CELL REGULATION, Vol. 1, 499–509, June 1990

Cellular mechanisms of antigen processing and the function of class I and II major histocompatibility complex molecules

Review

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Antigen recognition: an overview

Antigen recognition in humoral immunity has long been understood: the antigen-specific (Fab) portion of the antibody molecule binds directly to the antigen in its native state. The antibody molecules are found as antigen receptors on the plasma membrane of B cells and also as secreted proteins. In contrast, the biochemical basis for antigen recognition by T cells has only recently become clear. In general, T cells do not recognize antigens in their native conformation, but only after partial proteolysis within antigen processing or presenting cells (APC) into constituent peptides that bind to molecules encoded by genes in the major histocompatibility complex (MHC). The bimolecular complex of antigen peptide and MHC molecule is then displayed on the APC plasma membrane to be recognized by T-cell clones bearing a specific antigen receptor for it. This recognition leads to their stimulation and proliferation. The MHC molecules are, therefore, peptide carriers that serve to rescue peptides from extensive intracellular catabolism, making them available to the T-cell system (Unanue and Allen, 1987). They also constitute part of the antigenic determinant that T cells have evolved to recognize. Antigen presentation, therefore, includes a novel cell biological phenomenon: that a product from internal degradation of an exogenous protein becomes biologically active and recognized by another set of cells.

Two classes of MHC molecules function in antigen presentation. The class I molecules (MHC-I) include HLA-A, -B, and -C in humans and H-2K, D, and L in mice. The class II molecules (MHC-II) include HLA-DP, -DQ, and -DR in humans and the I-A and I-E molecules in mice. This review will focus primarily on MHC-II function, but it also discusses and compares the function of MHC-I molecules. More extensive historical background is available in previous reviews (Unanue and Allen, 1987), whereas some recent advances are summarized elsewhere (Moller, 1987, 1988).

Structure and function of MHC molecules

MHC-I molecules consist of a 44-kDa transmembrane heavy chain and a light chain, β_2-microglobulin (12 kDa), noncovalently associated with the extracellular domain of the heavy chain. The heavy chain includes three extracellular domains, α_1, α_2, and α_3. X-ray crystallography has revealed the structure of HLA-A molecules (Bjorkman *et al.*, 1987a,b; Garret *et al.*, 1989). The α_1 and α_2 domains form the antigen peptide binding site, which contains the amino acid residues responsible for the considerable allelic polymorphism. These domains are supported by the α_3 domain and β_2-microglobulin, both of which show homology to immunoglobulin constant domains. MHC-II molecules are composed of two noncovalently associated transmembrane polypeptide chains, α (34 kDa) and β (28 kDa), each of which contain two domains. Despite the differences in the polypeptide backbone, the tertiary structures of MHC-I and MHC-II molecules appear to be quite similar (Brown *et al.*, 1988; Gorga *et al.*, 1989). Models of MHC-II structure indicate that the polymorphic external α_1 and β_1 domains are homologous to the α_1 and α_2 domains of MHC-I. They may form a similar peptide-binding cleft, supported by the immunoglobulin-like α_2 and β_2 domains. In both molecules the peptide-binding site is envisioned as a groove on the external

aspect of the molecule, flanked by two α helices (one contributed by each constituent domain) and floored by a β pleated sheet structure. The groove can hold a peptide of 15–20 amino acids. The polymorphic residues of the MHC molecules cluster in the peptide-binding groove, which appears to contain "pockets" that may interact with particular amino acid residues (Todd *et al.,* 1988; Garret *et al.,* 1989).

The primary function of MHC molecules is to bind peptides and by doing this to create the bimolecular complex needed for engagement of the T cell (Babbitt *et al.,* 1985; Buus *et al.,* 1987). The binding of peptides has been documented chemically in detergent solutions of purified MHC-II proteins and labeled peptides. Peptides bind to MHC-II molecules in a saturable and homogeneous binding reaction with binding affinities in the micromolar range (Babbitt *et al.,* 1985). The binding is characterized by a slow on-rate and by a very slow rate of dissociation (Buus *et al.,* 1986; Allen *et al.,* 1989; Sadegh-Nasseri and McConnell, 1989; Roof *et al.,* 1990). Some peptides, once bound, will practically not dissociate unless the MHC-II molecules are drastically denatured. Binding kinetics and competition studies with unlabeled peptides established that there is a single binding site (Babbitt *et al.,* 1986; Buus *et al.,* 1987).

The binding affinity for MHC-II molecules varies among different peptides. Different MHC alleles bind varying spectra of peptides, and a given peptide will generally bind only to a subset of MHC alleles. The presentation of and immune response to a peptide is, accordingly, "restricted" to these alleles, and this may explain the fact that increased risk of various autoimmune disorders is restricted to particular MHC genotypes (Buus *et al.,* 1987; Todd *et al.,* 1988). Within a given peptide, certain amino acids appear to be MHC-binding residues that interact directly with the MHC molecule; amino acid substitutions at these sites tend to abrogate MHC binding as well as immunogenicity (Allen *et al.,* 1987b; Sette *et al.,* 1987). Other amino acid residues interact primarily with the T-cell receptor; substitutions of these residues alter immunogenicity despite continued MHC binding. Other amino acids are framework residues or may exhibit intermediate properties. The issue here is that the peptides appear to adopt novel conformations when bound to MHC molecules, so that the T-cell receptor engages the peptide amino acid side chains expressed at the combining site plus, most likely, some of the residues on the α helices of the MHC molecule. As expected, each MHC-II molecule can bind to many different peptides as a result of charge and hydrophobic interaction among amino acid side chains.

Recent studies suggest that, in solution, MHC-II molecules show considerable conformational flexibility that is influenced by the presence of phospholipids (Roof *et al.,* 1990). Binding kinetics indicate that only 10%–60% of purified MHC-II can bind peptides in vitro, a reflection of denaturation, occupation by other peptides, or the presence of different MHC-II conformers that vary in their binding affinities. Finally, binding studies with MHC-I molecules in solution have been very limited (Bouillot *et al.,* 1989; Chen and Parham, 1989). These studies indicate that an even smaller proportion (<0.3%) of solubilized MHC-I molecules is capable of binding peptides (Chen and Parham, 1989). This may reflect lesser dissociation of peptide–MHC-I complexes than of peptide–MHC-II complexes. The binding capacity of MHC-I molecules has been more extensively evaluated in cellular studies (below).

Why develop a system of protein recognition that requires partial proteolysis of the protein and its association with a transport molecule? The answer may lie in that such a system permits control of the T-cell response so that it is mainly focused on foreign proteins. Both B and T cells have developed by random combination of gene segments a wide library of receptors to recognize proteins. Among these are the receptors to self components that give rise to autoimmunity. The process whereby the autoreactive B and T cell clones remain unreactive is controlled at many stages, but the use of MHC proteins controls recognition at two key stages: 1) In the thymus, T cells are generated having receptors to self and non-self peptides. Self proteins that reach the thymus from the blood are processed by the stroma cells that bear MHC-II and MHC-I; many thymocytes that develop T-cell receptors for these self peptides undergo programmed cell death. Thus, many autoreactive T cells are eliminated during early differentiation in the thymus gland (reviewed in McDonald *et al.,* 1989; Pullen *et al.,* 1989). 2) MHC-II are primarily expressed on macrophages, B cells, and Langerhans cells, restricting immune recognition to these cells and decreasing the capacity of other tissues to stimulate autoimmune reactions.

MHC-II function and CD4+ T cells

Exogenous proteins (i.e., antigens from the extracellular space) are internalized by endocy-

tosis or phagocytosis and processed intracellularly to generate immunogenic peptides that bind to the MHC-II molecules of the APC. MHC-II–restricted antigen presentation is mediated primarily by three "professional" APC (macrophages, B lymphocytes, and Langerhans-dendritic cells). These cells express large numbers of MHC-II molecules (constitutively in B cells, dendritic cells, and some macrophages, or regulated by cytokines), as well as other important "costimulator" molecules (e.g., lymphokines and adhesion molecules) (reviewed in Weaver and Unanue, 1990) that must also be expressed to stimulate a T-cell response. MHC-II molecules can also be expressed by many other cells on stimulation by interferon γ, a cytokine elaborated by CD4 and CD8 T cells and by natural killer cells, after their stimulation. Other cell types, however, often fail to express costimulator molecules.

APC present peptide–MHC-II complexes almost exclusively to CD4+ T cells. (Mature T lymphocytes are divided into two mutually exclusive subsets that express the CD4 and CD8 molecules, respectively.) The CD4 protein appears to bind to a nonpolymorphic determinant on MHC-II molecules, furthering the APC–T-cell and MHC–T-cell receptor interactions (Doyle and Strominger, 1987; Gay *et al.,* 1988). The role of MHC-II–restricted antigen presentation is an essential one of regulation of the cellular immune response. CD4+ T cells provide helper function to stimulate specific antibody responses by B cells and specific responses of CD8+ lymphocytes (below); the battery of cytokines elaborated by these cells controls the function of many cells, e.g., macrophages in cellular immune responses such as delayed-type hypersensitivity (DTH). CD4+ cells can also exhibit cytolytic T cell effector functions (below), but this is more often observed with CD8+ T cells.

MHC-I function and CD8+ T lymphocytes

In contrast to the MHC-II molecules that present exogenous antigens, MHC-I molecules present primarily endogenous antigens synthesized within the presenting cell. Endogenous antigens are processed intracellularly to generate immunogenic peptides that appear to bind to MHC-I within the endoplasmic reticulum (ER) or a "pre-Golgi" compartment. Differences in the pathways for MHC-I versus MHC-II antigen processing will be discussed below.

MHC-I molecules are expressed by almost all cells and provide for the presentation of foreign antigens (e.g., viral proteins and tumor antigens) produced intracellularly within almost any cell of the body. MHC-I molecules are recognized primarily by CD8+ T cells (CD8 and MHC-I interact in a manner analogous to CD4 and MHC-II) (Norment *et al.,* 1988). CD8+ T cells can lyse target cells displaying the foreign peptide–MHC-I complexes. This leads to the lysis of virally infected cells that present viral peptides bound to MHC-I molecules. CD8+ T cells may also serve immune regulatory functions by secreting some cytokines like interferon γ.

Quantitative aspects of antigen processing and presentation

The MHC molecules expressed by a given APC at any one time contain a vast array of different peptides available for T-cell surveillance. This is important, because MHC molecules bind many peptides with little apparent specificity or discrimination between self and foreign epitopes (Babbitt *et al.,* 1986; Buus *et al.,* 1988; Lorenz and Allen, 1988). Thus, many different peptides must be presented simultaneously to ensure that immunogenic epitopes necessary for a particular immune response are included in the repertoire of expressed peptide complexes. A large number of self and foreign proteins are internalized and processed by APC. As a result, both foreign and self peptides compete for binding to MHC molecules, reducing the level of expression of any particular peptide complex. T cells, therefore, must be capable of recognizing the relatively low number of complexes of a particular species that can be generated in the face of antigen competition. T cell activation is initiated by ligation of only a few hundred T cell receptors by either peptide–MHC-II complexes (Harding and Unanue, unpublished observations) or antibodies to the T-cell receptor–CD3 complex (Braciale *et al.,* 1988; Kanagawa and Ahlem, 1989; Romani *et al.,* 1989). Thus, a T cell can respond to a peptide that occupies as few as 0.1% of the relevant MHC-II molecules on an APC. The presentation of a particular antigen is increased by mechanisms that facilitate its uptake by APC. For example, presentation of antigen by macrophages is increased by its opsonization and the formation of immune complexes, resulting in increased internalization via the Fc receptor. B cells also show increased uptake and processing of antigens that bind to their surface immunoglobulin (Rock *et al.,* 1984; Lanzavecchia, 1987).

Antigen catabolism produces immunogenic peptides that bind to MHC-II molecules

As stated previously, T cells recognize antigens only as peptides bound to MHC molecules. With some exceptions (Lee *et al.,* 1988; Sette *et al.,* 1989), antigens in their native conformation cannot bind to MHC molecules, but must first be processed, or subjected to partial proteolysis, within viable APC. A protein or a peptide needs to have sufficient flexibility to bind and mold into the MHC-II combining site. This is the biochemical basis for processing. Processing cannot occur at 4°C or within aldehyde-fixed cells. Fixed cells can, however, present antigens that are fragmented to peptides in vitro (by protease treatment) or synthetic peptides that contain immunogenic epitopes. These peptide antigens bind directly to MHC molecules on the surface of fixed cells, and the resulting peptide-MHC complexes can then be recognized by T cells (Shimonkevitz *et al.,* 1984; Babbitt *et al.,* 1985; Allen *et al.,* 1987a). By examining the presentation of synthetic peptides in this manner, it has been possible to define the immunogenic epitopes within the peptide sequence of many antigens recognized by T cells. For example, the T-cell response to the antigen hen egg lysozyme (HEL) in CBA/J (H-2^k) mice is mostly directed to the peptide sequence HEL(52–61), with a lesser response to other epitopes, including HEL(34–45) (Allen *et al.,* 1984, 1987a; Adorini *et al.,* 1988). The following general conclusions can be drawn from these studies. 1) Within a given genetic background, a particular protein antigen produces only one or a few restricted immunodominant peptide sequences that generate a T-cell response (Gammon *et al.,* 1987). There is, therefore, selection of the antigenic determinant during antigen presentation (Rosenthal *et al.,* 1977). 2) These core epitopes usually consist of ~10 amino acids, but peptides as short as 5 amino acids bind to MHC molecules (Reddehase *et al.,* 1989). 3) Peptides derived from both foreign antigens and self proteins bind to MHC molecules, i.e., these molecules do not discriminate between self and non-self (Babbitt *et al.,* 1986). This discrimination in the immune response is controlled by the repertoire of responding T cells, which is molded by events in the thymus and by mechanisms of peripheral tolerance. 4) Accordingly, not all peptides that bind to MHC molecules generate an immune response. 5) A given peptide will bind only a restricted number of MHC alleles, and a given MHC allele will bind only a restricted spectrum of peptides (the spectrum varies with different alleles). 6) Several algorithms predict sequences that are more likely to bind particular MHC molecules (DeLisi and Berzofsky, 1985; Rothbard and Taylor, 1988; Sette *et al.,* 1988; Reyes *et al.,* 1989), and 7) Peptides compete for binding to MHC molecules based on the affinity of each for the particular MHC allele. Clear competition among peptides for binding to the single MHC-II binding site can be documented in vitro (Babbitt *et al.,* 1986) and also in functional analyses using APC (Werdelin, 1982; Rock and Benacerraf, 1983).

The intracellular production of immunogenic peptides from native antigens requires internalization of the protein, its intracellular transport to sites of proteolysis, the coupling of peptides to MHC-II, and eventually the transport of the complex to the plasma membrane. Certain intracellular steps in this process are inhibited at 18°C (Harding and Unanue, 1990), by lysosomotropic amines (Ziegler and Unanue, 1982), or (in some cases) by the addition of certain protease inhibitors (Berzofsky *et al.,* 1988; Puri and Factorovich, 1988). It is presently unclear whether this proteolytic processing occurs within lysosomes or within prelysosomal endosomes that also contain proteolytic enzymes (albeit a more limited repertoire than in lysosomes) (Diment and Stahl, 1985). The first scenario is that of the protein being processed in endosomal vesicles where partial proteolysis takes place. The peptides may then bind to MHC-II molecules within these endosomal vesicles to form the complex. The remaining peptides that do not bind progress to lysosomes for extensive catabolism. The second scenario is that of the proteins first migrating to lysosomes, with some peptides traveling back to endosomes before complete catabolism. For this scenario to exist, a mechanism must be present to recycle peptides from lysosomes, which do not contain MHC-II molecules, to endosomes, which do contain them. A pathway for "retrograde" transport from lysosomes to endosomes and plasma membrane has been previously demonstrated for the transport of the membrane glycoprotein LEP100 (Lippincott-Schwartz and Fambrough, 1987).

Whether processing occurs in lysosomes or endosomes, peptides must bind to MHC molecules before they are completely degraded. Once bound to the MHC molecule, peptides appear to be relatively resistant to further proteolysis (Donermeyer and Allen, 1989). Indeed, antigen presentation is resistant to the treat-

ment of APC with proteolytic enzymes (Ellner *et al.,* 1977; Unanue, 1978). Studies with synthetic peptides (above) have defined the core epitopes required for MHC binding and T-cell stimulation, but the pattern of antigen catabolism and the exact sequence of naturally produced immunogenic peptides are still unclear. Recent studies indicate that the naturally processed peptides bound to MHC molecules are at least similar in size to the synthetic minimal core epitopes (Demotz *et al.,* 1989; Wallny and Rammensee, 1990).

Formation, turnover, and intracellular transport of peptide-MHC-II complexes

The exact subcellular compartment in which peptide–MHC-II complexes are formed is still unclear. Complexes can be formed on the surface of fixed cells on addition of exogenous peptides, but available evidence suggests that during processing of native protein antigens by viable APC, peptides bind to MHC-II molecules within endosomes. Internalized antigens, MHC-II molecules, and proteases such as cathepsin D are all found within endosomes (Guagliardi *et al.,* 1989; Harding *et al.,* 1990), suggesting that antigen catabolism and binding of resultant peptides to MHC-II may occur in the same compartment, although conceivably many antigens may first require delivery to a lysosomal compartment for catabolic processing (above).

The next question is the source of MHC-II molecules for complex formation. Are they derived exclusively from nascent molecules in transit from the Golgi process to the plasma membrane, or are preexisting MHC-II molecules "functionally" recycled to bind multiple peptides? The latter scenario is analogous to the recycling of other receptor molecules that can successively mediate the binding and internalization of numerous ligand molecules. MHC-II molecules are internalized by endocytosis in B cells, macrophages, and dendritic cells (Cantor *et al.,* 1985; Hanau *et al.,* 1987; Harding and Unanue, 1989). [Some B-cell lines exhibit little or no endocytosis of MHC-II molecules (Davis and Cresswell, 1990), perhaps reflecting a different stage of B-cell differentiation.] After endocytosis, MHC-II molecules are found in light density endosomes, similar to transferrin, without targeting to lysosomes (Harding and Unanue, 1989). These data demonstrate a "compartmental" recycling of MHC-II molecules that provides the basis for functional recycling of these molecules to successively present multiple antigen peptides.

Recent evidence supports the existence of functional recycling of MHC-II molecules, i.e., the turnover of peptide–MHC-II complexes (peptide exchange), at least in some B-lymphoma cell lines. These cells can present MHC-II–restricted antigens even after prolonged exposure to cycloheximide or Brefeldin A (Harding and Unanue, 1989, Unanue *et al.,* 1989), indicating that nascent MHC-II molecules are not required and implicating a recycling of preexisting molecules. Furthermore, enhanced dissociation and turnover of peptide–MHC-II complexes has been directly observed in viable B-lymphoma cells (Adorini *et al.,* 1989; Harding *et al.,* 1989). In these studies, peptide MHC-II complexes were generated by pulsing viable B-lymphoma cells with immunogenic peptides. The response of T-hybridoma cells specific for these peptides was used to monitor the number of specific peptide–MHC-II complexes remaining at various times after the immunogenic peptide was removed or a competing peptide was added. Under these conditions, in viable cells the number of specific peptide–MHC-II complexes declined rapidly, whereas the complexes were stably expressed on fixed cells. In addition, complexes formed in solution from purified MHC-II and peptide also had a very slow dissociation rate. These findings suggest a mechanism in viable cells for enhancing the dissociation and turnover of peptide–MHC-II complexes. The half-life of the complexes was very short relative to the half-life of MHC-II, allowing for the possibility of recycling of MHC-II molecules. In contrast to these results in B cells, antigen processing in macrophages is inhibited by cycloheximide and Brefeldin A (Harding and Unanue, 1989; Unanue *et al.,* 1989; Jensen, 1988), and these cells exhibit less turnover of existing peptide–MHC-II complexes (Harding *et al.,* 1989).

A discrepancy arises between the endocytic recycling of MHC-II molecules and the functional recycling of MHC-II–peptide complexes (i.e., turnover of the complexes to allow new peptide molecules to bind a given MHC molecule), between B cells and macrophages. The explanation may lie in differing properties of the endosomes containing MHC-II molecules in macrophages and B cells. Thus, B cells may recycle MHC-II molecules through a turnover-competent compartment that contains membrane proteins that facilitate the turnover of MHC-II-associated peptides, whereas intracellular MHC-II in macrophages may be largely

Table 1. Two pathways of antigen processing

	MHC-I	MHC-II
Primary antigen sources	Endogenous (cytosolic, membrane, or secretory proteins)	Exogenous (extracellular, also reinternalized endogenous proteins)
Site of antigen catabolism	Cytosol? ER?	endosomes vs. lysosomes
Site of peptide-MHC complexing	ER	endosomes
Turnover of peptide-MHC complexes	not known	varies among cells, prominent in some B cells
Inhibitors	cycloheximide Brefeldin A	chloroquine

confined to turnover-incompetent endosomes (Harding *et al.,* 1990).

Immunoprecipitation studies of biosynthetically labeled MHC-II molecules have revealed a nonpolymorphic "invariant" chain of 34 000 Da associated with the polymorphic α and β chains (Jones *et al.,* 1978). Invariant chain associates with nascent MHC-II molecules until they are transported to a protease-containing endosomal compartment, where it is proteolytically cleaved and dissociates from MHC-II (Blum and Cresswell, 1988). Invariant chain is not necessary for the transport of MHC-II molecules to the plasma membrane (Miller and Germain, 1986; Sekaly *et al.,* 1986). An interesting hypothesis has been proposed that invariant chain regulates the binding of peptides by directly associating with MHC-II proteins (possibly with the peptide-binding site itself). Thus, the invariant chain would have a protective role at the ER and Golgi; in endosomes the invariant chain is then released, leaving the class II molecules free to bind peptides (Elliott *et al.,* 1987). The experimental evidence so far is not definitive. L cells transfected with class II proteins together with or without invariant chain may or may not present antigens. In some cases (Stockinger *et al.,* 1989) but not in others (Sekaly *et al.,* 1988), lack of invariant chain led to impaired presentation. Finally, invariant chain could have a role in facilitation of the binding of peptides to MHC-II, perhaps initiated after its proteolytic cleavage, possibly including a function in the turnover of peptide–MHC-II complexes.

Different mechanisms and compartments involved in antigen processing for MHC-I–restricted presentation

Like MHC-II, MHC-I molecules bind and present immunogenic peptides derived from native antigens (Townsend *et al.,* 1985). Target cells can be lysed by CD8 T lymphocytes after the addition of peptides that bind to surface MHC-I molecules on the target cells. MHC-I can be directly shown to bind synthetic peptides in vitro (Chen and Parham, 1989; Bouillot *et al.,* 1989), although details of this binding urgently need to be worked out. However, significant differences exist in the pathways of antigen processing for MHC-I and MHC-II–restricted antigen presentation (Table 1) (Morrison *et al.,* 1986, 1988). As we have seen, MHC-II molecules serve primarily to bind and present peptides generated in endosomes or lysosomes from exogenous antigens internalized by endocytosis from the extracellular medium. In some examples, however, MHC-II may also present endogenous antigens (Sekaly *et al.,* 1988; Nuchtern *et al.,* 1990). In contrast, MHC-I appears to present almost exclusively endogenous antigens synthesized within the presenting cell.

In vitro study of the T-cell response to virally infected cells has clarified the distinction between the two processing pathways (Figures 1 and 2). Two basic sources of viral antigens exist during infection of a cell: 1) exogenous, preexisting components of the virion introduced into the cell during viral penetration; and 2) endogenous, viral infection resulting in the synthesis of viral antigens within the host cells, usually in vast excess of the amount of viral antigen introduced exogenously. Inactivated virus particles are presented exclusively by the exogenous pathway mediated by MHC-II; it is cycloheximide-resistant but inhibited by chloroquine (Morrison *et al.,* 1986). In contrast, after viral infection the presentation of endogenous viral antigens is chloroquine resistant but cycloheximide sensitive. Brefeldin A, which blocks ER-Golgi transport (Lippincott-Schwartz *et al.,* 1989), also

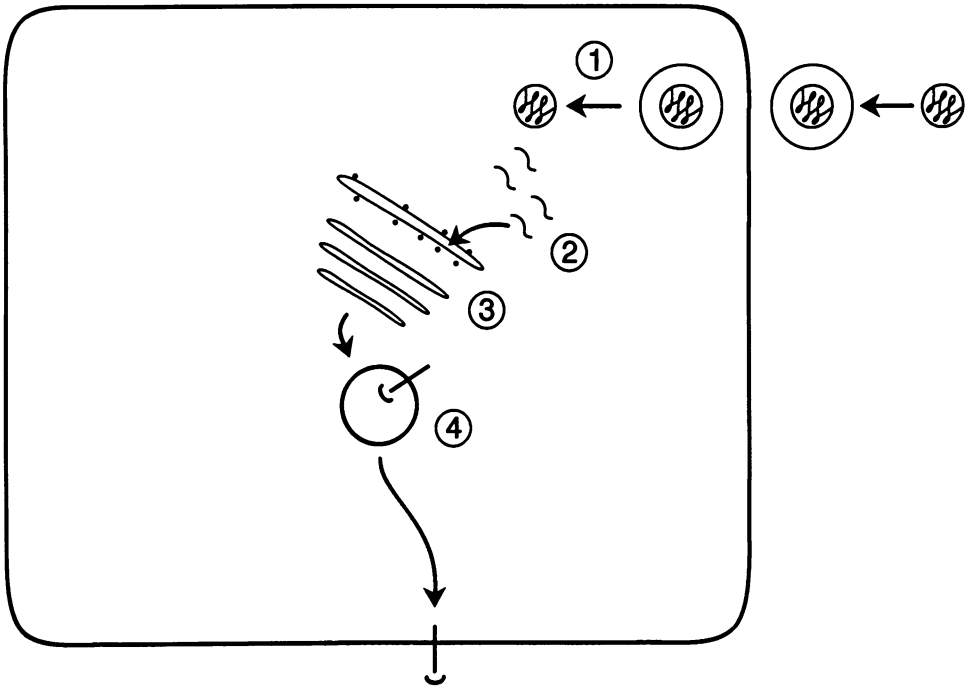

Figure 1. **Proposed mechanisms envisioned in presentation by MHC-I molecules.** The antigen is either synthesized endogenously or enters the cytosol from endocytic vesicles (1). It is then degraded to peptides (2) that cross into the ER (3). Eventually the peptides associate with MHC-I (4) and are transported to plasma membrane.

prevents the expression of new peptide–MHC-I complexes (Nuchtern *et al.*, 1989; Yewdell and Bennink, 1989), suggesting that newly synthesized MHC-I molecules must be utilized to form complexes. Furthermore, Townsend *et al.*, (1989) have recently shown that antigenic peptides may be required for the assembly of MHC-I, including the association of β_2-microglobulin with the class I heavy chain within the ER. This finding implies that peptide–MHC-I complexes

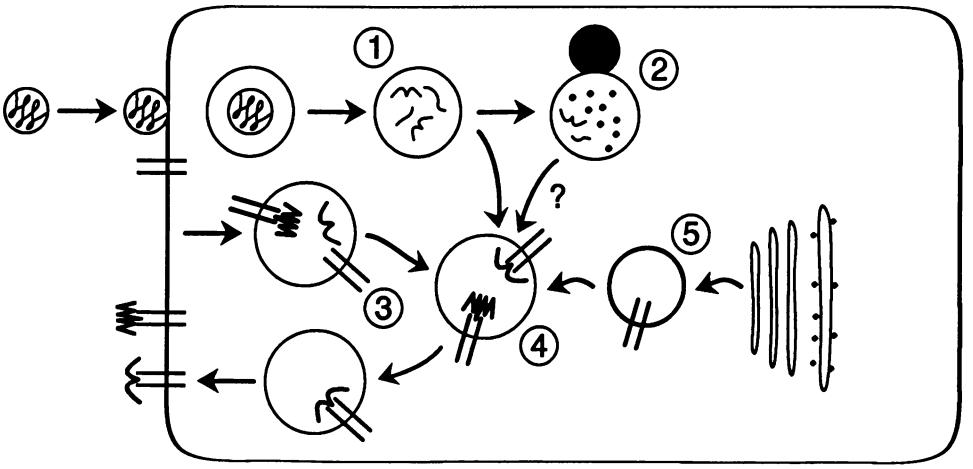

Figure 2. **Proposed mechanisms envisioned in presentation by MHC-II molecules.** A protein is internalized and then partially fragmented in endosomes (1) and extensively catabolized in lysosomes (2) where it is degraded to aminoacids (dots in 2). Some peptides are rescued and transported to a compartment that contains MHC-II (4). Vesicles containing MHC-II may derive from the plasma membrane (3) or from ER-Golgi (5). In 3 is depicted a mechanism of recycling and peptide exchange.

are actually formed there. It is possible that association of endogenous peptides with MHC-II may also occur within the ER in some instances.

Although peptide–MHC-I complexes may be formed in the ER, the pathway of antigen catabolism to produce immunogenic peptides is still unclear. It is important to note that endogenous antigens presented by MHC-I include cytosolic proteins (e.g., viral nucleoproteins) as well as secretory and membrane proteins. Exogenous proteins may enter the MHC-I processing pathway after their penetration into the cytosolic compartment (Yewdell *et al.,* 1988), which can be experimentally achieved by osmotic lysis of pinosomes (Moore *et al.,* 1988). Thus, a mechanism must exist for the transmembrane transport of cytosolic antigens or their constituent peptides into the ER for binding to MHC-I molecules. Studies of cytosolic influenza antigens expressed by infection of target cells with recombinant vaccinia virus show that constructs that lead to expression of less stable forms of the antigens produce enhanced presentation (Townsend *et al.,* 1988). Thus, antigen catabolism is also a central feature of antigen processing for MHC-I–restricted presentation.

Conclusion: a current model of events in antigen processing

A simplified model of antigen processing is indicated in Figures 1 and 2. Antigen processing for both the MHC-I pathway (Figure 1) and the MHC-II pathway (Figure 2) involves metabolism of antigen into constituent peptides. Endogenous antigens may be processed (within the ER or within the cytosol) and transported into the ER lumen, where the peptides bind to nascent MHC-I. Agents that penetrate into the cytosol, such as some intracellular pathogens, may be processed via the MHC-I pathway. Exogenous antigens are internalized via endocytosis or phagocytosis, and then proteolytically processed in endosomes and/or lysosomes to generate peptides that bind MHC-II molecules (either nascent or recycled by endocytosis and peptide turnover) within an endosomal compartment. In essence, we view the MHC-I proteins as the peptide-transport system that rescues peptides from ER-Golgi and transports them to the plasma membrane. The MHC-II proteins represent the rescue system for the endosomes. In both cases, the peptide-MHC complexes are then transported to the surface of the APC to be available for T-cell recognition.

Received: April 13, 1990.
Revised and accepted: May 11, 1990.

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