# IMMUNO TECHNOLOGY

## LECTURE 06: B-Cell DIFFERENTIATION

# Antigen Processing and Presentation

Recognition of foreign protein antigen 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.

### 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 TH cells occurred only in response to antigen presented by macrophages of the same MHC haplotype as the T cells.

### Role of Antigen-Presenting Cells

### 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 $CD8^+$ TC cells are referred to as *target cells;* cells that display peptides associated with class II MHC molecules to $CD4^+$ TH 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 costimulatory activity, they can activate naive TH 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 (see Table).

| 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 |

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.

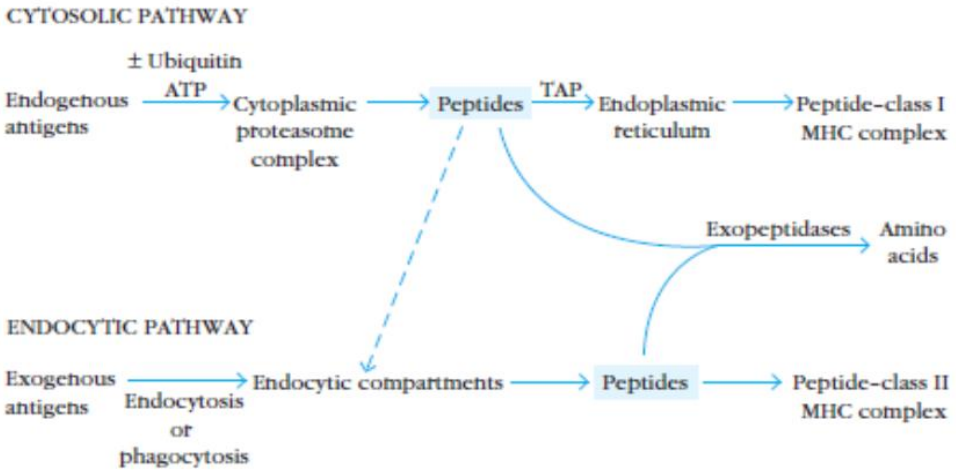
**Pathways - Processing and Presentation of antigens**

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 (see Figure).

**Endogenous Antigens: The Cytosolic Pathway**

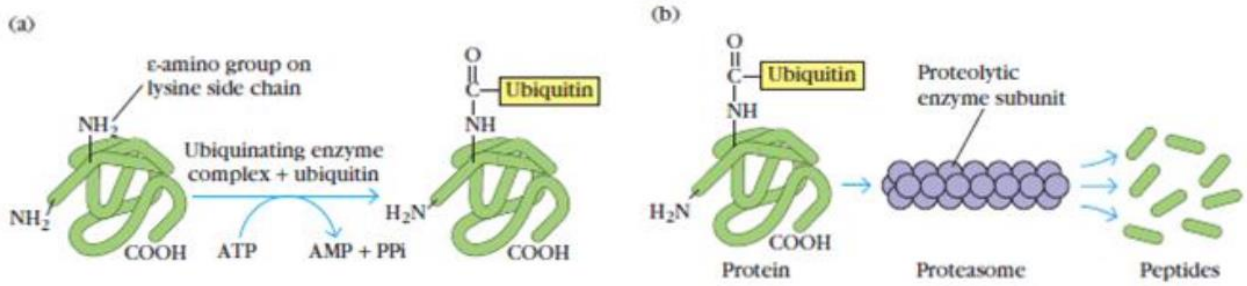
In eukaryotic cells, protein levels are carefully regulated. Every protein is subject to continuous turnover and is degraded at a rate that is generally expressed in terms of its half-life.

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 a). 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 b). Degradation of ubiquitin-protein complexes is thought to occur within the central hollow of the proteasome.

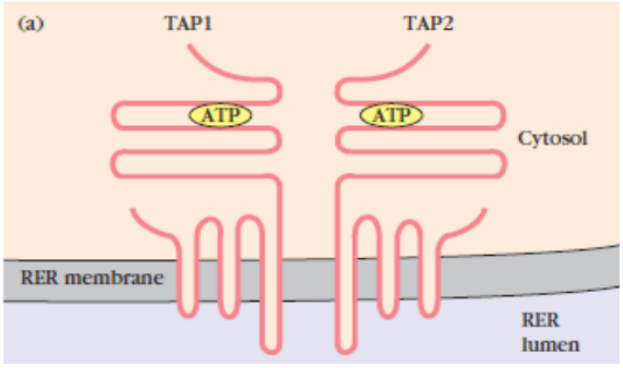



Experimental evidence indicates that the immune system utilizes this general pathway of protein degradation to produce small peptides for presentation with class I MHC molecules. The proteasomes involved in antigen processing include two subunits encoded within the MHC gene cluster, LMP2 and LMP7, and a third non-MHC protein, LMP10 (also called MECL-1). All three

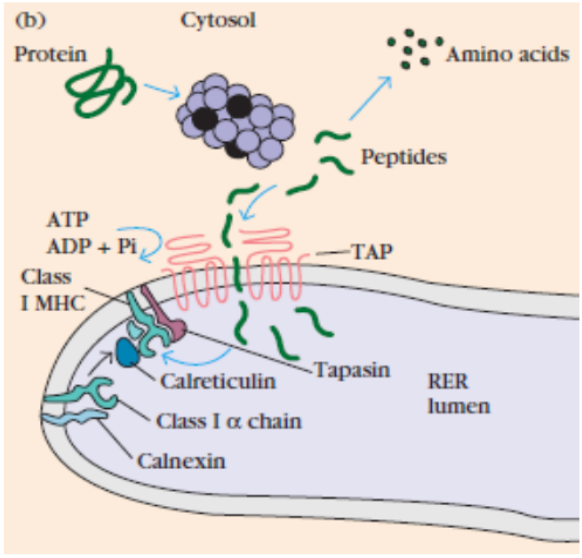
are induced by increased levels of the T-cell cytokine IFN-γ. The peptidase activities of proteasomes containing LMP2, LMP7, and LMP10 preferentially generate peptides that bind to MHC class I molecules. Such proteasomes, for example, show increased hydrolysis of peptide bonds that follow basic and/or hydrophobic residues. As described in earlier, peptides that bind to class I MHC molecules terminate almost exclusively with hydrophobic or basic residues.

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

The transporter protein, designated **TAP** (for **transporter associated with antigen processing**) is a membrane-spanning heterodimer consisting of two proteins: TAP1 and TAP2 (Figure a).




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 project 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 b).



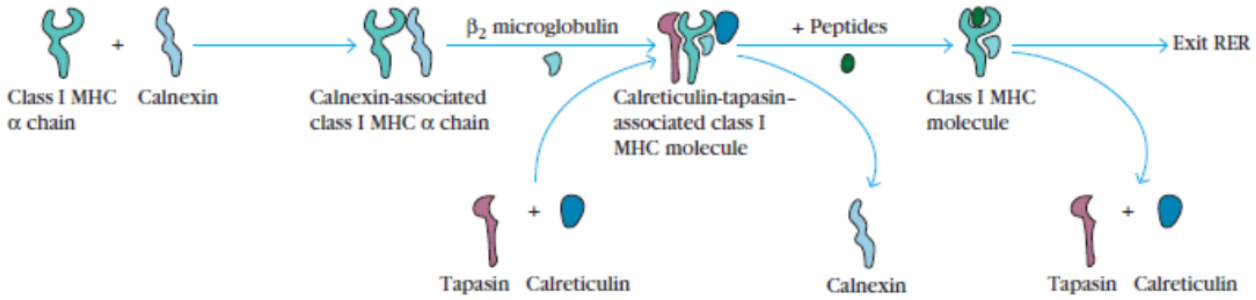
TAP has the highest affinity for peptides containing 8–10 amino acids, which is the optimal peptide length for class I MHC binding. In addition, TAP appears to favor peptides with hydrophobic or basic carboxyl-terminal amino acids, the preferred anchor residues for class I MHC molecules. Thus, TAP is 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. 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.

**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 (see Figure).



The physical association of the α chain– $\beta_2$-microglobulin heterodimer with the TAP protein promotes peptide capture by the class I molecule before the peptides are exposed to the luminal environment of the RER.

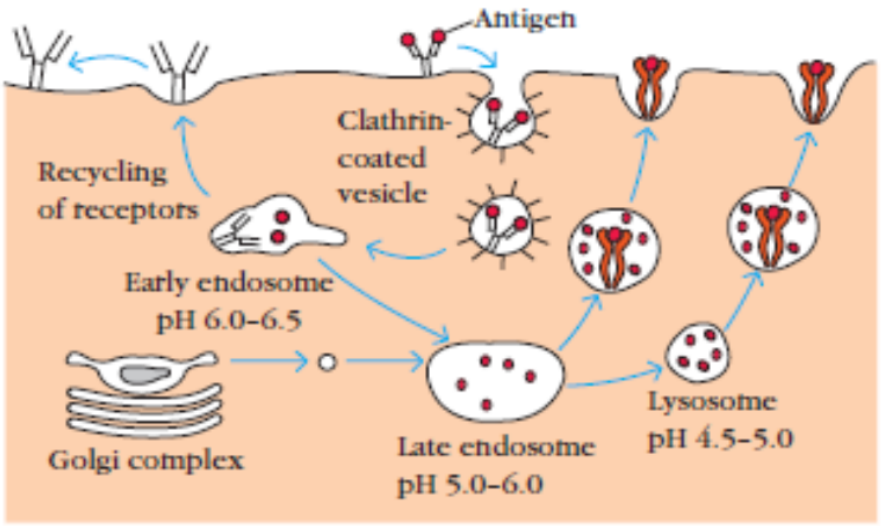
Peptides not bound by class I molecules are rapidly degraded. As a consequence of peptide binding, the class I molecule displays increased stability and can dissociate from calreticulin and tapasin, exit from the RER, and proceed to the cell surface via the Golgi. An additional chaperone protein, ERp57, 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.

**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 (see Figure). 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**

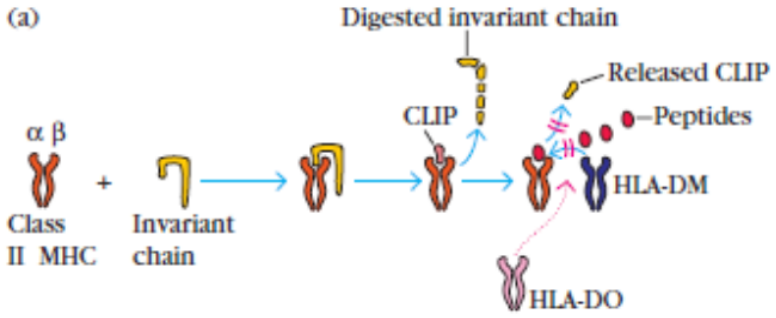
Since antigen-presenting cells express both class I and class II MHC molecules, some mechanism must exist to prevent class II MHC molecules from binding to the same set of antigenic peptides as the class I molecules. When class II MHC molecule are synthesized within the RER, three pairs of class II αβ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

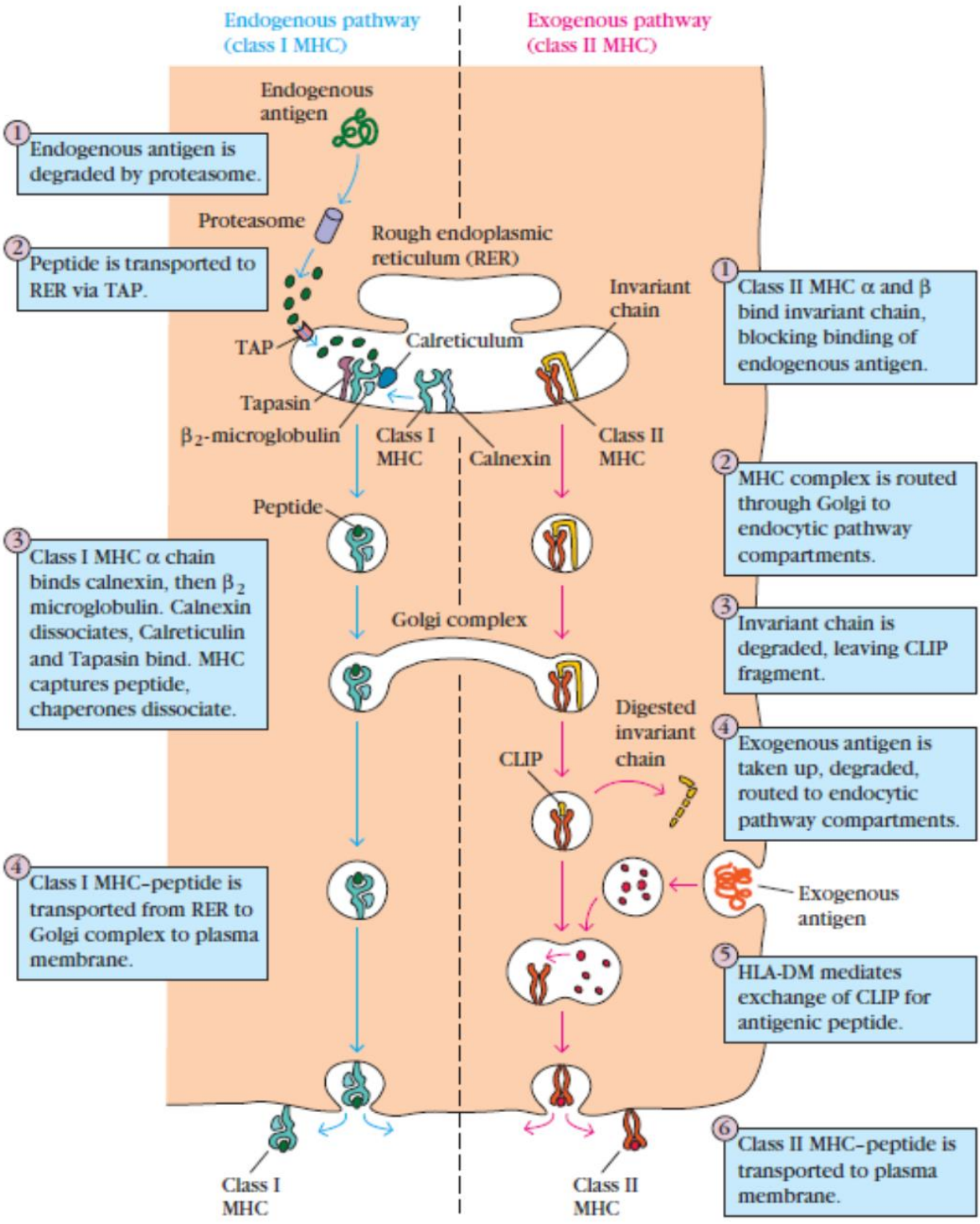
**Peptides Assemble with Class II MHC Molecules by Displacing CLIP**

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.

A nonclassical class II MHC molecule called *HLA-DM* is required to catalyze the exchange of CLIP with antigenic peptides (Figure a).




MHC class II genes encoding HLA-DM have been identified in the mouse and rabbit, indicating 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.




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

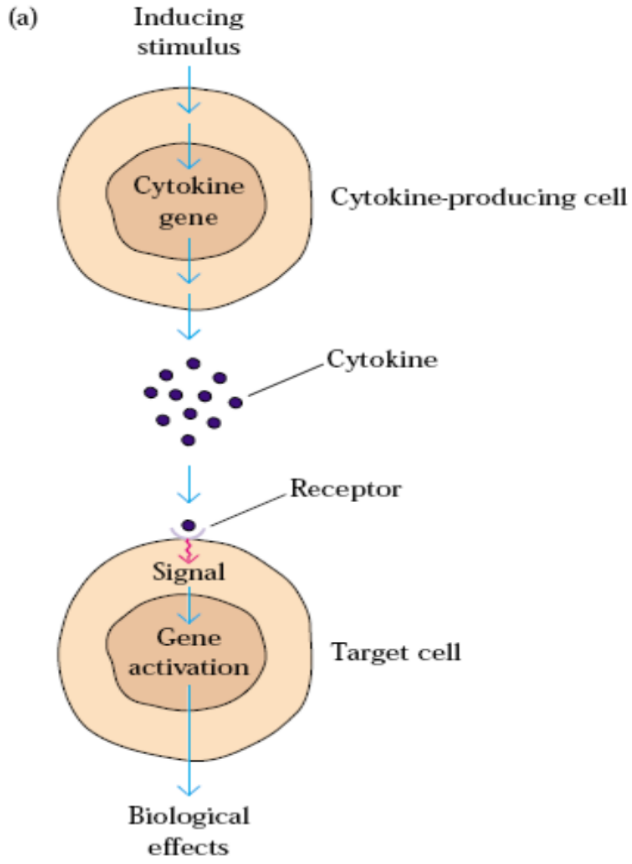
antigenic peptides associate with class I MHC molecules in the rough endoplasmic reticulum or with class II molecules in endocytic compartments.

# Cytokines – Action, Regulation and Signal Transduction

The development of an effective immune response involves lymphoid cells, inflammatory cells, and hematopoietic cells. The complex interactions among these cells are mediated by a group of proteins collectively designated **cytokines** to denote their role in cell-to-cell communication. Cytokines are low-molecular weight regulatory proteins or glycoproteins secreted by white blood cells and various other cells in the body in response to a number of stimuli. These proteins assist in regulating the development of immune effector cells, and some cytokines possess direct effector functions of their own.

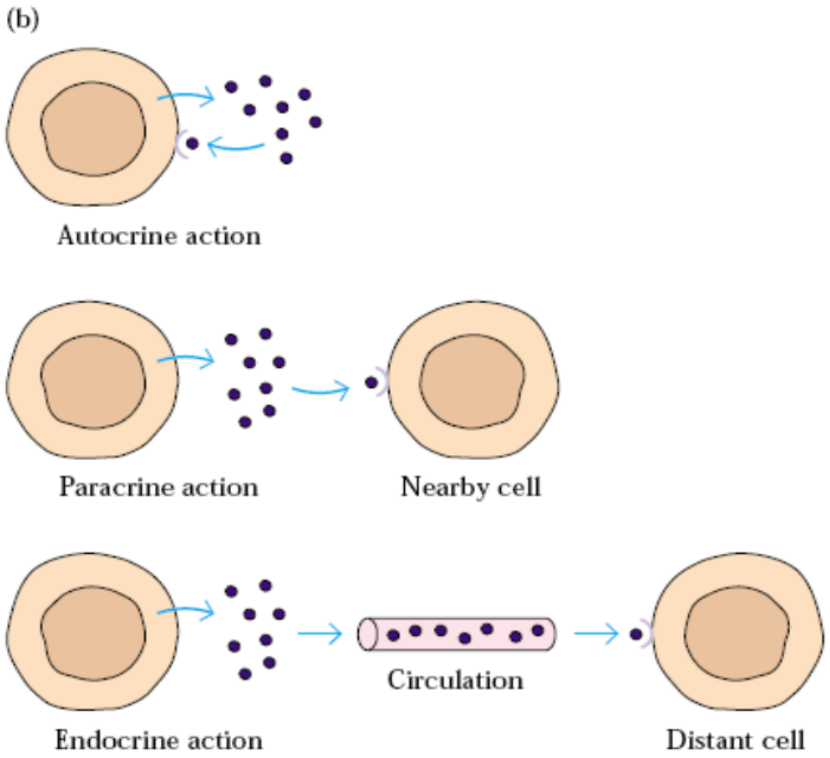
**Properties of Cytokines**

Cytokines bind to specific receptors on the membrane of target cells, triggering signal-transduction pathways that ultimately alter gene expression in the target cells (Figure a).




In general, the cytokines and their receptors exhibit very high affinity for each other, with dissociation constants ranging from $10^{-10}$ to $10^{-12}$ M. Because their affinities are so high, cytokines can mediate biological effects at picomolar concentrations.

A particular cytokine may bind to receptors on the membrane of the same cell that secreted it, exerting **autocrine** action; it may bind to receptors on a target cell in close proximity to the producer cell, exerting **paracrine** action; in a few cases, it may bind to target cells in distant parts of the body, exerting **endocrine** action.

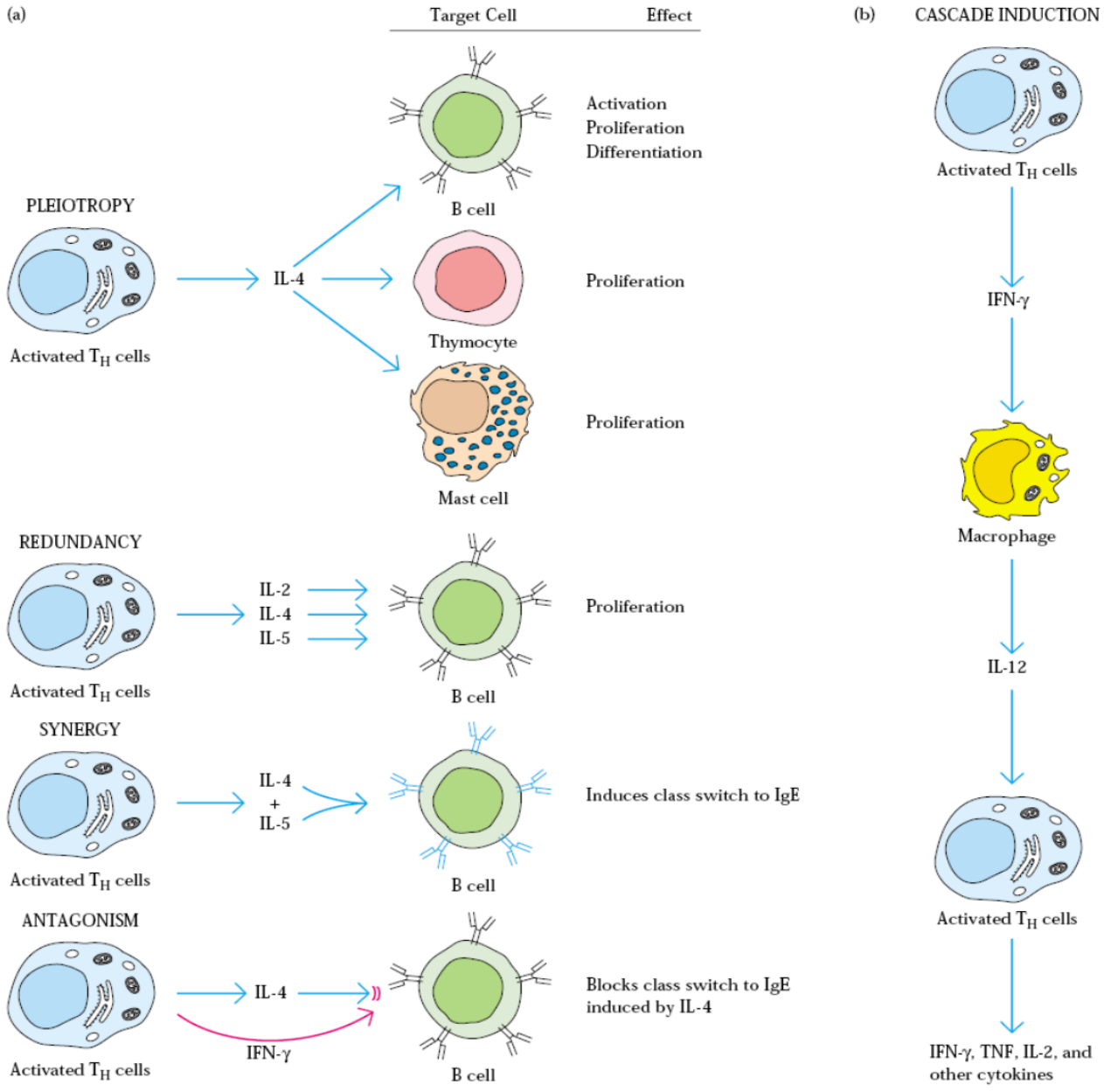



Cytokines regulate the intensity and duration of the immune response by stimulating or inhibiting the activation, proliferation, and / or differentiation of various cells and by regulating the secretion of antibodies or other cytokines.

Thus, the cytokines secreted by even a small number of lymphocytes activated by antigen can influence the activity of numerous cells involved in the immune response. For example, cytokines produced by activated $T_H$ cells can influence the activity of B cells, $T_C$ cells, natural killer cells, macrophages, granulocytes, and hematopoietic stem cells, thereby activating an entire network of interacting cells.

Cytokines exhibit the attributes of pleiotropy, redundancy; synergy, antagonism, and cascade induction, which permit them to regulate cellular activity in a coordinated, interactive way (see Figure). A given cytokine that has different biological effects on different target cells has a pleiotropic action. Two or more cytokines that mediate similar functions are said to be redundant; redundancy makes it difficult to ascribe a particular activity to a single cytokine. Cytokine

synergism occurs when the combined effect of two cytokines on cellular activity is greater than the additive effects of the individual cytokines. In some cases, cytokines exhibit antagonism; that is, the effects of one cytokine inhibit or offset the effects of another cytokine. Cascade induction occurs when the action of one cytokine on a target cell induces that cell to produce one or more other cytokines, which in turn may induce other target cells to produce other cytokines.




The term *cytokine* encompasses those cytokines secreted by lymphocytes, substances formerly known as **lymphokines,** and those secreted by monocytes and macrophages, substances formerly known as **monokines.** Although these other two terms continue to be used, they are misleading because secretion of many lymphokines and monokines is not limited to lymphocytes

and monocytes as these terms imply, but extends to a broad spectrum of cells and types. For this reason, the more inclusive term *cytokine* is preferred.

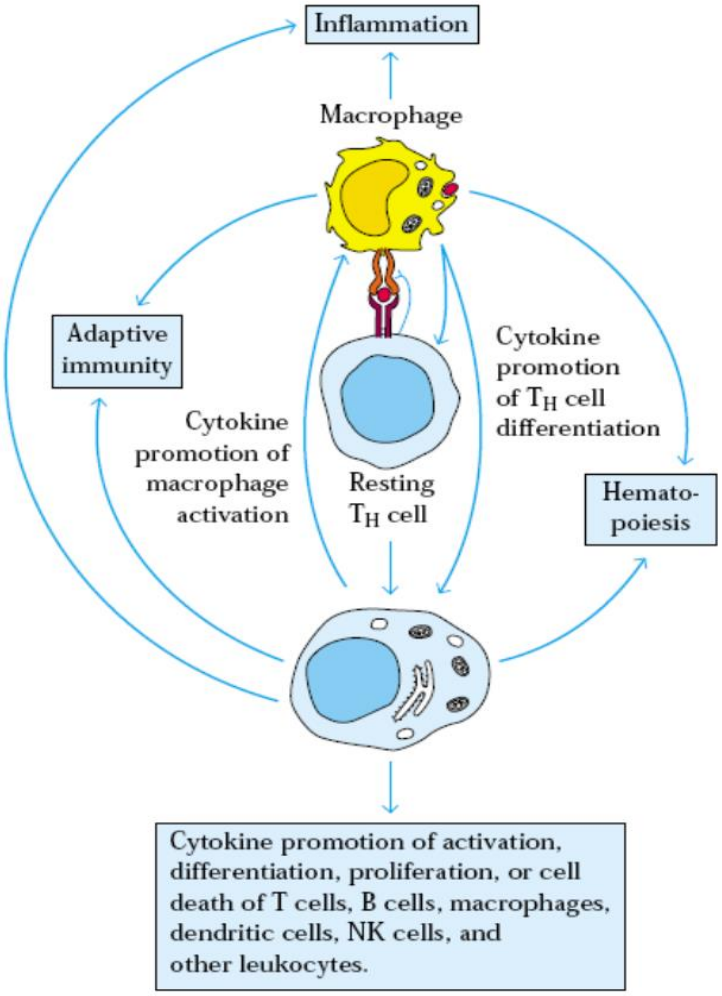
Many cytokines are referred to as **interleukins,** a name indicating that they are secreted by some leukocytes and act upon other leukocytes. Interleukins 1–25 have been identified. There is reason to suppose that still other cytokines will be discovered and that the interleukin group will expand further. Some cytokines are known by common names, including the interferons and tumor necrosis factors. Recently gaining prominence is yet another another subgroup of cytokines, the **chemokines,** a group of low-molecular weight cytokines that affect chemotaxis and other aspects of leukocyte behavior.

### Cytokines Belong to Four Structural Families

Once the genes encoding various cytokines had been cloned, sufficient quantities of purified preparations became available for detailed studies on their structure and function. Cytokines generally have a molecular mass of less than 30 kDa. Structural studies have shown that the cytokines share a similar polypeptide fold, with four α-helical regions (A–D) in which the first and second helices and the third and fourth helices run roughly parallel to one another and are connected by loops.

### Cytokines Have Numerous Biological Functions

| Cytokine* | Secreted by** | Targets and effects |
|---|---|---|
| SOME CYTOKINES OF INNATE IMMUNITY | | |
| Interleukin 1 (IL-1) | Monocytes, macrophages, endothelial cells, epithelial cells | Vasculature (inflammation); hypothalamus (fever); liver (induction of acute phase proteins) |
| Tumor Necrosis Factor-α (TNF-α) | Macrophages | Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophil activation |
| Interleukin 12 (IL-12) | Macrophages, dendritic cells | NK cells; influences adaptive immunity (promotes $T_H1$ subset) |
| Interleukin 6 (IL-6) | Macrophages, endothelial cells | Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B cell lineage) |
| Interferon α (IFN-α) (This is a family of molecules) | Macrophages | Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells |
| Interferon β (IFN-β) | Fibroblasts | Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells |
| SOME CYTOKINES OF ADAPTIVE IMMUNITY | | |
| Interleukin 2 (IL-2) | T cells | T-cell proliferation; can promote AICD. NK cell activation and proliferation; B-cell proliferation |
| Interleukin 4 (IL-4) | $T_H2$ cells; mast cells | Promotes $T_H2$ differentiation; isotype switch to IgE |
| Interleukin 5 (IL-5) | $T_H2$ cells | Eosinophil activation and generation |
| Interleukin 25 (IL-25) | Unknown | Induces secretion of $T_H2$ cytokine profile |
| Transforming growth factor β (TGF-β) | T cells, macrophages, other cell types | Inhibits T-cell proliferation and effector functions; inhibits B-cell proliferation; promotes isotype switch to IgE; inhibits macrophages |
| Interferon γ (IFN-γ) | $T_H1$ cells; $CD8^+$ cells; NK cells | Activates macrophages; increases expression MHC class I and class II molecules; increases antigen presentation |




Although a variety of cells can secrete cytokines, the two principal producers are the $T_H$ cell and the macrophage. Cytokines released from these two cell types activate an entire network of interacting cells (see Figure).

**Cytokine Receptors**

As noted already, to exert their biological effects, cytokines must first bind to specific receptors expressed on the membrane of responsive target cells. Because these receptors are expressed by many types of cells, the cytokines can affect a diverse array of cells. Biochemical characterization of cytokine receptors initially progressed at a very slow pace because their levels on the membrane of responsive cells are quite low. As with the cytokines themselves, cloning of the genes encoding cytokine receptors has led to rapid advances in the identification and characterization of these receptors.

**Cytokine Receptors Fall within Five Families**

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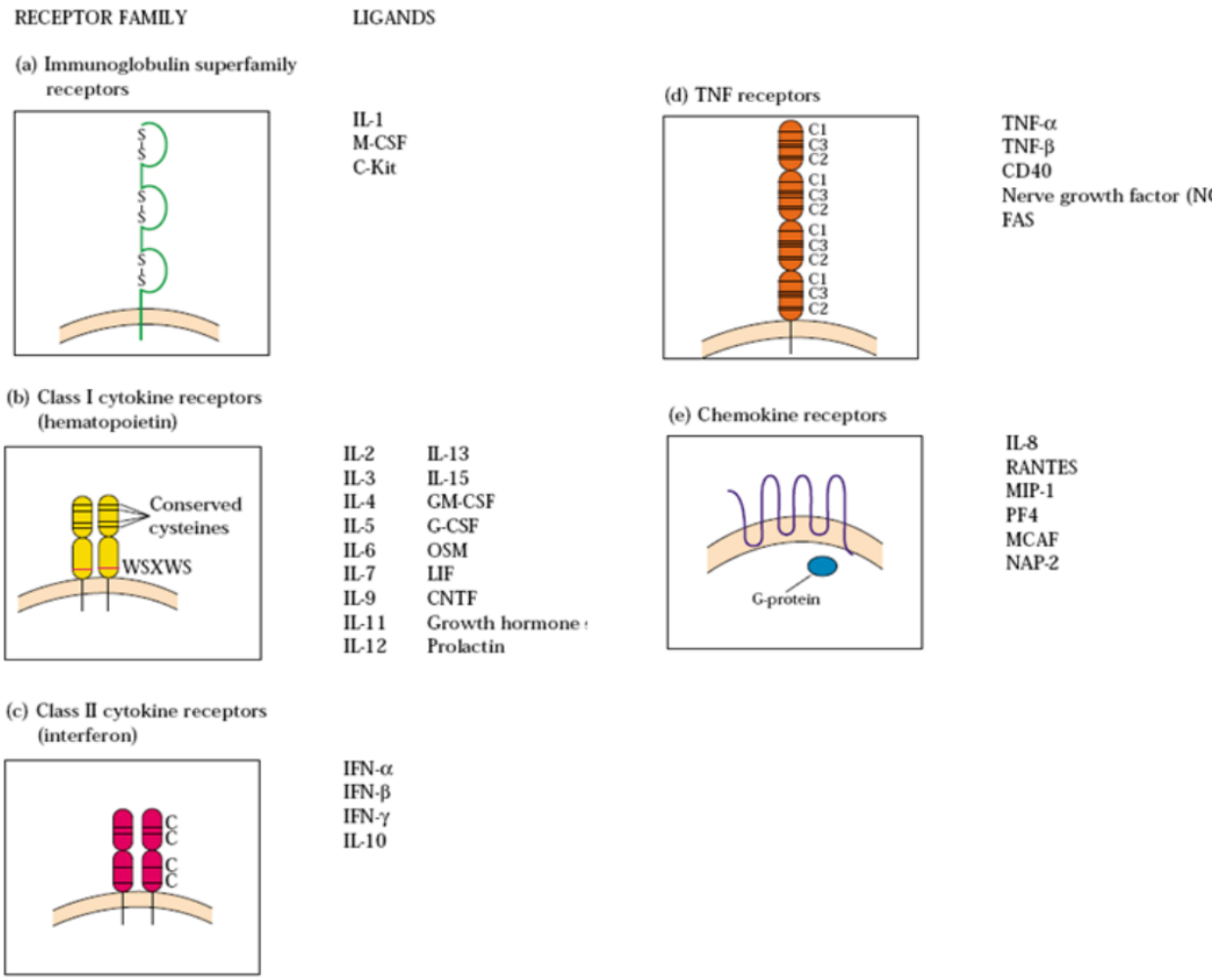
Receptors for the various cytokines are quite diverse structurally, but almost all belong to one of five families of receptor proteins (Figure 12-6):

1. Immunoglobulin superfamily receptors
2. Class I cytokine receptor family (also known as the hematopoietin receptor family)
3. Class II cytokine receptor family (also known as the interferon receptor family)
4. TNF receptor family
5. Chemokine receptor family




Many of the cytokine-binding receptors that function in the immune and hematopoietic systems belong to the class I cytokine receptor family. The members of this receptor family have conserved amino acid sequence motifs in the extracellular domain consisting of four positionally conserved cysteine residues (CCCC) and a conserved sequence of tryptophanserine-(any amino acid)-tryptophan-serine (WSXWS, where X is the nonconserved amino acid). The receptors for all the cytokines classified as hematopoietins belong to the class I cytokine receptor family, which also

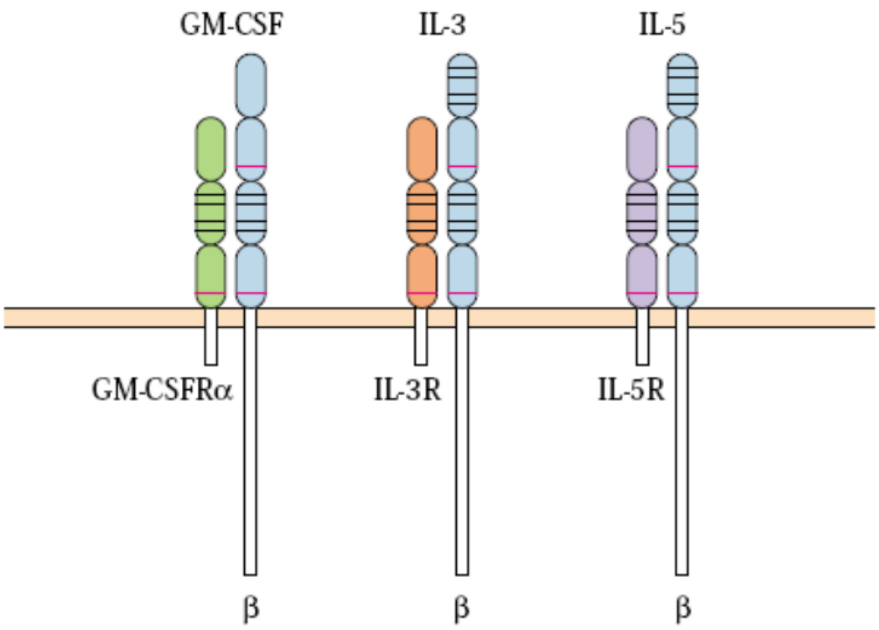
is called the hematopoietin receptor family. The class II cytokine receptors possess the conserved CCCC motifs, but lack the WSXWS motif presents in class I cytokine receptors. Initially only the three interferons, α, β, and γ, were thought to be ligands for these receptors. However, recent work has shown that the IL-10 receptor is also a member of this group.

Another feature common to most of the hematopoietin (class I cytokine) and the class II cytokine receptor families is multiple subunits, often including one subunit that binds specific cytokine molecules and another that mediates signal transduction. Note, however, that these two functions are not always confined to one subunit or the other. Engagement of all of the class I and class II cytokine receptors studied to date has been shown to induce tyrosine phosphorylation of the receptor through the activity of protein tyrosine kinases closely associated with the cytosolic domain of the receptors.

**Subfamilies of Class I Cytokine Receptors Have Signaling Subunits in Common**

Several subfamilies of class I cytokine receptors have been identified, with all the receptors in a subfamily having an identical signal-transducing subunit. The following figure schematically illustrates the members of three receptor subfamilies, named after GM-CSF, IL-2, and IL-6.

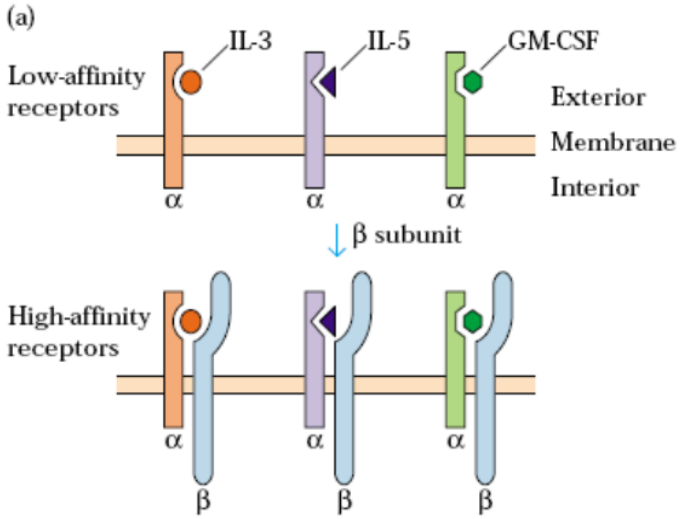
(a) GM-CSF receptor subfamily (common β subunit)



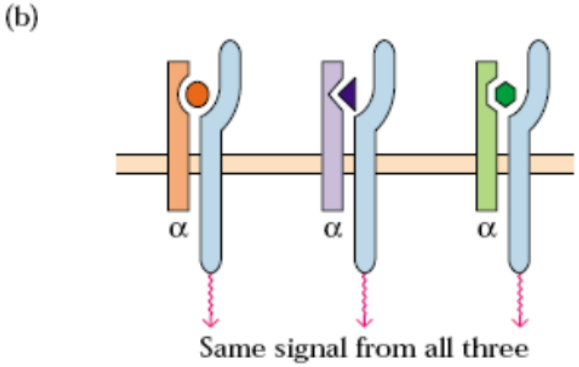

The sharing of signal-transducing subunits among receptors explains the redundancy and antagonism exhibited by some cytokines. Consider the GM-CSF receptor subfamily, which includes the receptors for IL-3, IL-5, and GM-CSF (see Figure a).

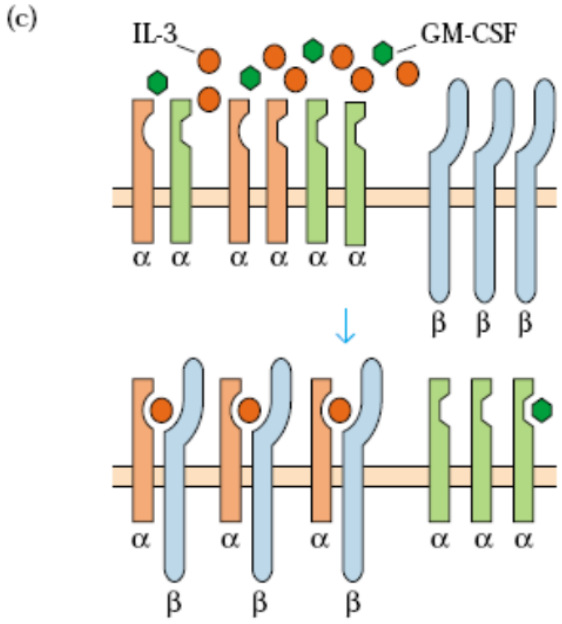
Each of these cytokines binds to a unique lowaffinity, cytokine-specific receptor consisting of an α subunit only. All three low-affinity subunits can associate noncovalently with a common signal-transducing β subunit. The resulting dimeric receptor not only exhibits increased affinity for the cytokine but also can transduce a signal across the membrane after binding the cytokine (see Figure a).

Interestingly, IL-3, IL-5, and GM-CSF exhibit considerable redundancy. IL-3 and GM-CSF both act upon hematopoietic stem cells and progenitor cells, activate monocytes, and induce megakaryocyte differentiation. All three of these cytokines induce eosinophil proliferation and basophil degranulation with release of histamine.




Since the receptors for IL-3, IL-5, and GM-CSF share a common signal-transducing β subunit, each of these cytokines would be expected to transduce a similar activation signal, accounting for the redundancy among their biological effects (see Figure b).





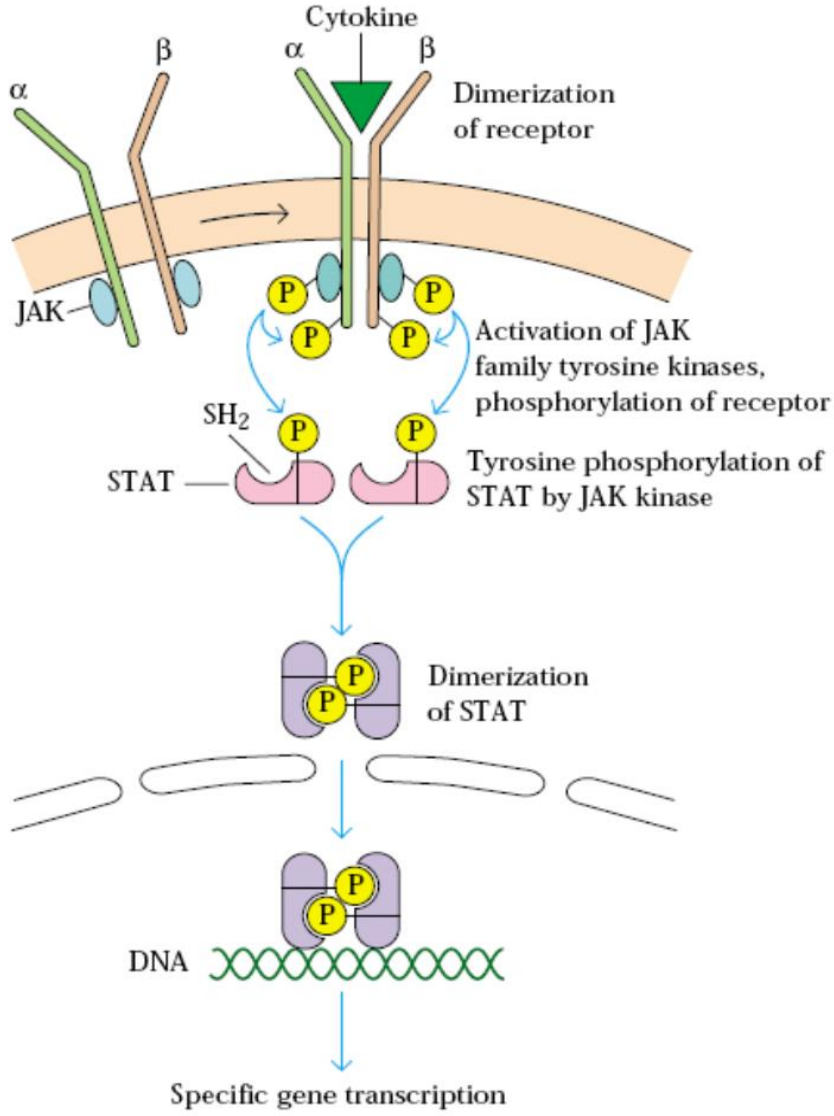

In fact, all three cytokines induce the same patterns of protein phosphorylation. Furthermore, IL-3 and GM-CSF exhibit antagonism; IL-3 binding has been shown to be inhibited by GM-CSF, and conversely, binding of GM-CSF has been shown to be inhibited by IL-3. Since the signal-transducing β subunit is shared between the receptors for these two cytokines, their antagonism is due to competition for a limited number of β subunits by the cytokine-specific α subunits of the receptors (Figure c).

**Engaged Cytokine Receptors Activate Signaling Pathways**

While some important cytokine receptors lie outside the class I and class II families, the majority are included within these two families. As mentioned previously, class I and class II cytokine receptors lack signaling motifs (e.g., intrinsic tyrosine kinase domains). Yet, early observations demonstrated that one of the first events after the interaction of a cytokine with one of these receptors is a series of protein tyrosine phosphorylations.While these results were initially puzzling, they were explained when a unifying model emerged from studies of the molecular events triggered by binding of interferon gamma (IFN-γ) to its receptor, a member of the class II family.

IFN-γ was originally discovered because of its ability to induce cells to block or inhibit the replication of a wide variety of viruses. Antiviral activity is a property it shares with IFN-α and IFN-β. However, unlike these other interferons, IFN-γ plays a central role in many immunoregulatory processes, including the regulation of mononuclear phagocytes, B-cell switching to certain IgG classes, and the support or inhibition of the development of $T_H$-cell subsets. The

discovery of the major signaling pathway invoked by interaction of IFN-γ with its receptor led to the realization that signal transduction through most, if not all, class I and class II cytokine receptors involves the following steps, which are the basis of a unifying signaling model (see Figure).




→ *The cytokine receptor is composed of separate subunits,* an α chain required for cytokine binding and for signal transduction and a β chain necessary for signaling but with only a minor role in binding.

→ *Different inactive protein tyrosine kinases are associated with different subunits of the receptor.* The α chain of the receptor is associated with a novel family of protein tyrosine kinases, the Janus kinase (JAK)* family. The association of the JAK and the receptor subunit occurs spontaneously and does not require the binding of cytokine. However, in the absence of cytokine, JAKs lack protein tyrosine kinase activity.

→ *Cytokine binding induces the association of the two separate cytokine receptor subunits and activation of the receptor-associated JAKs.* The ability of IFN-γ, which binds to a class II cytokine receptor, to bring about the association of the ligand-binding chains of its receptor has been directly demonstrated by x-ray crystallographic studies.

→ *Activated JAKs create docking sites for the STAT transcription factors by phosphorylation of specific tyrosine residues on cytokine receptor subunits.* Once receptorassociated JAKs are activated, they phosphorylate specific tyrosines in the receptor subunits of the *the transcription of specific genes.*While docked to receptor subunits, STATs undergo JAK-catalyzed phosphorylation of a key tyrosine. This is followed by the dissociation of the STATs from the receptor subunits and their dimerization. The STAT dimers then translocate into the nucleus and induce the expression of genes containing appropriate regulatory sequences in their promoter regions.

**Cytokine Antagonists**

A number of proteins that inhibit the biological activity of cytokines have been reported. These proteins act in one of two ways: either they bind directly to a cytokine receptor but fail to activate the cell, or they bind directly to a cytokine, inhibiting its activity. The best-characterized inhibitor is the IL-1 receptor antagonist (IL-1Ra), which binds to the IL-1 receptor but has no activity. Binding of IL-1Ra to the IL-1 receptor blocks binding of both IL-1α and IL-1β, thus accounting for its antagonistic properties. Production of IL-1Ra has been thought by some to play a role in regulating the intensity of the inflammatory response. It has been cloned and is currently being investigated as a potential treatment for chronic inflammatory diseases.

Cytokine inhibitors are found in the bloodstream and extracellular fluid. These soluble antagonists arise from enzymatic cleavage of the extracellular domain of cytokine receptors. Among the soluble cytokine receptors that have been detected are those for IL-2, -4, -6, and -7, IFN-γ and -α, TNF-β, and LIF. Of these, the soluble IL-2 receptor (sIL-2R), which is released in chronic T-cell activation, is the best characterized. A segment containing the amino-terminal 192 amino acids of the α subunit is released by proteolytic cleavage, forming a 45-kDa soluble IL-2 receptor. The shed receptor can bind IL-2 and prevent its interaction with the membrane-bound IL-2 receptor. The presence of sIL-2R has been used as a clinical marker of chronic T-cell activation and is observed in a number of diseases, including autoimmunity, transplant rejection, and AIDS.

Some viruses also produce cytokine-binding proteins or cytokine mimics. The evolution of such anti-cytokine strategies by microbial pathogens is good biological evidence of the importance of cytokines in organizing and promoting effective anti-microbial immune responses. The poxviruses, for example, have been shown to encode a soluble TNF-binding protein and a soluble IL-1 binding protein. Since both TNF and IL-1 exhibit a broad spectrum of activities in the inflammatory response, these soluble cytokine-binding proteins may prohibit or diminish the inflammatory effects of the cytokines, thereby conferring upon the virus a selective advantage.

Epstein-Barr virus produces an IL-10–like molecule (viral IL-10 or vIL-10) that binds to the IL-10 receptor and, like cellular IL-10, suppresses $T_H1$-type cell-mediated responses, which are effective against many intracellular parasites such as viruses.

| Virus | Product |
|---|---|
| Leporipoxvirus (a myxoma virus) | Soluble IFN-γ receptor |
| Several poxviruses | Soluble IFN-γ receptor |
| Vaccinia, smallpox virus | Soluble IL-1 β receptor |
| Epstein-Barr | IL-10 homolog |
| Human herpesvirus-8 | IL-6 homolog; also homologs of the chemokines MIP-I and MIP-II |
| Cytomegalovirus | Three different chemokine receptor homologs, one of which binds three different soluble chemokines (RANTES, MCP-1, and MIP-1α) |

Molecules produced by viruses that mimic cytokines allow the virus to manipulate the immune response in ways that aid the survival of the pathogen. This is an interesting and powerful modification some viruses have undergone in their continuing struggle to overcome the formidable barrier of host immunity. The above Table lists a number of viral products that mimic cytokines or their receptors.