected target cells were incubated in vitro with synthetic peptides corresponding to sequences of internal influenza proteins, these cells could be recognized by $T_C$ cells and subsequently lysed just as well as target cells that had been infected with live influenza virus. These findings along with those presented in Figure 8-3 suggest that antigen processing is a metabolic process that digests proteins into peptides, which can then be displayed on the cell membrane together with a class I or class II MHC molecule.

## Most Cells Can Present Antigen with Class I MHC; Presentation with Class II MHC Is Restricted to APCs

Since all cells expressing either class I or class II MHC molecules can present peptides to T cells, strictly speaking they all could be designated as antigen-presenting cells. However, by convention, cells that display peptides associated with class I MHC molecules to $CD8^+$ $T_C$ cells are referred to as *target cells;* cells that display peptides associated with class II MHC molecules to $CD4^+$ $T_H$ cells are called **antigen-presenting cells (APCs).** This convention is followed throughout this text.

A variety of cells can function as antigen-presenting cells. Their distinguishing feature is their ability to express class II MHC molecules and to deliver a co-stimulatory signal. Three cell types are classified as *professional* antigen-presenting cells: dendritic cells, macrophages, and B lymphocytes. These cells differ from each other in their mechanisms of antigen uptake, in whether they constitutively express class II MHC molecules, and in their co-stimulatory activity:

- Dendritic cells are the most effective of the antigen-presenting cells. Because these cells constitutively express a high level of class II MHC molecules and co-stimulatory activity, they can activate naive $T_H$ cells.
- Macrophages must be activated by phagocytosis of particulate antigens before they express class II MHC molecules or the co-stimulatory B7 membrane molecule.
- B cells constitutively express class II MHC molecules but must be activated before they express the co-stimulatory B7 molecule.

Several other cell types, classified as *nonprofessional* antigen-presenting cells, can be induced to express class II MHC molecules or a co-stimulatory signal (Table 8-1). Many of these cells function in antigen presentation only for short periods of time during a sustained inflammatory response.

Because nearly all nucleated cells express class I MHC molecules, virtually any nucleated cell is able to function as a target cell presenting endogenous antigens to $T_C$ cells. Most often, target cells are cells that have been infected by a virus or some other intracellular microorganism. However, altered self-cells such as cancer cells, aging body cells, or allogeneic cells from a graft can also serve as targets.

# Evidence for Two Processing and Presentation Pathways

The immune system uses two different pathways to eliminate intracellular and extracellular antigens. Endogenous antigens (those generated within the cell) are processed in the *cytosolic pathway* and presented on the membrane with class I MHC molecules; exogenous antigens (those taken up by endocytosis) are processed in the *endocytic pathway* and presented on the membrane with class II MHC molecules (Figure 8-4).

Experiments carried out by L. A. Morrison and T. J. Braciale provided early evidence that the antigenic peptides presented by class I and class II MHC molecules are derived from different processing pathways. These researchers based their experimental protocol on the properties of two clones of $T_C$ cells, one that recognized influenza hemagglutinin (HA) associated with a class I MHC molecule, and an atypical $T_C$ line that recognized the same antigen associated with a class II MHC molecule. (In this case, and in some

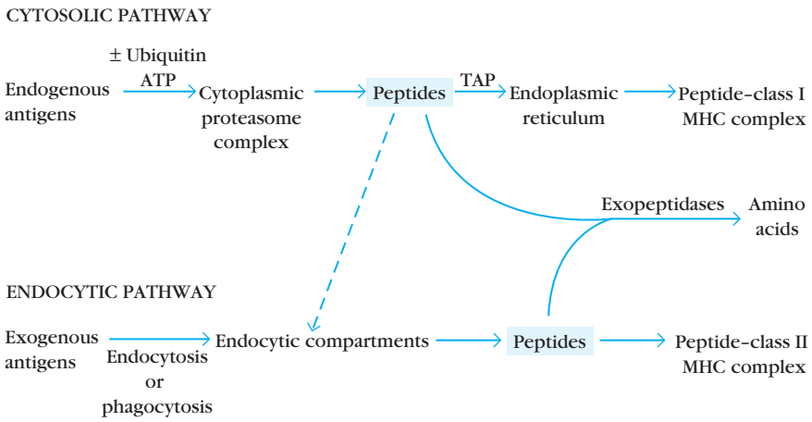



**FIGURE 8-4** Overview of cytosolic and endocytic pathways for processing antigen. The proteasome complex contains enzymes that cleave peptide bonds, converting proteins into peptides. The antigenic peptides from proteasome cleavage and those from endocytic compartments associate with class I or class II MHC molecules, and the peptide-MHC complexes are then transported to the cell membrane. TAP (*t*ransporter of *a*ntigenic *p*eptides) transports the peptides to the endoplasmic reticulum. It should be noted that the ultimate fate of most peptides in the cell is neither of these pathways, but rather to be degraded completely into amino acids.

others as well, the association of T-cell function with MHC restriction is not absolute). In one set of experiments, target cells that expressed both class I and class II MHC molecules were incubated with infectious influenza virus or with UV-inactivated influenza virus. (The inactivated virus retained its antigenic properties but was no longer capable of replicating within the target cells.) The target cells were then incubated with the class I–restricted or the atypical class II–restricted $T_C$ cells and subsequent lysis of the target cells was determined. The results of their experiments, presented in Table 8-2, show that the class II–restricted $T_C$ cells responded to target cells treated with either infectious or noninfectious influenza virions. The class I–restricted $T_C$ cells responded only to target cells treated with infectious virions. Similarly, target cells that had been treated with infectious influenza virions in the presence of emetine, which inhibits viral protein synthesis, stimulated the class II–restricted $T_C$ cells but not the class I–restricted $T_C$ cells. Conversely, target cells that had been treated with infectious virions in the presence of chloroquine, a drug that blocks the endocytic processing pathway, stimulated class I– but not class II–restricted $T_C$ cells.

These results support the distinction between the processing of exogenous and endogenous antigens, including the preferential association of exogenous antigens with class II MHC molecules and of endogenous antigens with class I

**TABLE 8-2** Effect of antigen presentation on activation of class I and class II MHC-restricted $T_C$ cells

| | CTL ACTIVITY[†] | |
|---|---|---|
| Treatment of target cells[*] | Class I restricted | Class II restricted |
| Infectious virus | + | + |
| UV-inactivated virus (noninfectious) | − | + |
| Infectious virus + emetine | − | + |
| Infectious virus + chloroquine | + | − |

[*]Target cells, which expressed both class I and class II MHC molecules, were treated with the indicated preparations of influenza virus and other agents. Emetine inhibits viral protein synthesis, and chloroquine inhibits the endocytic processing pathway.

[†]Determined by lysis (+) and no lysis (−) of the target cells.

SOURCE: Adapted from T. J. Braciale et al., 1987, *Immunol. Rev.* **98:**95.

MHC molecules. Association of viral antigen with class I MHC molecules required replication of the influenza virus and viral protein synthesis within the target cells; association with class II did not. These findings suggested that the peptides presented by class I and class II MHC molecules are trafficked through separate intracellular compartments; class I MHC molecules interact with peptides derived from cytosolic degradation of endogenously synthesized proteins, class II molecules with peptides derived from endocytic degradation of exogenous antigens. The next two sections examine these two pathways in detail.

## Endogenous Antigens: The Cytosolic Pathway

In eukaryotic cells, protein levels are carefully regulated. Every protein is subject to continuous turnover and is degraded at a rate that is generally expressed in terms of its half-life. Some proteins (e.g., transcription factors, cyclins, and key metabolic enzymes) have very short half-lives; denatured, misfolded, or otherwise abnormal proteins also are degraded rapidly. The pathway by which endogenous antigens are degraded for presentation with class I MHC molecules utilizes the same pathways involved in the normal turnover of intracellular proteins.

### Peptides for Presentation Are Generated by Protease Complexes Called Proteasomes

Intracellular proteins are degraded into short peptides by a cytosolic proteolytic system present in all cells. Those proteins targeted for proteolysis often have a small protein, called *ubiquitin,* attached to them (Figure 8-5a). Ubiquitin-protein conjugates can be degraded by a multifunctional protease complex called a **proteasome.** Each proteasome is a large (26S), cylindrical particle consisting of four rings of protein subunits with a central channel of diameter 10–50 Å. A proteasome can cleave peptide bonds between 2 or 3 different amino acid combinations in an ATP-dependent process (Figure 8-5b). Degradation of ubiquitin-protein complexes is thought to occur within the central hollow of the proteasome.

Experimental evidence indicates that the immune system utilizes this general pathway of protein degradation to produce small peptides for presentation with class I MHC molecules. The proteasomes involved in antigen processing include two subunits encoded within the MHC gene cluster, LMP2 and LMP7, and a third non-MHC protein, LMP10 (also called MECL-1). All three are induced by increased levels of the T-cell cytokine IFN-γ. The peptidase activities of proteasomes containing LMP2, LMP7, and LMP10 preferentially generate peptides that bind to MHC class I molecules. Such proteasomes, for example, show increased hydrolysis of peptide bonds that follow basic and/or hydrophobic residues. As described in Chapter 7, peptides that bind to class I MHC molecules terminate almost exclusively with hydrophobic or basic residues.

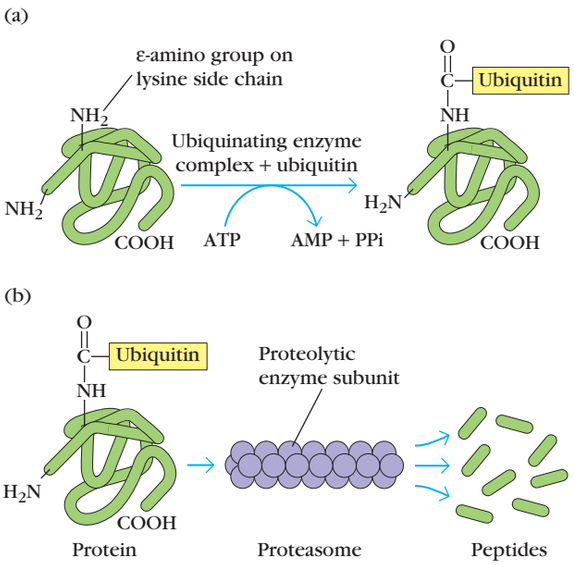



**FIGURE 8-5** Cytosolic proteolytic system for degradation of intracellular proteins. (a) Proteins to be degraded are often covalently linked to a small protein called ubiquitin. In this reaction, which requires ATP, a ubiquinating enzyme complex links several ubiquitin molecules to a lysine-amino group near the amino terminus of the protein. (b) Degradation of ubiquitin-protein complexes occurs within the central channel of proteasomes, generating a variety of peptides. Proteasomes are large cylindrical particles whose subunits catalyze cleavage of peptide bonds.

### Peptides Are Transported from the Cytosol to the Rough Endoplasmic Reticulum

Insight into the role that peptide transport, the delivery of peptides to the MHC molecule, plays in the cytosolic processing pathway came from studies of cell lines with defects in peptide presentation by class I MHC molecules. One such mutant cell line, called RMA-S, expresses about 5% of the normal levels of class I MHC molecules on its membrane. Although RMA-S cells synthesize normal levels of class I α chains and $\beta_2$-microglobulin, neither molecule appears on the membrane. A clue to the mutation in the RMA-S cell line was the discovery by A. Townsend and his colleagues that "feeding" these cells peptides restored their level of membrane-associated class I MHC molecules to normal. These investigators suggested that peptides might be required to stabilize the interaction between the class I α chain and $\beta_2$-microglobulin. The ability to restore expression of class I MHC molecules on the membrane by feeding the cells predigested peptides suggested that the RMA-S cell line might have a defect in peptide transport.

Subsequent experiments showed that the defect in the RMA-S cell line occurs in the protein that transports peptides from the cytoplasm to the RER, where class I molecules are synthesized. When RMA-S cells were transfected with a functional gene encoding the transporter protein, the cells began to express class I molecules on the membrane. The transporter protein, designated **TAP** (for **transporter associated with antigen processing**) is a membrane-spanning heterodimer consisting of two proteins: TAP1 and TAP2 (Figure 8-6a). In addition to their multiple transmembrane segments, the TAP1 and TAP2 proteins each have a domain projecting into the lumen of the RER, and an ATP-binding domain that projects into the cytosol. Both TAP1 and TAP2 belong to the family of ATP-binding cassette proteins found in the membranes of many cells, including bacteria; these proteins mediate ATP-dependent transport of amino acids, sugars, ions, and peptides.

Peptides generated in the cytosol by the proteasome are translocated by TAP into the RER by a process that requires the hydrolysis of ATP (Figure 8-6b). TAP has the highest affinity for peptides containing 8–10 amino acids, which is the optimal peptide length for class I MHC binding. In addition, TAP appears to favor peptides with hydrophobic or basic carboxyl-terminal amino acids, the preferred anchor residues for class I MHC molecules. Thus, TAP is optimized to transport peptides that will interact with class I MHC molecules.

The *TAP1* and *TAP2* genes map within the class II MHC region, adjacent to the *LMP2* and *LMP7* genes (see Figure 7-15). Both the transporter genes and these *LMP* genes are polymorphic; that is, different allelic forms of these genes exist within the population. Allelic differences in LMP-mediated proteolytic cleavage of protein antigens or in the transport of different peptides from the cytosol into the RER may contribute to the observed variation among individuals in their response to different endogenous antigens. TAP deficiencies can lead to a disease syndrome that has aspects of both immunodeficiency and autoimmunity (see Clinical Focus).

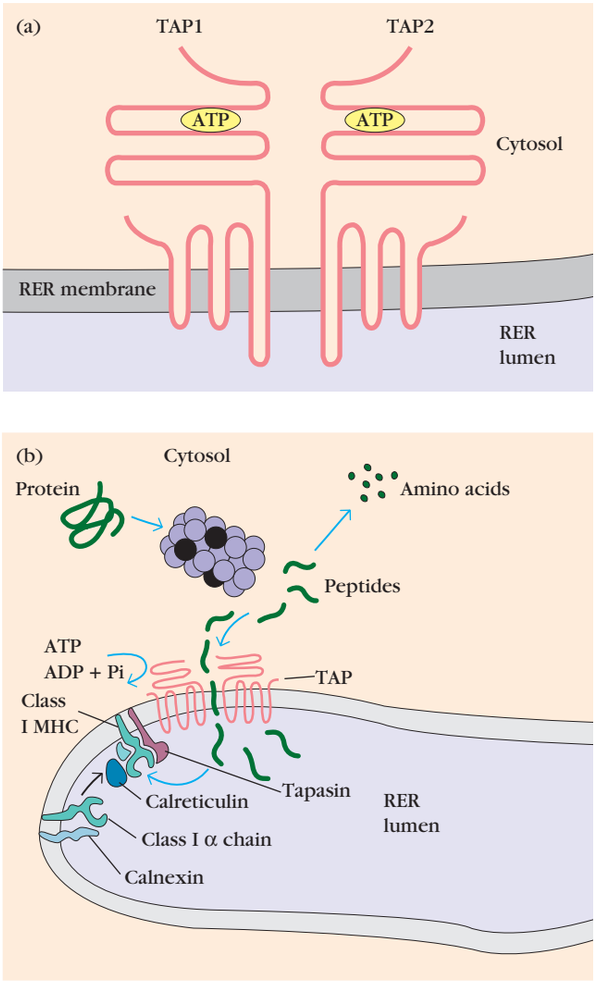



**FIGURE 8-6** Generation of antigenic peptide–class I MHC complexes in the cytosolic pathway. (a) Schematic diagram of TAP, a heterodimer anchored in the membrane of the rough endoplasmic reticulum (RER). The two chains are encoded by *TAP1* and *TAP2*. The cytosolic domain in each TAP subunit contains an ATP-binding site, and peptide transport depends on the hydrolysis of ATP. (b) In the cytosol, association of LMP2, LMP7, and LMP10 (black spheres) with a proteasome changes its catalytic specificity to favor production of peptides that bind to class I MHC molecules. Within the RER membrane, a newly synthesized class I α chain associates with calnexin until $\beta_2$-microglobulin binds to the α chain. The class I α chain/$\beta_2$-microglobulin heterodimer then binds to calreticulin and the TAP-associated protein tapasin. When a peptide delivered by TAP is bound to the class I molecule, folding of MHC class I is complete and it is released from the RER and transported through the Golgi to the surface of the cell.

## Peptides Assemble with Class I MHC Aided by Chaperone Molecules

Like other proteins, the α chain and $\beta_2$-microglobulin components of the class I MHC molecule are synthesized on polysomes along the rough endoplasmic reticulum. Assembly of these components into a stable class I MHC molecular complex that can exit the RER requires the presence of a peptide in the binding groove of the class I molecule. The assembly process involves several steps and includes the participation of *molecular chaperones,* which facilitate the folding of polypeptides. The first molecular chaperone involved in class I MHC assembly is *calnexin,* a resident membrane protein of the endoplasmic reticulum. Calnexin associates with the free class I α chain and promotes its folding. When $\beta_2$-microglobulin binds to the α chain, calnexin is released and the class I molecule associates with the chaperone *calreticulin* and with *tapasin.* Tapasin (TAP-associated protein) brings the TAP transporter into proximity with the class I molecule and allows it to acquire an antigenic peptide (Figure 8-7). The physical association of the α chain–$\beta_2$-microglobulin

CLINICAL FOCUS

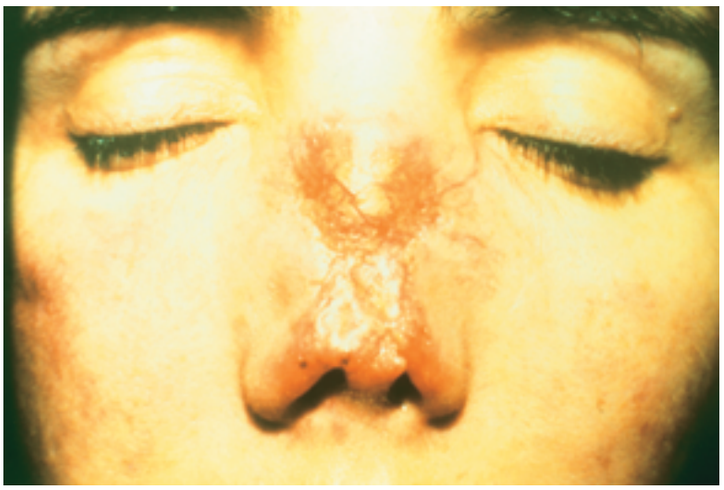
# Deficiency in Transporters Associated with Antigen Presentation (TAP) Leads to a Diverse Disease Spectrum

A relatively rare condition known as bare lymphocyte syndrome, or BLS, has been recognized for more than 22 years. The lymphocytes in BLS patients express MHC molecules at below-normal levels and, in some cases, not at all. In type 1 BLS, a deficiency in MHC class I molecules exists; in type 2 BLS, expression of class II molecules is impaired. The pathogenesis of one type of BLS underscores the importance of the class I family of MHC molecules in their dual roles of preventing autoimmunity as well as defending against pathogens.

Defects in promoter sequences that preclude MHC gene transcription were found for some type 2 BLS cases, but in many instances the nature of the underlying defect is not known. A recent study has identified a group of patients with type 1 BLS due to defects in *TAP1* or *TAP2* genes. Manifestations of the TAP deficiency were consistent in this patient group and define a unique disease. As described earlier in this chapter, TAP proteins are necessary for the loading of peptides onto class I molecules, a step that is essential for expression of class I MHC molecules on the cell surface. Lymphocytes in individuals with TAP deficiency express levels of class I molecules significantly lower than normal controls. Other cellular abnormalities include increased numbers of NK and γδ T cells, and decreased levels of $CD8^+$ αβ T cells. As we shall see, the disease manifestations are reasonably well explained by these deviations in the levels of certain cells involved in immune function.

In early life the TAP-deficient individual suffers frequent bacterial infections of the upper respiratory tract, and in the second decade begins to have chronic infection of the lungs. It is thought that a post-nasal-drip syndrome common in younger patients promotes the bacterial lung infections in later life. Noteworthy is the absence of any severe viral infection, which is common in immunodeficiencies with T-cell involvement (see Chapter 19). Bronchiectasis (dilation of the bronchial tubes) often occurs and recurring infections can lead to lung damage that may be fatal. The most characteristic mark of the deficiency is the occurrence of necrotizing skin lesions on the extremities and the midface. These lesions ulcerate and may cause disfigurement (see figure). The skin lesions are probably due to activated NK cells and γδ T cells; NK cells were isolated from biopsied skin from several patients, supporting this possibility. Normally, the activity of NK cells is limited through the action of killer-cell-inhibitory receptors (KIRs), which deliver a negative signal to the NK cell following interaction with class I molecules (see Chapter 14). The deficiency of class I molecules in TAP-related BLS patients explains the excessive activity of the NK cells. Activation of NK cells further explains the absence of severe virus infections, which are limited by NK and γδ cells.

The best treatment for the characteristic lung infections appears to be antibiotics and intravenous immunoglobulin. Attempts to limit the skin disease by immunosuppressive regimens, such as steroid treatment or cytotoxic agents, can lead to exacerbation of lesions and is therefore contraindicated. Mutations in the promoter region of *TAP* that preclude expression of the gene were found for several patients, suggesting the possibility of gene therapy, but the cellular distribution of class I is so widespread that it is not clear what cells would need to be corrected to alleviate all symptoms.



Necrotizing granulomatous lesions in the midface of patient with TAP-deficiency syndrome. TAP deficiency leads to a condition with symptoms characteristic of autoimmunity, such as the skin lesions that appear on the extremities and the midface, as well as immunodeficiency that causes chronic sinusitis, leading to recurrent lung infection. *[From S. D. Gadola et al., 1999*, Lancet ***354:****1598, and 2000,* Clinical and Experimental Immunology ***121:****173.]*

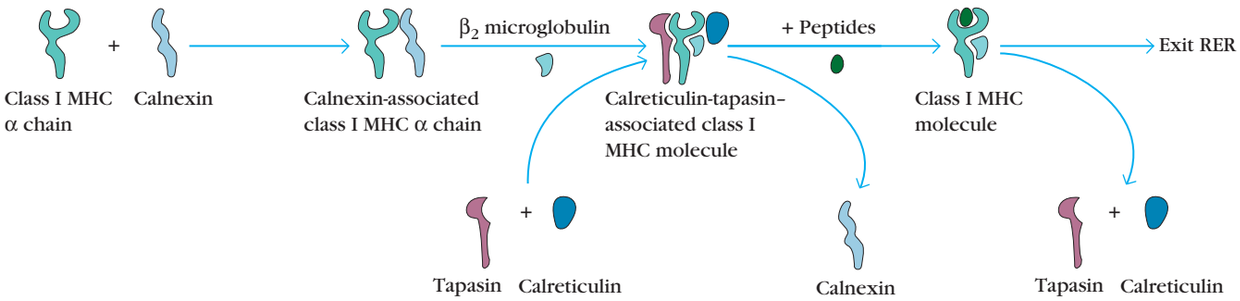



**FIGURE 8-7** Assembly and stabilization of class I MHC molecules. Newly formed class I α chains associate with calnexin, a molecular chaperone, in the RER membrane. Subsequent binding to $\beta_2$-microglobulin releases calnexin and allows binding to the chaperonin calreticulin and to tapasin, which is associated with the peptide transporter TAP. This association promotes binding of an antigenic peptide, which stabilizes the class I molecule–peptide complex, allowing its release from the RER.

heterodimer with the TAP protein (see Figure 8-6b) promotes peptide capture by the class I molecule before the peptides are exposed to the luminal environment of the RER. Peptides not bound by class I molecules are rapidly degraded. As a consequence of peptide binding, the class I molecule displays increased stability and can dissociate from calreticulin and tapasin, exit from the RER, and proceed to the cell surface via the Golgi. An additional chaperone protein, ERp57, has been observed in association with calnexin and calreticulin complexes. The precise role of this resident endoplasmic reticulum protein in the class I peptide assembly and loading process has not yet been defined, but it is thought to contribute to the formation of disulfide bonds during the maturation of class I chains. Because its role is not clearly defined, ERp57 is not shown in Figures 8-6 and 8-7.

## Exogenous Antigens: The Endocytic Pathway

Figure 8-8 recapitulates the endogenous pathway discussed previously (left side), and compares it with the separate exogenous pathway (right), which we shall now consider. Whether an antigenic peptide associates with class I or with class II molecules is dictated by the mode of entry into the cell, either exogenous or endogenous, and by the site of processing.

Antigen-presenting cells can internalize antigen by phagocytosis, endocytosis, or both. Macrophages internalize antigen by both processes, whereas most other APCs are not phagocytic or are poorly phagocytic and therefore internalize exogenous antigen only by endocytosis (either receptor-mediated endocytosis or pinocytosis). B cells, for example, internalize antigen very effectively by receptor-mediated endocytosis using antigen-specific membrane antibody as the receptor.

### Peptides Are Generated from Internalized Molecules in Endocytic Vesicles

Once an antigen is internalized, it is degraded into peptides within compartments of the endocytic processing pathway. As the experiment shown in Figure 8-3 demonstrated, internalized antigen takes 1–3 h to transverse the endocytic pathway and appear at the cell surface in the form of peptide–class II MHC complexes. The endocytic pathway appears to involve three increasingly acidic compartments: early endosomes (pH 6.0–6.5); late endosomes, or endolysosomes (pH 5.0–6.0); and lysosomes (pH 4.5–5.0). Internalized antigen moves from early to late endosomes and finally to lysosomes, encountering hydrolytic enzymes and a lower pH in each compartment (Figure 8-9). Lysosomes, for example, contain a unique collection of more than 40 acid-dependent hydrolases, including proteases, nucleases, glycosidases, lipases, phospholipases, and phosphatases. Within the compartments of the endocytic pathway, antigen is degraded into oligopeptides of about 13–18 residues, which bind to class II MHC molecules. Because the hydrolytic enzymes are optimally active under acidic conditions (low pH), antigen processing can be inhibited by chemical agents that increase the pH of the compartments (e.g., chloroquine) as well as by protease inhibitors (e.g., leupeptin).

The mechanism by which internalized antigen moves from one endocytic compartment to the next has not been conclusively demonstrated. It has been suggested that early endosomes from the periphery move inward to become late endosomes and finally lysosomes. Alternatively, small transport vesicles may carry antigens from one compartment to the next. Eventually the endocytic compartments, or portions of them, return to the cell periphery, where they fuse with the plasma membrane. In this way, the surface receptors are recycled.

### The Invariant Chain Guides Transport of Class II MHC Molecules to Endocytic Vesicles

Since antigen-presenting cells express both class I and class II MHC molecules, some mechanism must exist to prevent class II MHC molecules from binding to the same set of antigenic peptides as the class I molecules. When class II MHC molecule are synthesized within the RER, three pairs of class II αβ chains associate with a preassembled trimer of a

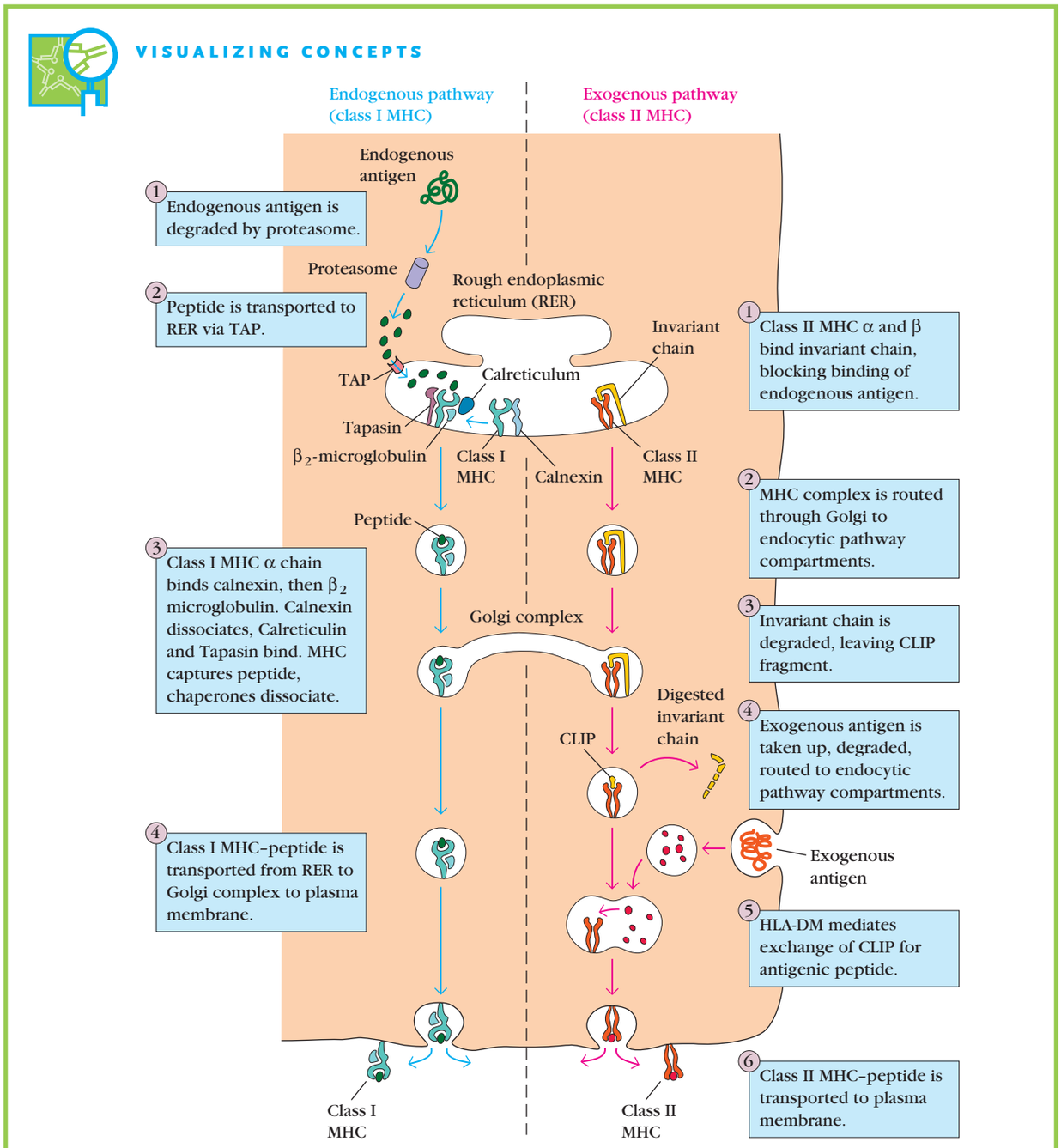



**FIGURE 8-8** Separate antigen-presenting pathways are utilized for endogenous (green) and exogenous (red) antigens. The mode of antigen entry into cells and the site of antigen processing determine whether antigenic peptides associate with class I MHC molecules in the rough endoplasmic reticulum or with class II molecules in endocytic compartments.

protein called **invariant chain (Ii, CD74).** This trimeric protein interacts with the peptide-binding cleft of the class II molecules, preventing any endogenously derived peptides from binding to the cleft while the class II molecule is within the RER (see right side of Figure 8-8). The invariant chain also appears to be involved in the folding of the class II α and

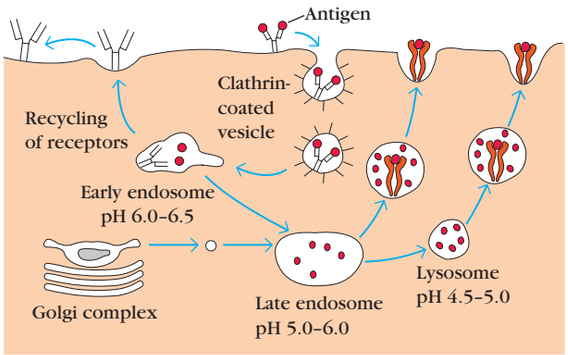



**FIGURE 8-9** Generation of antigenic peptides in the endocytic processing pathway. Internalized exogenous antigen moves through several acidic compartments, in which it is degraded into peptides that ultimately associate with class II MHC molecules transported in vesicles from the Golgi complex. The cell shown here is a B cell, which internalizes antigen by receptor-mediated endocytosis, with the membrane-bound antibody functioning as an antigen-specific receptor.

β chains, their exit from the RER, and the subsequent routing of class II molecules to the endocytic processing pathway from the trans-Golgi network.

The role of the invariant chain in the routing of class II molecules has been demonstrated in transfection experiments with cells that lack the genes encoding class II MHC molecules and the invariant chain. Immunofluorescent labeling of such cells transfected only with class II MHC genes revealed class II molecules localized within the Golgi complex. However, in cells transfected with both the class II MHC genes and invariant-chain gene, the class II molecules were localized in the cytoplasmic vesicular structures of the endocytic pathway. The invariant chain contains sorting signals in its cytoplasmic tail that directs the transport of the class II MHC complex from the trans-Golgi network to the endocytic compartments.

## Peptides Assemble with Class II MHC Molecules by Displacing CLIP

Recent experiments indicate that most class II MHC–invariant chain complexes are transported from the RER, where they are formed, through the Golgi complex and trans-Golgi network, and then through the endocytic pathway, moving from early endosomes to late endosomes, and finally to lysosomes. As the proteolytic activity increases in each successive compartment, the invariant chain is gradually degraded. However, a short fragment of the invariant chain termed *CLIP* (for *class II–associated invariant chain peptide*) remains bound to the class II molecule after the invariant chain has been cleaved within the endosomal compartment. CLIP physically occupies the peptide-binding groove of the class II MHC molecule, presumably preventing any premature binding of antigenic peptide (see Figure 8-8).

A nonclassical class II MHC molecule called *HLA-DM* is required to catalyze the exchange of CLIP with antigenic peptides (Figure 8-10a). MHC class II genes encoding HLA-DM have been identified in the mouse and rabbit, indicating

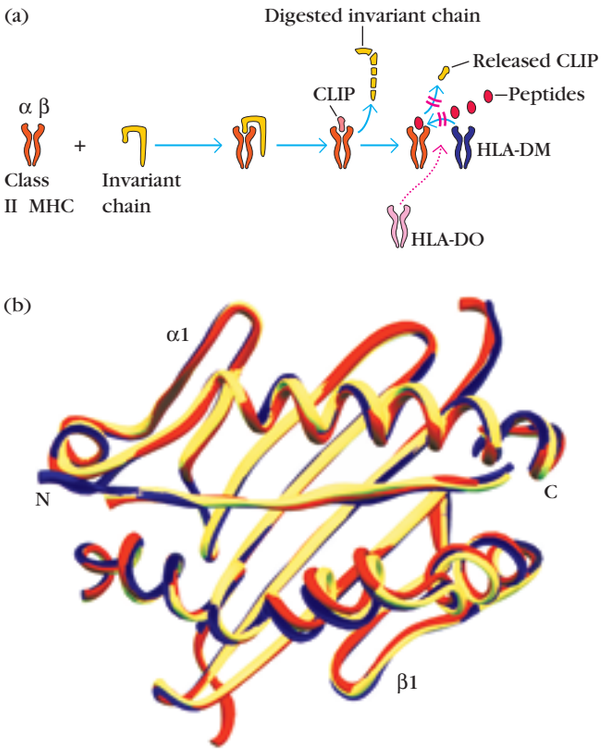



**FIGURE 8-10** (a) Assembly of class II MHC molecules. Within the rough endoplasmic reticulum, a newly synthesized class II MHC molecule binds an invariant chain. The bound invariant chain prevents premature binding of peptides to the class II molecule and helps to direct the complex to endocytic compartments containing peptides derived from exogenous antigens. Digestion of the invariant chain leaves CLIP, a small fragment remaining in the binding groove of the class II MHC molecule. HLA-DM, a nonclassical MHC class II molecule expressed within endosomal compartments, mediates exchange of antigenic peptides for CLIP. The nonclassical class II molecule HLA-DO may act as a negative regulator of class II antigen processing by binding to HLA-DM and inhibiting its role in the dissociation of CLIP from class II molecules. (b) Comparison of three-dimensional structures showing the binding groove of HLA class II molecules (α1 and β1) containing different antigenic peptides or CLIP. The red lines show DR4 complexed with collagen II peptide, yellow lines are DR1 with influenza hemagglutinin peptide, and blue lines are DR3 associated with CLIP. (N indicates the amino terminus and C the carboxyl terminus of the peptides.) No major differences in the structures of the class II molecules or in the conformation of the bound peptides are seen. This comparison shows that CLIP binds the class II molecule in a manner identical to that of antigenic peptides. *[Part (b) from Dessen et al., 1997,* Immunity ***7:****473–481; courtesy of Don Wiley, Harvard University.]*

that HLA-DM is widely conserved among mammalian species. Like other class II MHC molecules, HLA-DM is a heterodimer of α and β chains. However, unlike other class II molecules, HLA-DM is not polymorphic and is not expressed at the cell membrane but is found predominantly within the endosomal compartment. The *DMα* and *DMβ* genes are located near the *TAP* and *LMP* genes in the MHC complex of humans and DM is expressed in cells that express classical class II molecules.

The reaction between HLA-DM and the class II CLIP complex facilitating exchange of CLIP for another peptide is impaired in the presence of HLA-DO, which binds to HLA-DM and lessens the efficiency of the exchange reaction. HLA-DO, like HLA-DM, is a nonclassical and nonpolymorphic class II molecule that is also found in the MHC of other species. HLA-DO differs from HLA-DM in that it is expressed only by B cells and the thymus, and unlike other class II molecules, its expression is not induced by IFN-γ. An additional difference is that the genes encoding the α and the β chains of HLA-DO are not adjacent in the MHC as are all other class II α and β pairs (see Fig 7-15).

An HLA-DR3 molecule associated with CLIP was isolated from a cell line that did not express HLA-DM and was therefore defective in antigen processing. Superimposing the structure of HLA-DR3–CLIP on another DR molecule bound to antigenic peptide reveals that CLIP binds to class II in the same stable manner as the antigenic peptide (Figure 8-10b). The discovery of this stable complex in a cell with defective HLA-DM supports the argument that HLA-DM is required for the replacement of CLIP.

Although it certainly modulates the activity of HLA-DM, the precise role of HLA-DO remains obscure. One possibility is that it acts in the selection of peptides bound to class II MHC molecules in B cells. DO occurs in complex with DM in these cells and this association continues in the endosomal compartments. Conditions of higher acidity weaken the association of DM/DO and increase the possibility of antigenic peptide binding despite the presence of DO. Such a pH-dependent interaction could lead to preferential selection of class II-bound peptides from lysosomal compartments in B cells as compared with other APCs.

As with class I MHC molecules, peptide binding is required to maintain the structure and stability of class II MHC molecules. Once a peptide has bound, the peptide–class II complex is transported to the plasma membrane, where the neutral pH appears to enable the complex to assume a compact, stable form. Peptide is bound so strongly in this compact form that it is difficult to replace a class II–bound peptide on the membrane with another peptide at physiologic conditions.

## Presentation of Nonpeptide Antigens

To this point the discussion has been limited to peptide antigens and their presentation by classical class I and II MHC molecules. It is well known that nonprotein antigens also are recognized by the immune system, and there are reports dating back to the 1980s of T cell proliferation in the presence of nonprotein antigens derived from infectious agents. More recent reports indicate that T cells that express the γδ TCR (T-cell receptors are dimers of either αβ or γδ chains) that react with glycolipid antigens derived from bacteria such as *Mycobacterium tuberculosis*. These nonprotein antigens are presented by members of the CD1 family of nonclassical class I molecules.

The CD1 family of molecules associates with $\beta_2$-microglobulin and has general structural similarity to class I MHC molecules. There are five genes encoding human CD1 molecules (*CD1A-E,* encoding the gene products CD1a-d, with no product yet identified for *E*). These genes are located not within the MHC but on chromosome 1 (Figure 8-11a). The genes are classified into two groups based on sequence homology. Group 1 includes *CD1A, B, C,* and *E*; *CD1D* is in group 2. All mammalian species studied have CD1 genes, although the number varies. Rodents have only group 2 *CD1* genes, the counterpart of human *CD1D,* whereas rabbits, like humans, have five genes, including both group 1 and 2 types. Sequence identity of CD1 with classical class I molecules is considerably lower than the identity of the class I molecules with each other. Comparison of the three-dimensional structure of the mouse CD1d1 with the class I MHC molecule H-$2k^b$ shows that the antigen-binding groove of the CD1d1 molecules is deeper and more voluminous than that of the classical class I molecule (Fig 8-11b).

Expression of CD1 molecules varies according to subset; *CD1D1* genes are expressed mainly in nonprofessional APCs and on certain B-cell subsets. The mouse CD1d1 is more widely distributed and found on T cells, B cells, dendritic cells, hepatocytes, and some epithelial cells. The *CD1A, B,* and *C* genes are expressed on immature thymocytes and professional APCs, mainly those of the dendritic type. *CD1C* gene expression is seen on B cells, whereas the *CD1A* and *B* products are not. *CD1* genes can be induced by exposure to certain cytokines such as GM-CSF or IL-3. The intracellular trafficking patterns of the CD1 molecules differ; for example, CD1a is found mostly in early endosomes or on the cell surface; CD1b and CD1d localize to late endosomes; and CD1c is found throughout the endocytic system.

Certain CD1 molecules are recognized by T cells in the absence of foreign antigens, and self restriction can be demonstrated in these reactions. Examination of antigens presented by CD1 molecules revealed them to be lipid components (mycolic acid) of the *M. tuberculosis* cell wall. Further studies of CD1 presentation indicated that a glycolipid (lipoarabinomannan) from *Mycobacterium leprae* could also be presented by these molecules. The data concerning CD1 antigen presentation point out the existence of a third pathway for the processing of antigens, a pathway with distinct intracellular steps that do not involve the molecules found to facilitate class I antigen processing. For example, CD1 molecules are able to process antigen in TAP-deficient cells. Recent data indicate that the CD1a and 1b molecules traffic differently,

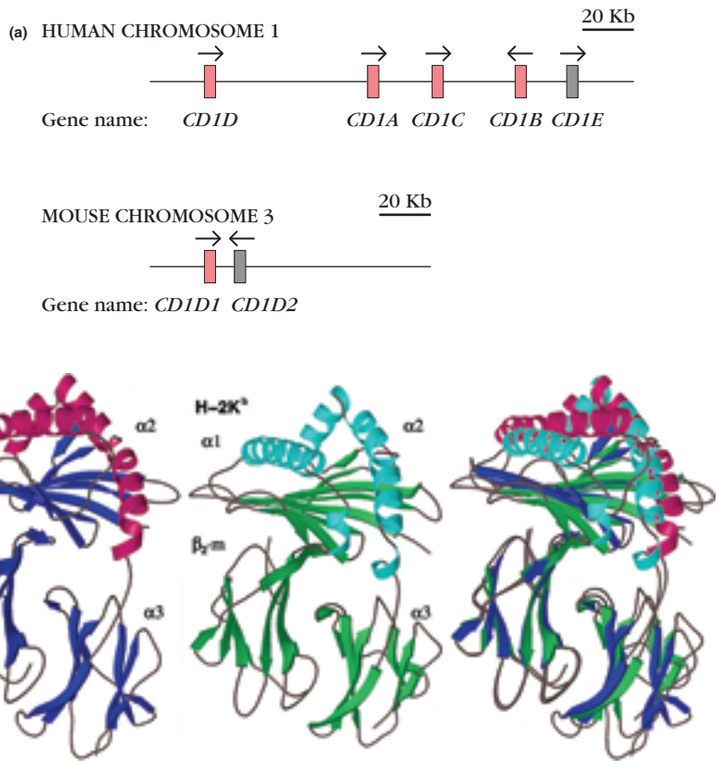



**FIGURE 8-11** The *CD1* family of genes and structure of a CD1d molecule. (a) The genes encoding the CD1 family of molecules in human (top) and mouse (bottom). The genes are separated into two groups based on sequence identity; *CD1A, B, C,* and *E* are group 1, *CD1D* genes are group 2. The products of the pink genes have been identified; products of grey genes have not yet been detected. (b) Comparison of the crystal structures of mouse nonclassical CD1 and classical class I molecule H-2$k^b$. Note the differences in the antigen binding grooves. *[Part (b) reprinted from* Trends in Immunology *(formerly* Immunology Today*), Vol. 19, S. A. Porcelli and R. L. Modlin, The CD1 family of lipid antigen presenting molecules, pp. 362–368, 1998, with permission from Elsevier Science.]*

with CD1a at the surface or in the recycling endocytic compartments and CD1b and CD1d in the lysomal compartments. Exactly how the CD1 pathway complements or intersects the better understood class I and class II pathways remains an open question. The T-cell types reactive to CD1 were first thought to be limited to T cells expressing the γδ TCR and lacking both CD4 and CD8, or T cells with a single TCR α chain, but recent reports indicate that a wider range of T-cell types will recognize CD1-presenting cells. Recent evidence indicates that natural killer T cells recognize CD1d molecules presenting autologous antigen. This may represent a mechanism for eliminating cells that are altered by stress, senescence, or neoplasia.

## SUMMARY

- T-cells recognize antigen displayed within the cleft of a self-MHC molecule on the membrane of a cell.
- In general, $CD4^+$ $T_H$ cells recognize antigen with class II MHC molecules on antigen-processing cells.
- $CD8^+$ $T_C$ cells recognize antigen with class I MHC molecules on target cells.
- Complexes between antigenic peptides and MHC molecules are formed by degradation of a protein antigen in one of two different antigen-processing pathways.
- Endogenous antigens are degraded into peptides within the cytosol by proteasomes and assemble with class I molecules in the RER.
- Exogenous antigens are internalized and degraded within the acidic endocytic compartments and subsequently pair with class II molecules.
- Peptide binding to class II molecules involves replacing a fragment of invariant chain in the binding cleft by a process catalyzed by nonclassic MHC molecule HLA-DM.
- Presentation of nonpeptide (lipid and glycolipid) antigens derived from bacteria involves the class I–like CD1 molecules.

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## Study Questions

**Clinical Focus Question** Patients with TAP deficiency have partial immunodeficiency as well as autoimmune manifestations. How do the profiles for patients' immune cells explain the partial immunodeficiency? Why is it difficult to design a gene therapy treatment for this disease, despite the fact that a single gene defect is implicated?

1. Explain the difference between the terms *antigen-presenting cell* and *target cell*, as they are commonly used in immunology.

2. Define the following terms:

   a. Self-MHC restriction
   b. Antigen processing
   c. Endogenous antigen
   d. Exogenous antigen

3. L. A. Morrison and T. J. Braciale conducted an experiment to determine whether antigens presented by class I or II MHC molecules are processed in different pathways. Their results are summarized in Table 8-2.

   a. Explain why the class I–restricted $T_C$ cells did not respond to target cells infected with UV-inactivated influenza virus.
   b. Explain why chloroquine inhibited the response of the class II–restricted $T_C$ cells to live virus.
   c. Explain why emetine inhibited the response of class I–restricted but not class II–restricted $T_C$ cells to live virus.

4. For each of the following cell components or processes, indicate whether it is involved in the processing and presentation of exogenous antigens (EX), endogenous antigens (EN), or both (B). Briefly explain the function of each item.

   a. _____Class I MHC molecules
   b. _____Class II MHC molecules
   c. _____Invariant (Ii) chains
   d. _____Lysosomal hydrolases
   e. _____TAP1 and TAP2 proteins
   f. _____Transport of vesicles from the RER to the Golgi complex
   g. _____Proteasomes
   h. _____Phagocytosis or endocytosis
   i. _____Calnexin
   j. _____CLIP
   k. _____Tapasin

5. Antigen-presenting cells have been shown to present lysozyme peptide 46–61 together with the class II $IA^k$ molecule. When $CD4^+T_H$ cells are incubated with APCs and native lysozyme or the synthetic lysozyme peptide 46–61, $T_H$-cell activation occurs.

   a. If chloroquine is added to the incubation mixture, presentation of the native protein is inhibited, but the peptide continues to induce $T_H$-cell activation. Explain why this occurs.
   b. If chloroquine addition is delayed for 3 h, presentation of the native protein is not inhibited. Explain why this occurs.

6. Cells that can present antigen to $T_H$ cells have been classified into two groups—professional and nonprofessional APCs.

   a. Name the three types of professional APCs. For each type indicate whether it expresses class II MHC molecules and a co-stimulatory signal constitutively or must be activated before doing so.
   b. Give three examples of nonprofessional APCs. When are these cells most likely to function in antigen presentation?

7. Predict whether $T_H$-cell proliferation or CTL-mediated cytolysis of target cells will occur with the following mixtures of cells. The $CD4^+$ $T_H$ cells are from lysozyme-primed mice, and the $CD8^+$ CTLs are from influenza-infected mice. Use R to indicate a response and NR to indicate no response.

   a. _____$H\text{-}2^k$ $T_H$ cells + lysozyme-pulsed $H\text{-}2^k$ macrophages
   b. _____$H\text{-}2^k$ $T_H$ cells + lysozyme-pulsed $H\text{-}2^{b/k}$ macrophages

c. _____H-2$^k$ $T_H$ cells + lysozyme-primed H-2$^d$ macrophages
d. _____H-2$^k$ CTLs + influenza-infected H-2$^k$ macrophages
e. _____H-2$^k$ CTLs + influenza-infected H-2$^d$ macrophages
f. _____H-2$^d$ CTLs + influenza-infected H-2$^{d/k}$ macrophages

**8.** HLA-DM and HLA-DO are termed nonclassical MHC class II molecules. How do they differ from the classical MHC class II? How do they differ from each other?

**9.** Molecules of the CD1 family were recently shown to present nonpeptide antigens.

a. What is a major source of nonpeptide antigens?
b. Why are CD1 molecules not classified as members of the MHC family even though they associate with $\beta_2$-microglobulin?
c. What evidence suggests that the CD1 pathway is different from that utilized by classical class I MHC molecules?