IMMUNO TECHNOLOGY

LECTURE 07: CELL MEDIATED IMMUNE RESPONSE

Cell mediated immune response

The cell mediated and humoral branches of the immune system assume different roles in protecting the host. The effectors of the humoral branch are secreted antibodies, highly specific molecules that can bind and neutralize antigens on the surface of cells and in the extracellular spaces. The primary domain of antibody protection lies outside the cell. If antibodies were the only agents of immunity, pathogens that managed to evade them and colonize the intracellular environment would escape the immune system. This is not the case. The principal role of cell-mediated immunity is to detect and eliminate cells that harbor intracellular pathogens. Cell-mediated immunity also can recognize and eliminate cells, such as tumor cells, that have undergone genetic modifications so that they express antigens not typical of normal cells. Both antigen-specific and -nonspecific cells can contribute to the cell-mediated immune response. Specific cells include $CD8^+$ cytotoxic T lymphocytes (T_C cells or CTLs) and cytokine-secreting $CD4^+$ T_H cells that mediate delayed-type hypersensitivity (DTH).

Cell-mediated immune responses can be divided into two major categories according to the different effector populations that are mobilized. One group comprises effector cells that have direct cytotoxic activity. These effectors eliminate foreign cells and altered self-cells by mounting a cytotoxic reaction that lyses their target. The various cytotoxic effector cells can be grouped into two general categories: one comprises antigen-specific cytotoxic T lymphocytes (CTLs) and nonspecific cells, such as natural killer (NK) cells and macrophages. The target cells to which these effectors are directed include allogeneic cells, malignant cells, virus-infected cells, and chemically conjugated cells. The other group is a subpopulation of effector $CD4^+$ T cells that mediates delayed type hypersensitivity reactions.

General Properties of Effector T Cells

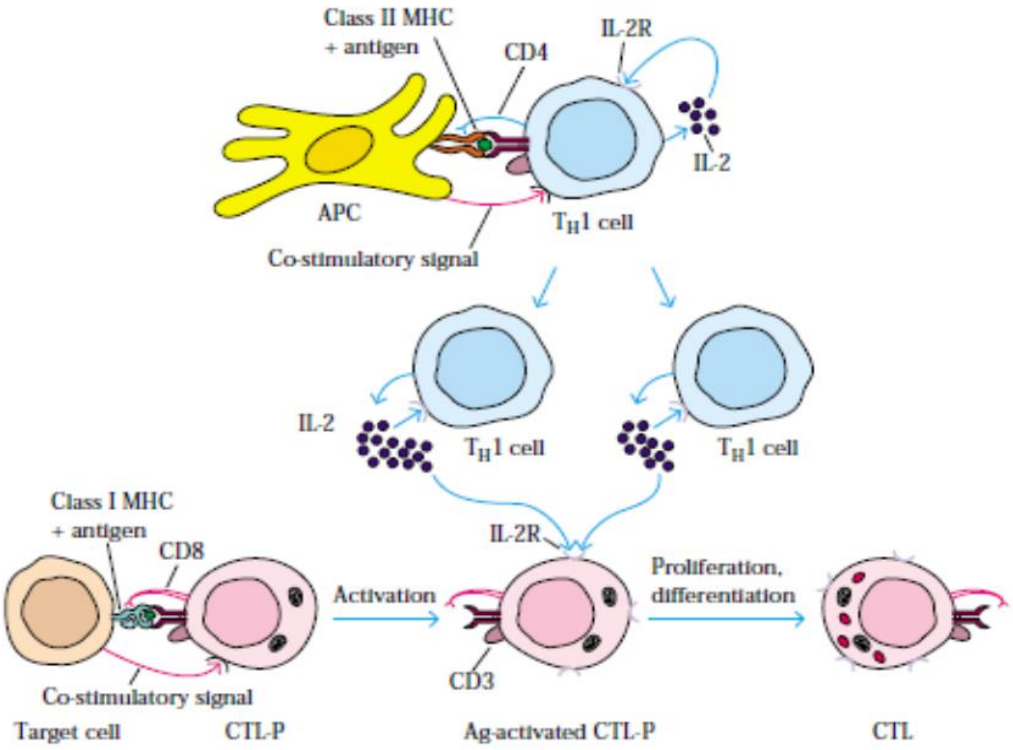
The three types of effector T cells—$CD4^+$, T_H1 and T_H2 cells, and $CD8^+$ CTLs—exhibit several properties that set them apart from naive helper and cytotoxic T cells (see Table). In particular, effector cells are characterized by their less stringent activation requirements, increased expression of cell adhesion molecules, and production of both membrane bound and soluble effector molecules.

Property	Naive T cells	Effector T cells
Co-stimulatory signal (CD28-B7 interaction)	Required for activation	Not required for activation
CD45 isoform	CD45RA	CD45RO
Cell-adhesion molecules (CD2 and LFA-1)	Low	High
Trafficking patterns	HEVs* in secondary lymphoid tissue	Tertiary lymphoid tissues; inflammatory sites

Effector CTLs Are Generated from CTL Precursors

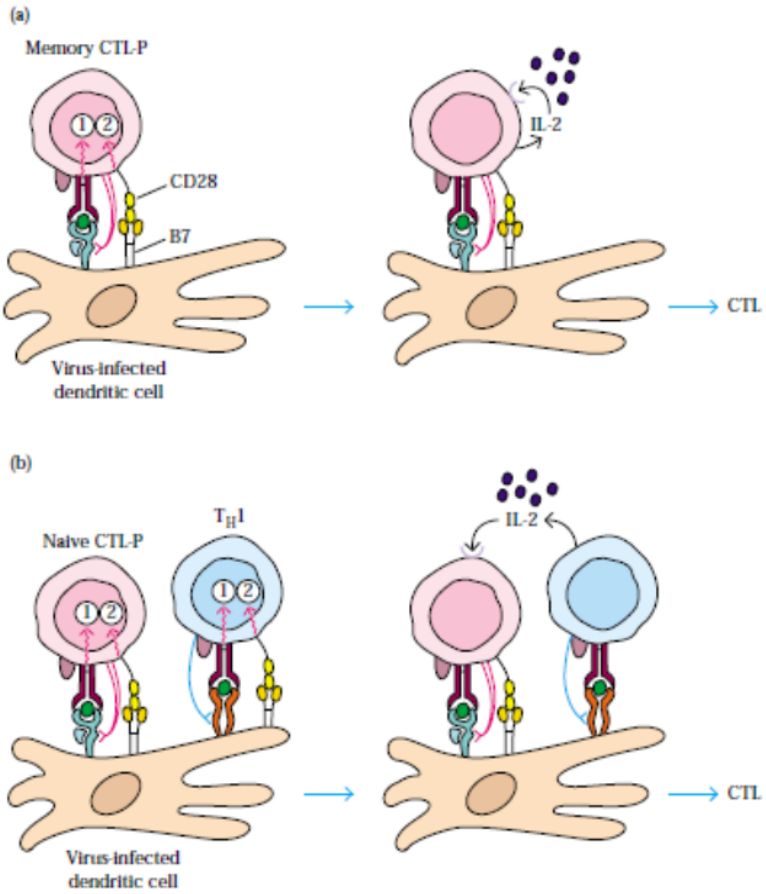
Naive TC cells are incapable of killing target cells and are therefore referred to as CTL precursors (CTL-Ps) to denote their functionally immature state. Only after a CTL-P has been activated will the cell differentiate into a functional CTL with cytotoxic activity. Generation of CTLs from CTL-Ps appears to require at least three sequential signals (Figure 14-1):

- An antigen-specific signal 1 transmitted by the TCR complex upon recognition of a peptide–class I MHC molecule complex
- A co-stimulatory signal transmitted by the CD28-B7 interaction of the CTL-P and the antigen-presenting cell
- A signal induced by the interaction of IL-2 with the high-affinity IL-2 receptor, resulting in proliferation and differentiation of the antigen-activated CTL-P into effector CTLs



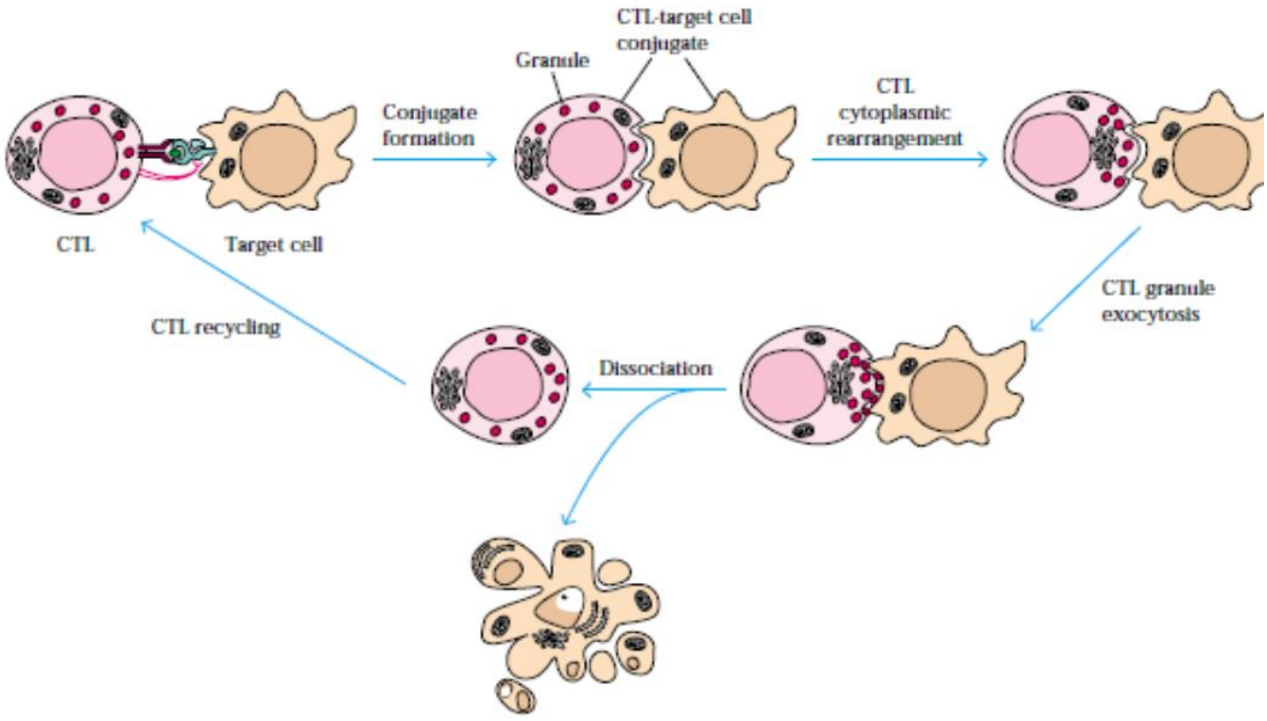
Unactivated CTL-Ps do not express IL-2 or IL-2 receptors, do not proliferate, and do not display cytotoxic activity. Antigen activation induces a CTL-P to begin expressing the IL-2 receptor and to a lesser extent IL-2, the principal cytokine required for proliferation and differentiation of activated CTL-Ps into effector CTLs. In some cases, the amount of IL-2 secreted by an antigen-activated CTL-P may be sufficient to induce its own proliferation and differentiation; this is particularly true of memory CTL-Ps, which have lower activation requirements than naive cells do (see Figure a). In general, though, most activated CTL-Ps requires additional IL-2 produced by proliferating TH1 cells to proliferate and differentiate into effector CTLs. The fact that the IL-2

receptor is not expressed until after a CTL-P has been activated by antigen plus a class I MHC molecule favors the clonal expansion and acquisition of cytotoxicity by only the antigen-specific CTL-Ps. The proliferation and differentiation of both antigen activated T_H1 cells and CTL-Ps depend on IL-2.



Proliferation of memory CTL-Ps may not require help from T_H cells. (a) Antigen-activated memory CTL-Ps appear to secrete sufficient IL-2 to stimulate their own proliferation and differentiation into effector CTLs. They also may not require the CD28-B7 co-stimulatory signal for activation. (b) A TH cell may provide the IL-2 necessary for proliferation of an antigen-activated naive CTL-P when it binds to the same APC as the CTL-P. Also, T_H cells may alter the behavior of APCs in a number of ways, such as increasing the display of co-stimulatory molecules by the APC.

The primary events in CTL-mediated death are conjugate formation, membrane attack, CTL dissociation, and target cell destruction.

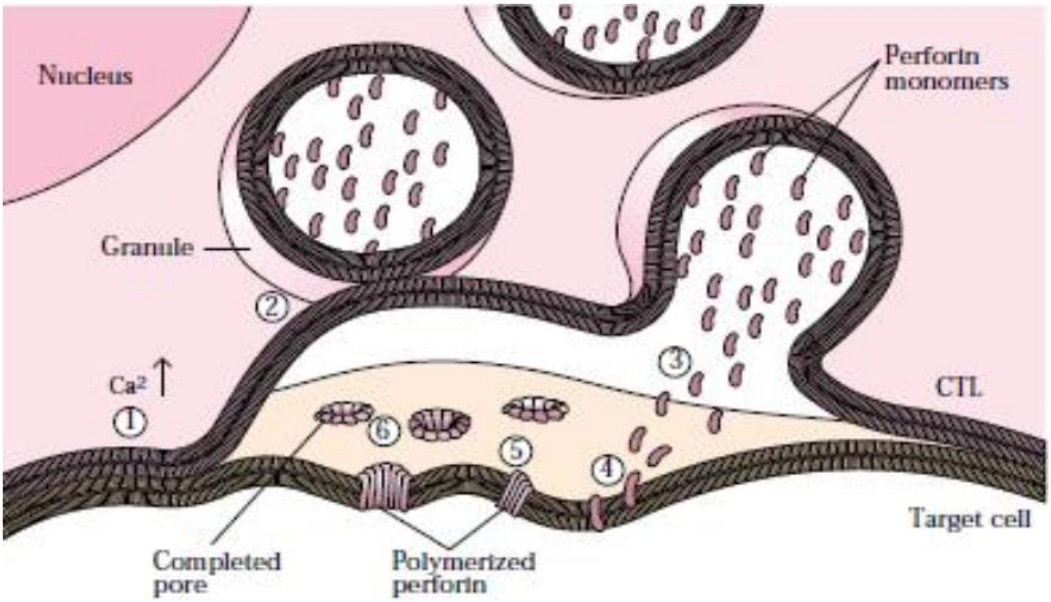


CTLs Kill Cells in Two Ways

The effector phase of a CTL-mediated response involves a carefully orchestrated sequence of events that begin with the embrace of the target cell by the attacking cell (Figure 14-5). Long-term cultures of CTL clones have been used to identify many of the membrane molecules and membrane events involved in this process. As described below, studies with receptor LFA-1 on the CTL membrane binds to ICAMs on the target-cell membrane, resulting in the formation of a conjugate. Antigen-mediated CTL activation converts LFA-1 from a low-avidity state to a high-avidity state. Because of this phenomenon, CTLs adhere to and form conjugates only with appropriate target cells that display antigenic peptides associated with class I MHC molecules. LFA-1 persists in the high-avidity state for only 5–10 min after antigen mediated activation, and then it returns to the low-avidity state. This downshift in LFA-1 avidity may facilitate dissociation of the CTL from the target cell.

Electron microscopy of cultured CTL clones reveals the presence of intracellular electron-dense storage granules. These granules have been isolated by fractionation and shown to mediate target-cell damage by themselves. Analysis of their contents revealed 65-kDa monomers of a pore-forming protein called **perforin** and several serine proteases called **granzymes** (or **fragmentins**). CTL-Ps lack cytoplasmic granules and perforin; upon activation, cytoplasmic granules appear, bearing newly expressed perforin monomers.

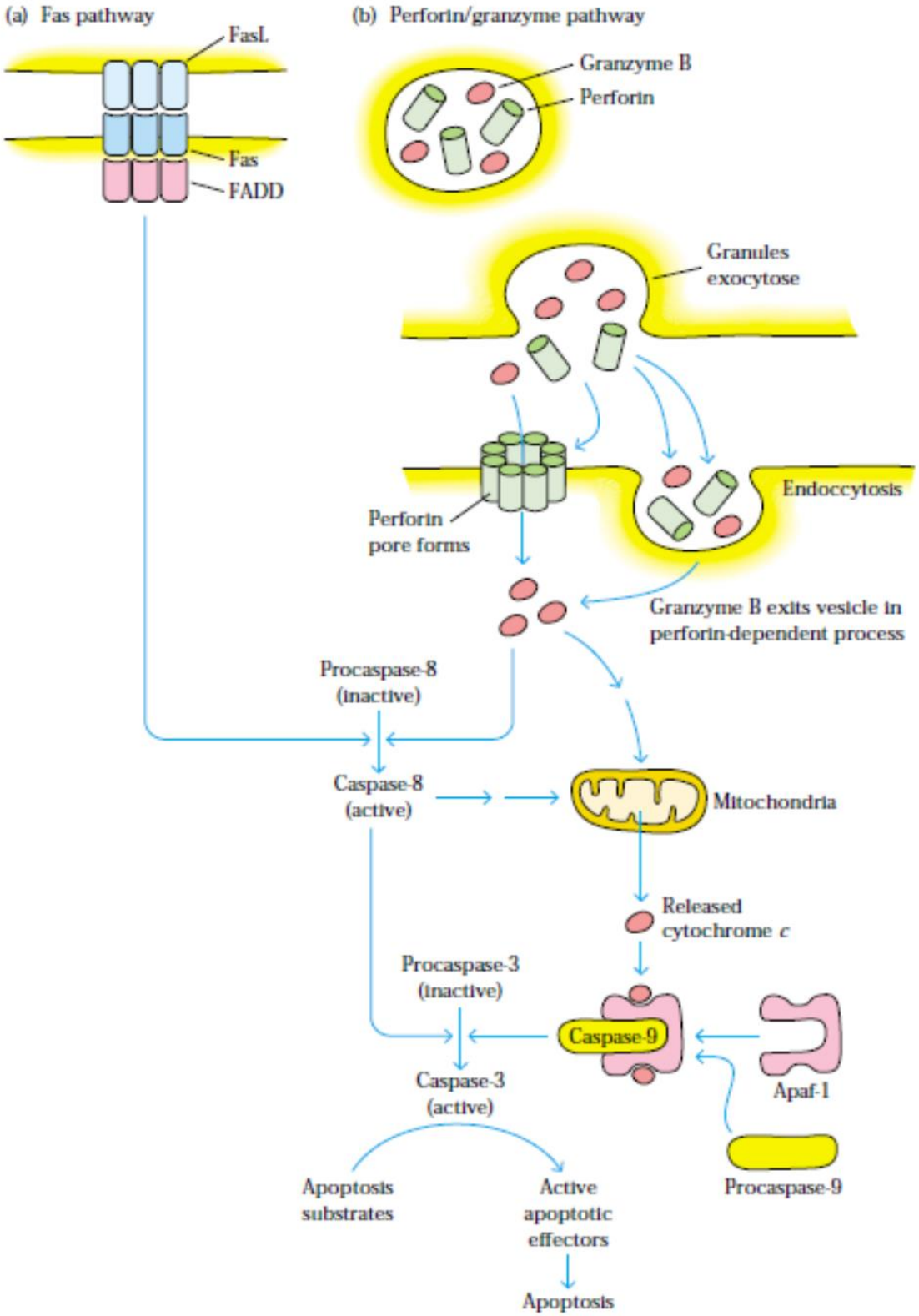
Immediately after formation of a CTL–target cell conjugate, the Golgi stacks and storage granules reorient within the cytoplasm of the CTL to concentrate near the junction with the target cell (Figure 14-8). Evidence suggests that perforin monomers and the granzyme proteases are then released from the granules by exocytosis into the junctional space between the two cells. As the perforin monomers contact the target-cell membrane, they undergo a conformational change, exposing an amphipathic domain that inserts into the target-cell membrane; the monomers then polymerize (in the presence of Ca2+) to form cylindrical pores with an internal diameter of 5–20 nm (see Figure).



A large number of perforin pores are visible on the target-cell membrane in the region of conjugate formation (Figure 14-9b). Interestingly, perforin exhibits some sequence homology with the terminal C9 component of the complement system, and the membrane pores formed by perforin are similar to those observed in complement mediated lysis. The importance of perforin to CTL-mediated killing is demonstrated by perforin-deficient knockout mice, which are unable to eliminate lymphocytic choriomeningitis virus (LCMV) even though they mount a significant CD8+ immune response to the virus.

Pore formation in the cell membrane of the target is one way that perforin mediates granzyme entry; another is the perforin-assisted pathway.Many target cells have a molecule known as the mannose 6-phosphate receptor on their surface that also binds to granzyme B. Granzyme B/mannose 6-phosphate receptor complexes are internalized and appear inside vesicles. In this case, perforin is necessary for releasing granzyme B from the vesicle into the cytoplasm of the target cell. Once it enters the cytoplasm of the target cell, granzyme B initiates a cascade of reactions that result in the fragmentation of the target-cell DNA into oligomers of 200 bp; this type of DNA

fragmentation is typical of apoptosis. Since granzymes are proteases, they cannot directly mediate DNA fragmentation.



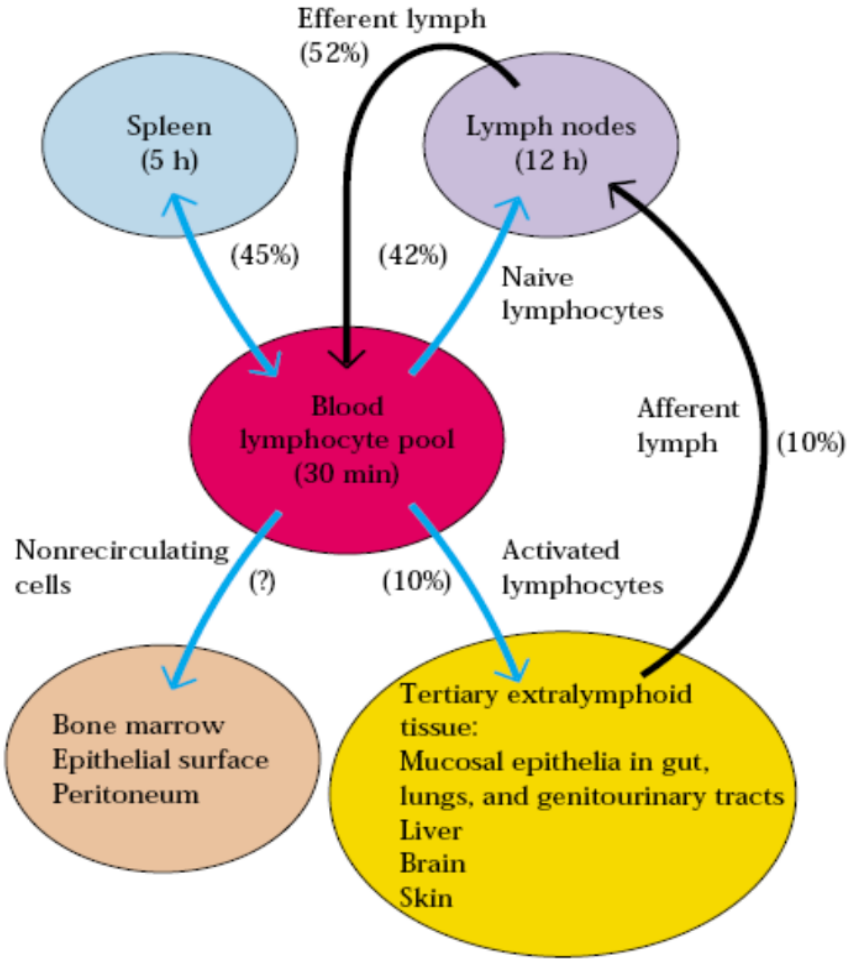
Rather, they activate an apoptotic pathway within the target cell. This apoptotic process does not require mRNA or protein synthesis in either the CTL or the target cell. Within 5 min of CTL contact, target cells begin to exhibit DNA fragmentation. Interestingly, viral DNA within infected target cells has also been shown to be fragmented during this process. This observation shows that CTL-mediated killing not only kills virus-infected cells but can also destroy the viral DNA in those cells. It has been suggested that the rapid onset of DNA fragmentation after CTL contact may prevent continued viral replication and assembly in the period before the target cell is destroyed.

Some potent CTL lines have been shown to lack perforin and granzymes. In these cases, cytotoxicity is mediated by Fas. This transmembrane protein, which is a member of the TNF-receptor family, can deliver a death signal when crosslinked by its natural ligand, a member of the tumor necrosis family called Fas ligand. Fas ligand (FasL) is found on the membrane of CTLs, and the interaction of FasL with Fas on a target cell triggers apoptosis.

Leukocyte Migration and Inflammation

Many types of leucocytes move from one part of the body to another. This is especially true of lymphocytes, which circulate continually in the blood and lymph and, in common with other types of leukocytes, migrate into the tissues at sites of infection or tissue injury. This recirculation not only increases the chance that lymphocytes specific for a particular antigen will encounter that antigen but also is critical to development of an **inflammatory response.** Inflammation is a complex response to local injury or other trauma; it is characterized by redness, heat, swelling, and pain. Inflammation involves various immune-system cells and numerous mediators. Assembling and regulating inflammatory responses would be impossible without the controlled migration of leukocyte populations.

Lymphocyte Recirculation



Lymphocytes are capable of a remarkable level of recirculation, continually moving through the blood and lymph to the various lymphoid organs (see Figure). After a brief transit time of

approximately 30 min in the bloodstream, nearly 45% of all lymphocytes are carried from the blood directly to the spleen, where they reside for approximately 5 h.

Almost equal numbers (42%) of lymphocytes exit from the blood into various peripheral lymph nodes, where they reside for about 12 h. A smaller number of lymphocytes (10%) migrate to tertiary extra lymphoid tissues by crossing between endothelial cells that line the capillaries. These tissues normally have few, if any, lymphoid cells but can import them during an inflammatory response. The most immunologically active tertiary extra lymphoid tissues are those that interface with the external environment, such as the skin and various mucosal epithelia of the gastrointestinal, pulmonary, and genitourinary tracts. The process of continual lymphocyte recirculation allows maximal numbers of antigenically committed lymphocytes to encounter antigen. An individual lymphocyte may make a complete circuit from the blood to the tissues and lymph and back again as often as 1–2 times per day. Since only about one in 105 lymphocytes recognize a particular antigen, it would appear that a large number of T or B cells must contact antigen on a given antigen-presenting cell within a short time in order to generate a specific immune response. The odds of the small percentage of lymphocytes committed to a given antigen actually making contact with that antigen when it is present are elevated by the extensive recirculation of lymphocytes. The likelihood of such contacts is profoundly increased also by factors that regulate, organize, and direct the circulation of lymphocytes and antigen presenting cells.

Cell-Adhesion Molecules

The vascular endothelium serves as an important “gatekeeper,” regulating the movement of blood-borne molecules and leukocytes into the tissues. In order for circulating leukocytes to enter inflamed tissue or peripheral lymphoid organs, the cells must adhere to and pass between the endothelial cells lining the walls of blood vessels, a process called **extravasation.** Endothelial cells express leukocyte-specific **cell adhesion molecules (CAMs).** Some of these membrane proteins are expressed constitutively; others are expressed only in response to local concentrations of cytokines produced during an inflammatory response. Recirculating lymphocytes, monocytes, and granulocytes bear receptors that bind to CAMs on the vascular endothelium, enabling these cells to extravasate into the tissues.

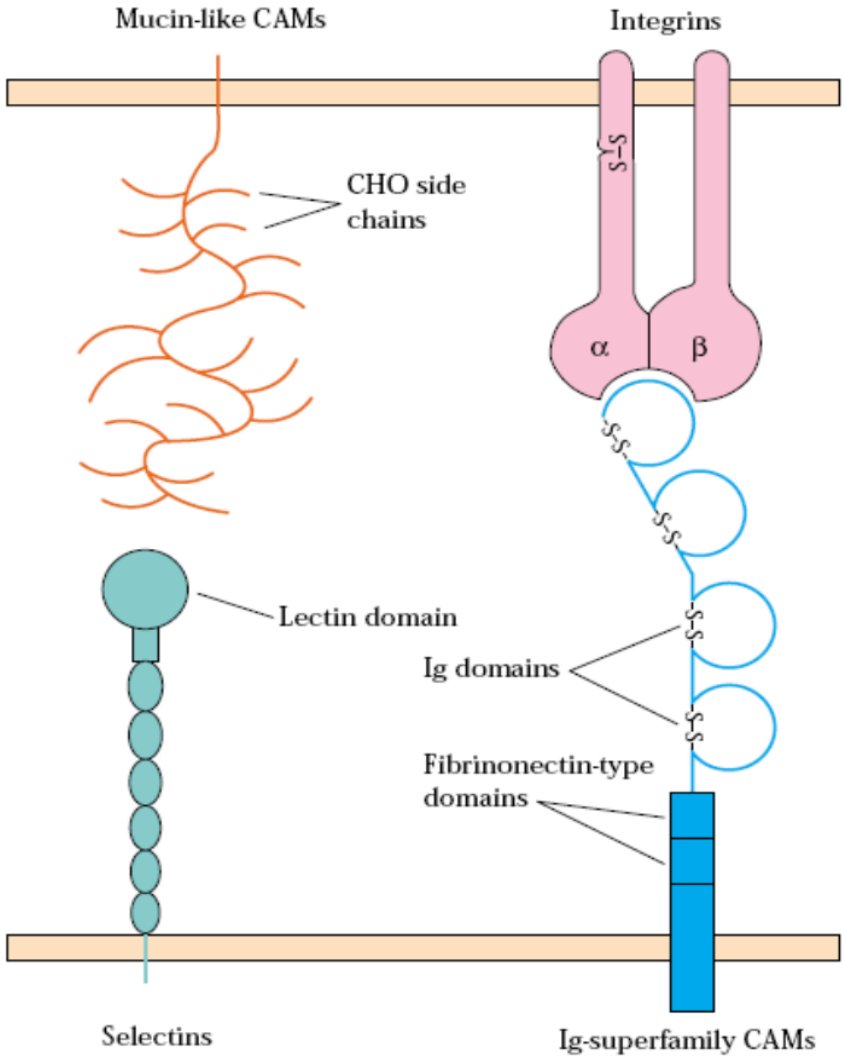
In addition to their role in leukocyte adhesion to vascular endothelial cells, CAMs on leukocytes also serve to increase the strength of the functional interactions between cells of the immune system. Various adhesion molecules have been shown to contribute to the interactions between T_H cells and APCs, T_H and B cells, and CTLs and target cells.

Most of these CAMs belong to four families of proteins:

1. The selectin family,
2. The mucin-like family,
3. The integrin family, and
4. The immunoglobulin (Ig) superfamily.

Selectins

The **selectin** family of membrane glycoproteins has a distal lectin-like domain that enables these molecules to bind to specific carbohydrate groups. Selectins interact primarily with sialylated carbohydrate moieties, which are often linked to mucin-like molecules. The selectin family includes three molecules, designated l, e, and p. Most circulating leukocytes express l-selectin, whereas e-selectin and p-selectin are expressed on vascular endothelial cells. Selectin molecules are responsible for the initial stickiness of leukocytes to vascular endothelium.



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Mucins

The **mucins** are a group of serine- and threonine rich proteins that are heavily glycosylated. Their extended structure allows them to present sialylated carbohydrate ligands to selectins. For example, l-selectin on leukocytes recognizes sialylated carbohydrates on two mucin-like molecules (CD34 and GlyCAM-1) expressed on certain endothelial cells of lymph nodes. Another mucin-like molecule (PSGL-1) found on neutrophils interacts with e- and p-selectin expressed on inflamed endothelium.

Integrins

The **integrins** are heterodimeric proteins (consisting of an α and a β chain) that are expressed by leukocytes and facilitate both adherence to the vascular endothelium and other cell-to-cell interactions. The integrins are grouped into categories according to which β subunit they contain. Different integrins are expressed by different populations of leukocytes, allowing these cells to bind to different cams that belong to the immunoglobulin superfamily expressed along the vascular endothelium. As described later, some integrins must be activated before they can bind with high affinity to their ligands. The importance of integrin molecules in leukocyte extravasation is demonstrated by **leukocyte-adhesion deficiency (LAD),** an autosomal recessive disease. It is characterized by recurrent bacterial infections and impaired healing of wounds.

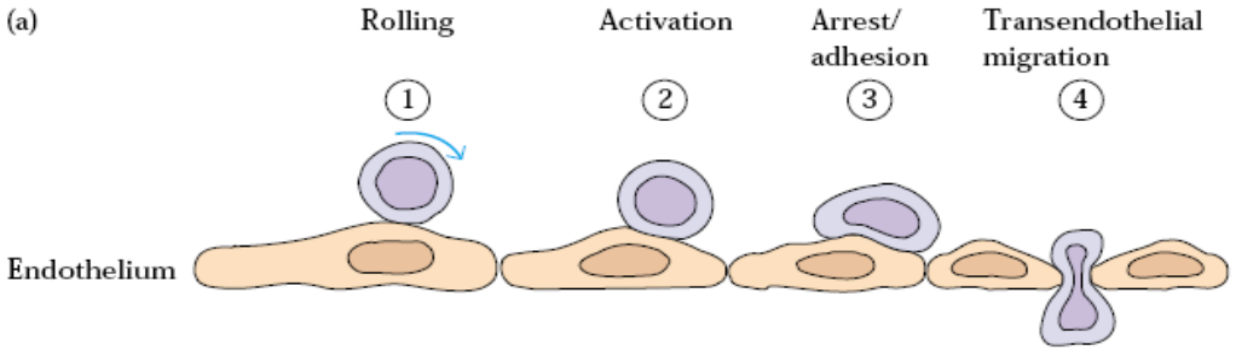
ICAMS

Several adhesion molecules contain a variable number of immunoglobulin-like domains and thus are classified in the **immunoglobulin superfamily.** Included in this group are ICAM-1, ICAM-2, ICAM-3, and VCAM, which are expressed on vascular endothelial cells and bind to various integrin molecules. An important cell-adhesion molecule called MAdCAM-1 has both Ig-like domains and mucin-like domains. This molecule is expressed on mucosal endothelium and directs lymphocyte entry into mucosa. It binds to integrins by its immunoglobulin-like domain and to selectins by its mucin-like domain.

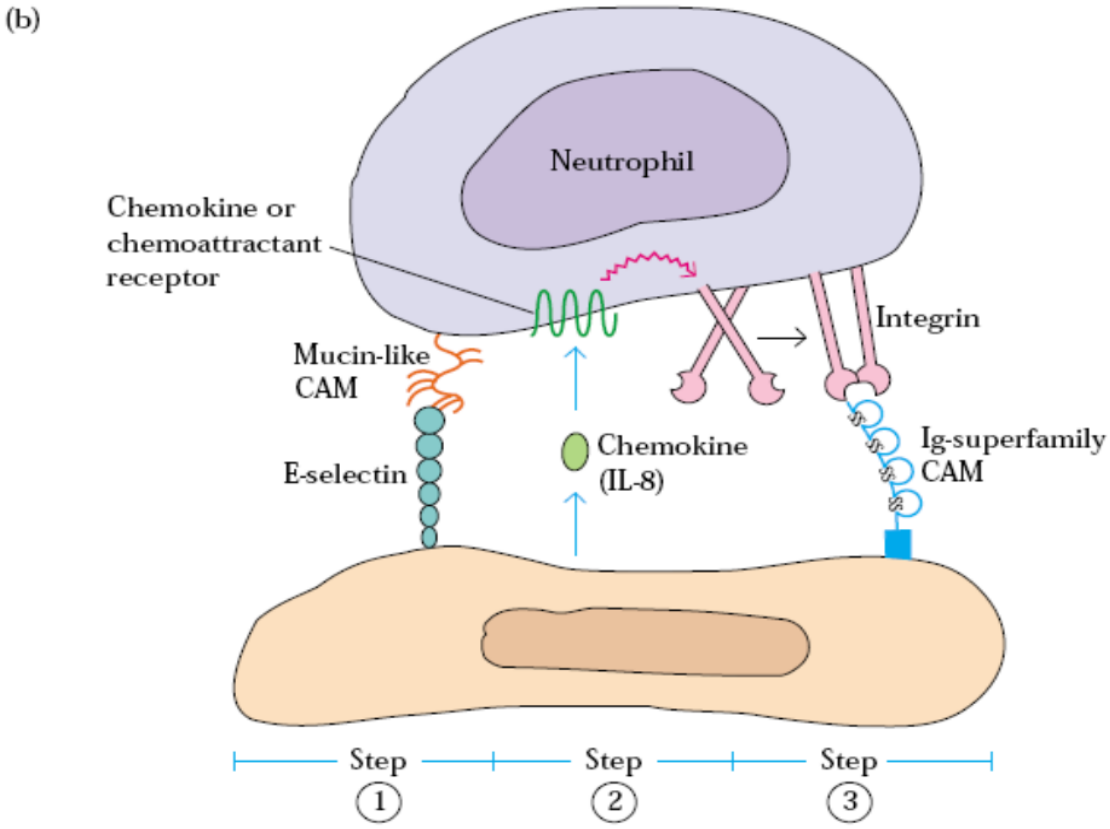
Neutrophil Extravasation

As an inflammatory response develops, various cytokines and other inflammatory mediators act upon the local blood vessels, inducing increased expression of endothelial CAMs. The vascular endothelium is then said to be **activated,** or **inflamed.** Neutrophils are generally the first cell type to bind to inflamed endothelium and extravasate into the tissues. To accomplish this, neutrophils must recognize the inflamed endothelium and adhere strongly enough so that they are not swept away by the flowing blood. The bound neutrophils must then penetrate the endothelial layer and migrate into

the underlying tissue. Monocytes and eosinophils extravasate by a similar process, but the steps have been best established for the neutrophil, so we focus on neutrophils here.



The process of neutrophil extravasation can be divided into four sequential steps: (1) rolling, (2) activation by chemoattractant stimulus, (3) arrest and adhesion, and (4) transendothelial migration (Figure a).



In the first step, neutrophils attach loosely to the endothelium by a low-affinity selectincarbohydrate interaction. During an inflammatory response, cytokines and other mediators act upon the local endothelium, inducing expression of adhesion molecules of the selectin family. These E- and P-selectin molecules bind to mucinlike cell-adhesion molecules on the neutrophil membrane or with a sialylated lactosaminoglycan called sialyl Lewisx (Figure b).

This interaction tethers the neutrophil briefly to the endothelial cell, but the shear force of the circulating blood soon detaches the neutrophil. Selectin molecules on another endothelial cell again tether the neutrophil; this process is repeated so that the neutrophil tumbles end-over-end along the endothelium, a type of binding called *rolling.* As the neutrophil rolls, it is activated by various **chemoattractants;** these are either permanent features of the endothelial cell surface or secreted locally by cells involved in the inflammatory response.

Among the chemoattractants are members of a large family of chemoattractive cytokines called **chemokines.** Two chemokines involved in the activation process are interleukin 8 (IL-8) and macrophage inflammatory protein (MIP-1β). However, not all chemoattractants belong to the chemokine group.

Other chemoattractants are platelet-activating factor (PAF), the complement split products C5a, C3a, and C5b67 and various *N*-formyl peptides produced by the breakdown of bacterial proteins during an infection. Binding of these chemoattractants to receptors on the neutrophil membrane triggers an activating signal mediated by G proteins associated with the receptor. This signal induces a conformational change in the integrin molecules in the neutrophil membrane, increasing their affinity for the Ig-superfamily adhesion molecules on the endothelium.

Subsequent interaction between integrins and Ig-superfamily CAMs stabilizes adhesion of the neutrophil to the endothelial cell, enabling the cell to adhere firmly to the endothelial cell. Subsequently, the neutrophil migrates through the vessel wall into the tissues. The steps in transendothelial migration and how it is directed are still largely unknown; they may be mediated by further activation by chemoattractants and subsequent integrin–Ig-superfamily interactions or by a separate migration stimulus.

Lymphocyte Extravasation

Various subsets of lymphocytes exhibit directed extravasation at inflammatory sites and secondary lymphoid organs. The recirculation of lymphocytes thus is carefully controlled to ensure that appropriate populations of B and T cells are recruited into different tissues.

High-Endothelial Venules Are Sites of Lymphocyte Extravasation

Some regions of vascular endothelium in postcapillary venules of various lymphoid organs are composed of specialized cells with a plump, cuboidal (“high”) shape; such regions are called **high-endothelial venules,** or **HEVs**. Their cells contrast sharply in appearance with the flattened

endothelial cells that line the rest of the capillary. Each of the secondary lymphoid organs, with the exception of the spleen, contains HEVs.

It has been estimated that as many as 1.4 X 10^4 lymphocytes extravasate every second through HEVs into a single lymph node. The development and maintenance of HEVs in lymphoid organs is influenced by cytokines produced in response to antigen capture. For example, HEVs fail to develop in animals raised in a germ-free environment.

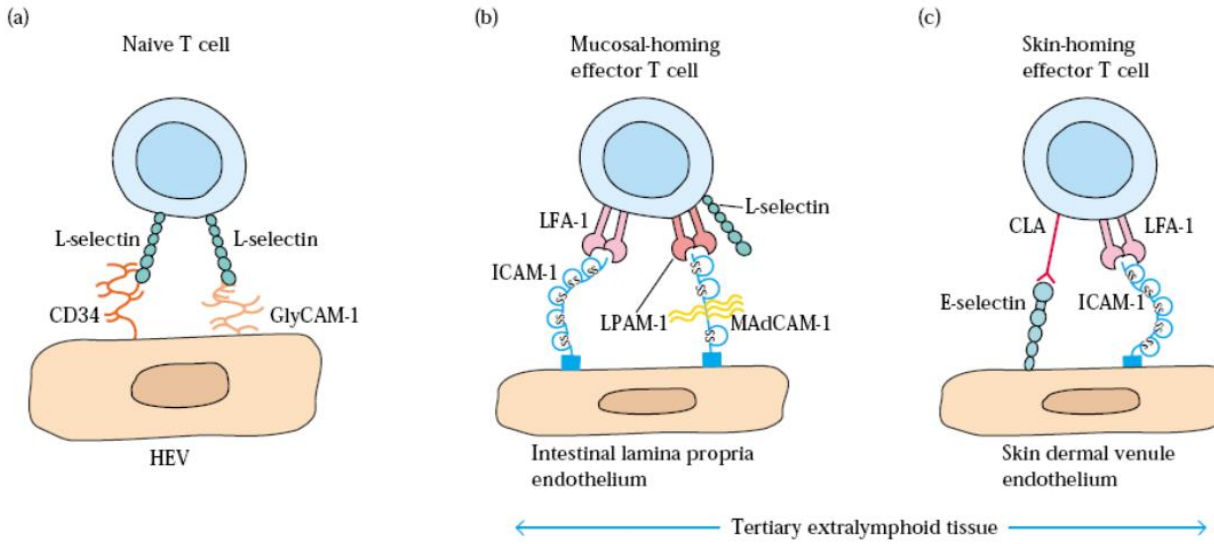
High-endothelial venules express a variety of cell-adhesion molecules. Like other vascular endothelial cells, HEVs express CAMs of the selectin family (E- and P-selectin), the mucinlike family (GlyCAM-1 and CD34), and the immunoglobulin superfamily (ICAM-1, ICAM-2, ICAM-3, VCAM-1, and MAdCAM-1). Some of these adhesion molecules are distributed in a tissue-specific manner. These tissue-specific adhesion molecules have been called **vascular addressins (VAs)** because they serve to direct the extravasation of different populations of recirculating lymphocytes to particular lymphoid organs.

Lymphocyte Homing Is Directed by Receptor Profiles and Signals

The general process of lymphocyte extravasation is similar to neutrophil extravasation. An important feature distinguishing the two processes is that different subsets of lymphocytes migrate differentially into different tissues. This process is called **trafficking,** or **homing.** The different trafficking patterns of lymphocyte subsets are mediated by unique combinations of adhesion molecules and chemokines; receptors that direct the circulation of various populations of lymphocytes to particular lymphoid and inflammatory tissues is called **homing receptors.** Researchers have identified a number of lymphocyte and endothelial cell-adhesion molecules that participate in the interaction of lymphocytes with HEVs and with endothelium at tertiary sites or sites of inflammation.

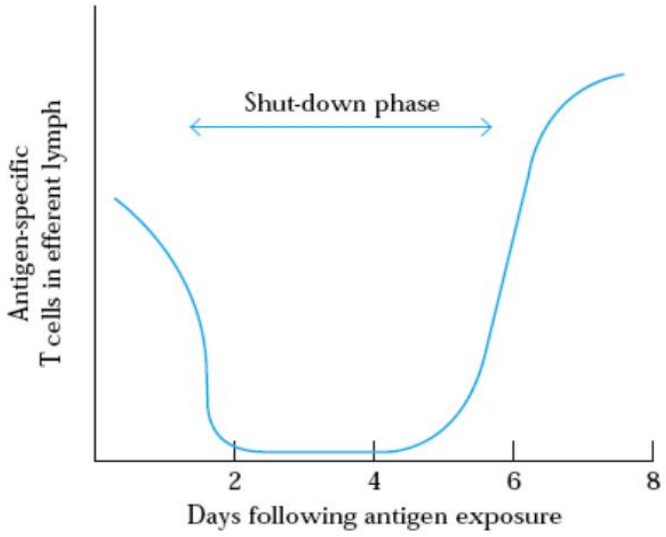
Naive Lymphocytes Recirculate to Secondary Lymphoid Tissue

A naive lymphocyte is not able to mount an immune response until it has been activated to become an effector cell. Activation of a naive cell occurs in specialized microenvironments within secondary lymphoid tissue (e.g., peripheral lymph nodes, Peyer's patches, tonsils, and spleen). Within these microenvironments, dendritic cells capture antigen and present it to the naive lymphocyte, resulting in its activation.



Naive cells do not exhibit a preference for a particular type of secondary lymphoid tissue but instead circulate indiscriminately to secondary lymphoid tissue throughout the body by recognizing adhesion molecules on HEVs. The initial attachment of naive lymphocytes to HEVs is generally mediated by the binding of the homing receptor L-selectin to adhesion molecules such as GlyCAM-1 and CD34 on HEVs (Figure a). The trafficking pattern of naive cells is designed to keep these cells constantly recirculating through secondary lymphoid tissue, whose primary function is to trap blood-borne or tissue-borne antigen.

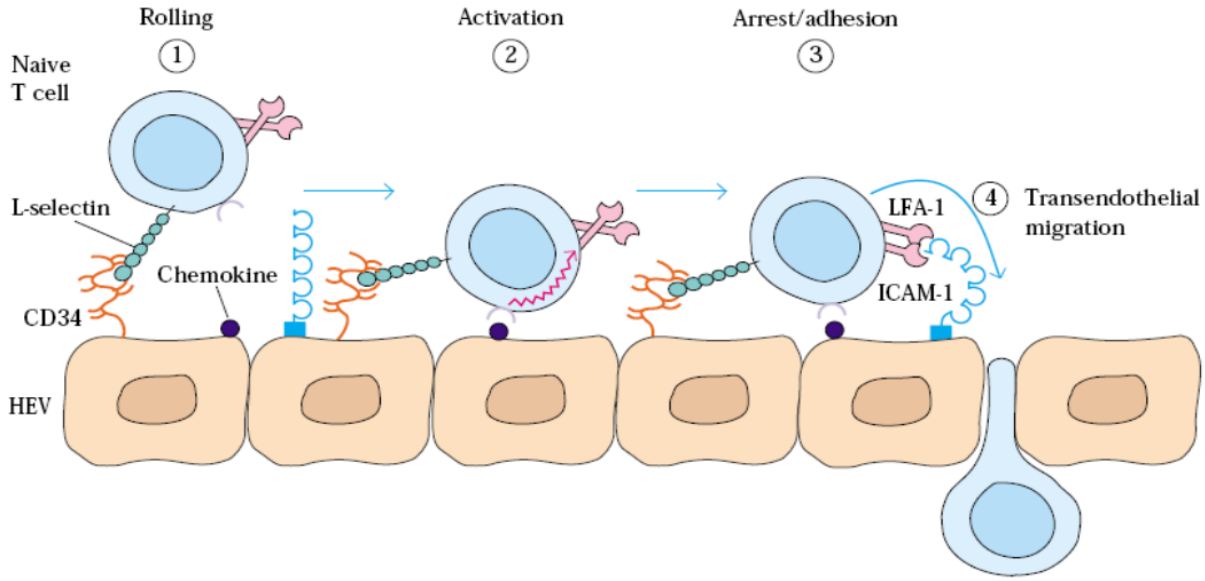
Once naive lymphocytes encounter antigen trapped in a secondary lymphoid tissue, they become activated and enlarge into lymphoblasts. Activation takes about 48 h, and during this time the blast cells are retained in the paracortical region of the secondary lymphoid tissue. During this phase, called the shut-down phase, the antigen-specific lymphocytes cannot be detected in the circulation (see Figure).



Rapid proliferation and differentiation of naive cells occurs during the shut-down phase. The effector and memory cells that are generated by this process then leave the lymphoid tissue and begin to recirculate.

Adhesion-Molecule Interactions Play Critical Roles in Extravasation

The extravasation of lymphocytes into secondary lymphoid tissue or regions of inflammation is a multistep process involving a cascade of adhesion-molecule interactions similar to those involved in neutrophil emigration from the bloodstream. The following figure depicts the typical interactions in extravasation of naive T cells across HEVs into lymph nodes. The first step is usually a selectin-carbohydrate interaction similar to that seen with neutrophil adhesion.Naive lymphocytes initially bind to HEVs by L-selectin, which serves as a homing receptor that directs the lymphocytes to particular tissues expressing a corresponding mucin-like vascular addressin such as CD34 or GlyCAM-1. Lymphocyte rolling is less pronounced than that of neutrophils. Although the initial selectin-carbohydrate interaction is quite weak, the slow rate of blood flow in postcapillary venules, particularly in regions of HEVs, reduces the likelihood that the shear force of the flowing blood will dislodge the tethered lymphocyte.



In the second step, an integrin-activating stimulus is mediated by chemokines that are either localized on the endothelial surface or secreted locally. The thick glycocalyx covering of the HEVs may function to retain these soluble chemoattractant factors on the HEVs. If, as some have proposed, HEVs secrete lymphocyte-specific chemoattractants, it would explain why neutrophils do not extravasate into lymph nodes at the HEVs even though they express L-selectin. Chemokine binding to G-protein–coupled receptors on the lymphocyte leads to activation of integrin molecules on the membrane, as occurs in neutrophil extravasation. Once activated, the integrin molecules

interact with Ig-superfamily adhesion molecules (e.g., ICAM-1), so the lymphocyte adheres firmly to the endothelium. The molecular mechanisms involved in the final step, transendothelial migration, are poorly understood.

Chemokines—Key Mediators of Inflammation

Chemokines are a superfamily of small polypeptides, most of which contain 90–130 amino acid residues. They selectively, and often specifically, control the adhesion, chemotaxis, and activation of many types of leukocyte populations and subpopulations. Consequently, they are major regulators of leukocyte traffic. Some chemokines are primarily involved in inflammatory processes, others are constitutively expressed and play important homeostatic or developmental roles. “Housekeeping” chemokines are produced in lymphoid organs and tissues or in non-lymphoid sites such as skin, where they direct normal trafficking of lymphocytes, such as determining the correct positioning of leukocytes newly generated by hematopoiesis and arriving from bone marrow. The thymus constitutively expresses chemokines, and normal B cell lymphopoiesis is also dependent on appropriate chemokine expression. Chemokine-mediated effects are not limited to the immune system.

The inflammatory chemokines are typically induced in response to infection. Contact with pathogens or the action of proinflammatory cytokines, such as TNF-α, up-regulate the expression of inflammatory cytokines at sites of developing inflammation. Chemokines cause leukocytes to move into various tissue sites by inducing the adherence of these cells to the vascular endothelium.After migrating into tissues, leukocytes are attracted toward high localized concentrations of chemokines resulting in the targeted recruitment of phagocytes and effector lymphocyte populations to inflammatory sites. The assembly of leukocytes at sites of infection, orchestrated by chemokines, is an essential part of mounting an appropriately focused response to infection.

More than 50 chemokines and at least 15 chemokine receptors have been described. The chemokines possess four conserved cysteine residues and based on the position of two of the four invariant cysteine residues, almost all fall into one or the other of two distinctive subgroups:

- **C-C subgroup** chemokines, in which the conserved cysteines are contiguous;
- **C-X-C subgroup** chemokines, in which the conserved cysteines are separated by some other amino acid (X).

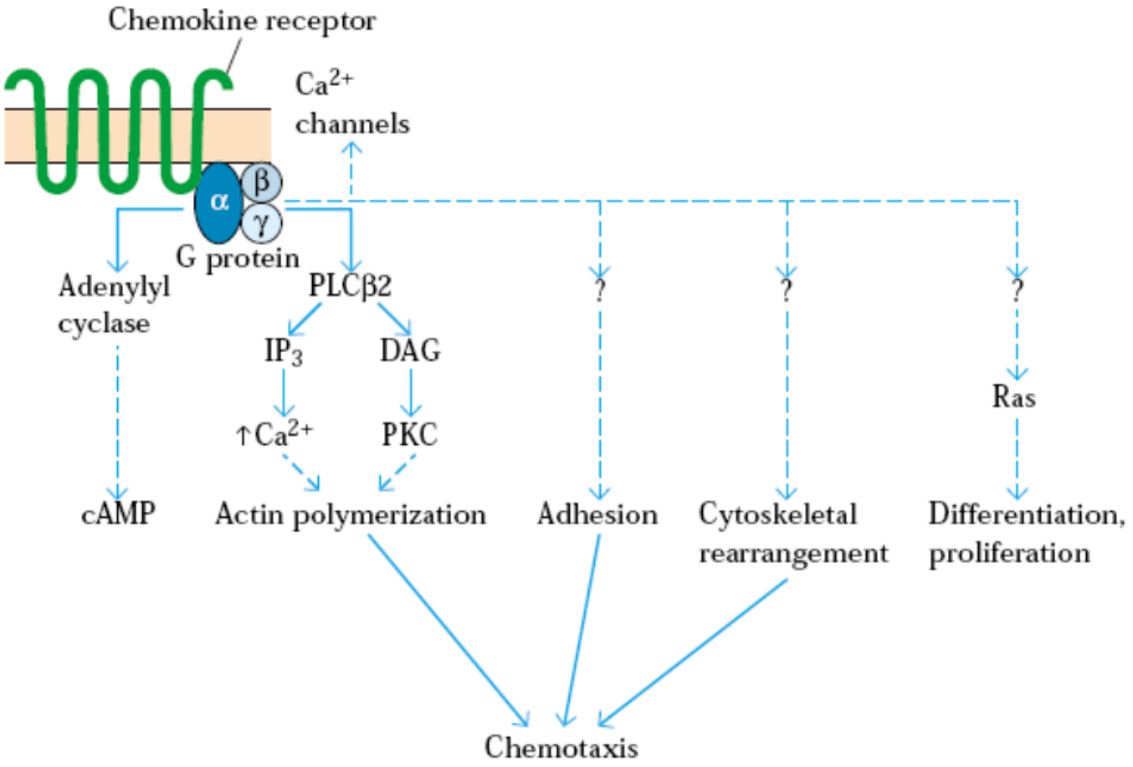
Chemokine action is mediated by receptors whose polypeptide chain traverses the membrane seven times. There are two subgroups of receptors, CC receptors (CCRs), which

recognize CC chemokines, and CXC receptors (CXCRs), which recognize CXC chemokines. As with cytokines, the interaction between chemokines and their receptors is of high affinity (*K*a > 109) and high specificity.

Chemokine receptors	Chemokines bound by receptor
CXC SUBGROUP	
CXCR1	IL-8, GCP-2
CXCR2	IL-8, Gro-α, Gro-β, Gro-γ, NAP-2, ENA-78
CXCR3	IP-10, Mig, I-TAC
CXCR4	SDF-1, PBSF
CXCR5	BCA-1
CC SUBGROUP	
CCR1	MIP-1, RANTES, MCP-2, MIP-5
CCR2	MCP-1, MCP-2, MCP-3
CCR3	Eotaxin, RANTES, MCP-2, MCP-3, MCP-4, Eotaxin-2, MIP-5
CCR4	TARC, RANTES
CCR5	MIP-1α RANTES, MIP-1β
CCR6	Exodus-1
CCR7	ELC
CCR8	1-309
CCR10	MCP-1, MCP-2, MCP-3, RANTES
BOTH CC AND CXC SUBGROUPS	
DARC (the Duffy antigen of RBCs)	Binds to a number of CC and CXC chemokines

However, in the above table, shows, most receptors bind more than one chemokine. For example, CXCR2 recognizes at least six different chemokines, and many chemokines can bind to more than one receptor.

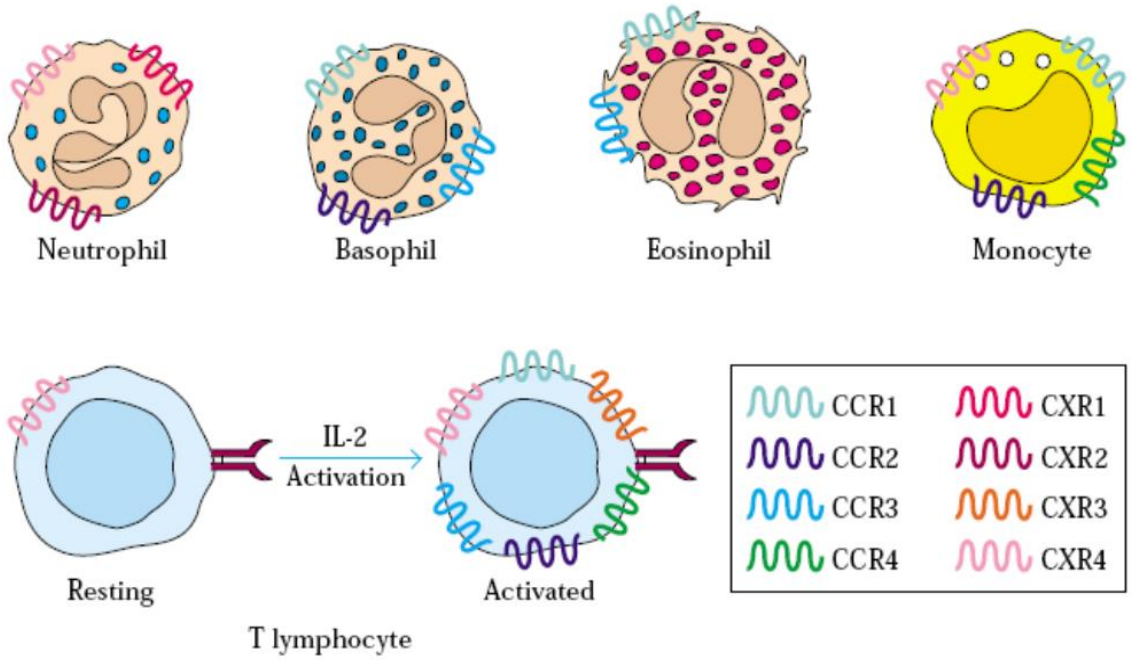
When a receptor binds an appropriate chemokine, it activates heterotrimeric large G proteins, initiating a signaltransduction process that generate such potent second messengers as cAMP, IP3, Ca2+, and activated small G proteins (see Figure). Dramatic changes are effected by the chemokine-initiated activation of these signal transduction pathways. Within seconds, the addition of an appropriate chemokine to leukocytes causes abrupt and extensive changes in shape, the promotion of greater adhesiveness to endothelial walls by activation of leukocyte integrins, and the generation of microbicidal oxygen radicals in phagocytes. These signal-transduction pathways promote other changes such as the release of granular contents, proteases in neutrophils and macrophages, histamine from basophils, and cytotoxic proteins from eosinophils.



Chemokines signal through receptors coupled with heterotrimeric large G proteins. Binding of a chemokine to its receptor activates many signal-transduction pathways, resulting in a variety of modifications in the physiology of the target cell.

Chemokine-Receptor Profiles Mediate Leukocyte Activity

Among major populations of human leukocytes, neutrophils express CXCR1, -2, and -4; eosinophils have CCR1 and CCR3 (see Figure).While resting naive T cells display few types of chemokine receptors, some activated T cells have CCR1, -2, -3, and -5, CXCR3 and -4, and possibly others. Clearly, a cell can respond to a chemokine only if it possesses a receptor that recognizes it. Consequently, differences in the expression of chemokine receptors by leukocytes coupled with the production of distinctive profiles of chemokines by destination tissues and sites provide rich opportunities for the differential regulation of activities of different leukocyte populations. Indeed, differences in patterns of chemokine-receptor expression occur within leukocyte populations as well as between different ones. Recall that T_H1 and T_H2 subsets of T_H cells can be distinguished by their different patterns of cytokine production. These subsets also display different profiles of chemokine receptors. T_H2 cells express CCR3 and -4, and a number of other receptors not expressed by T_H1 cells. On the other hand, T_H1 cells express CCR1, -3, and -5, but most T_H2 cells do not.



Other Mediators of Inflammation

In addition to chemokines, a variety of other mediators released by cells of the innate and acquired immune systems trigger or enhances specific aspects of the inflammatory response. They are released by,

- Tissue mast cells,
- Blood platelets, and
- A variety of leukocytes, including neutrophils, monocytes/macrophages, eosinophils, basophils, and lymphocytes.

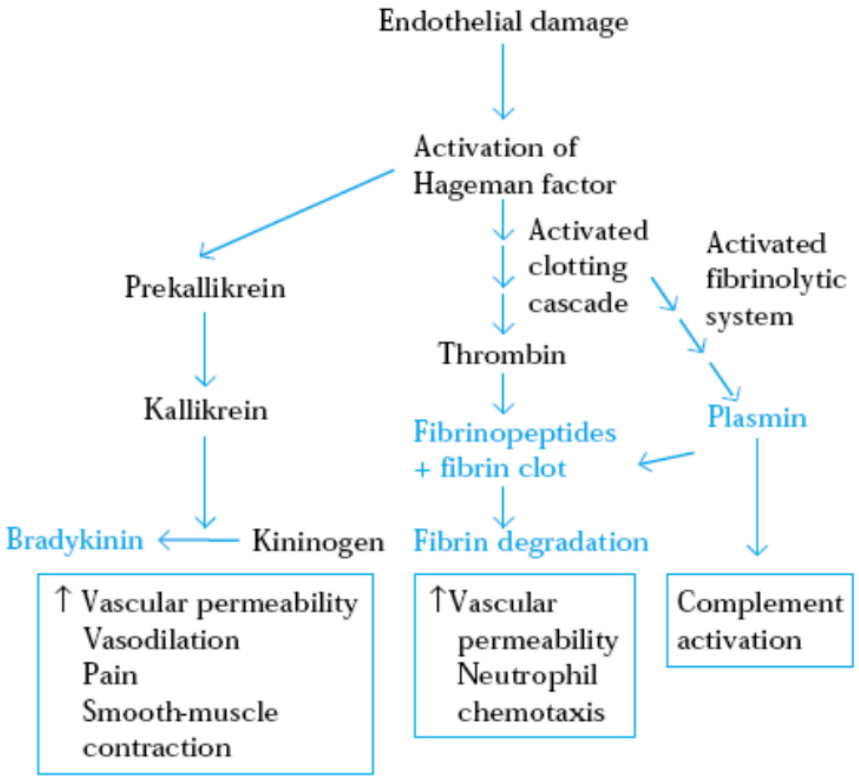
In addition to these sources, plasma contains four interconnected mediator-producing systems:

- The kinin system,
- The clotting system,
- The fibrinolytic system, and
- The complement system.

The first three systems share a common intermediate, Hageman factor, as illustrated in Figure 15-10. When tissue damage occurs, these four systems are activated to form a web of interacting systems that generate a number of mediators of inflammation.

The Kinin System Is Activated by Tissue Injury

The kinin system is an enzymatic cascade that begins when a plasma clotting factor, called Hageman factor, is activated following tissue injury. The activated Hageman factor then activates prekallikrein to form kallikrein, which cleaves kininogen to produce **bradykinin** (see Figure). This inflammatory mediator is a potent basic peptide that increases vascular permeability, causes vasodilation, induces pain, and induces contraction of smooth muscle. Kallikrein also acts directly on the complement system by cleaving C5 into C5a and C5b. The C5a complement component is an anaphylatoxin that induces mast-cell degranulation, resulting in the release of a number of inflammatory mediators from the mast cell.



The Clotting System Yields Fibrin-Generated Mediators of Inflammation

Another enzymatic cascade that is triggered by damage to blood vessels yields large quantities of thrombin. Thrombin acts on soluble fibrinogen in tissue fluid or plasma to produce insoluble strands of **fibrin** and **fibrinopeptides.** The insoluble fibrin strands crisscross one another to form **a clot,** which serves as a barrier to the spread of infection. The clotting system is triggered very rapidly after tissue injury to prevent bleeding and limit the spread of invading pathogens into the bloodstream. The fibrinopeptides act as inflammatory mediators, inducing increased vascular permeability and neutrophil chemotaxis.

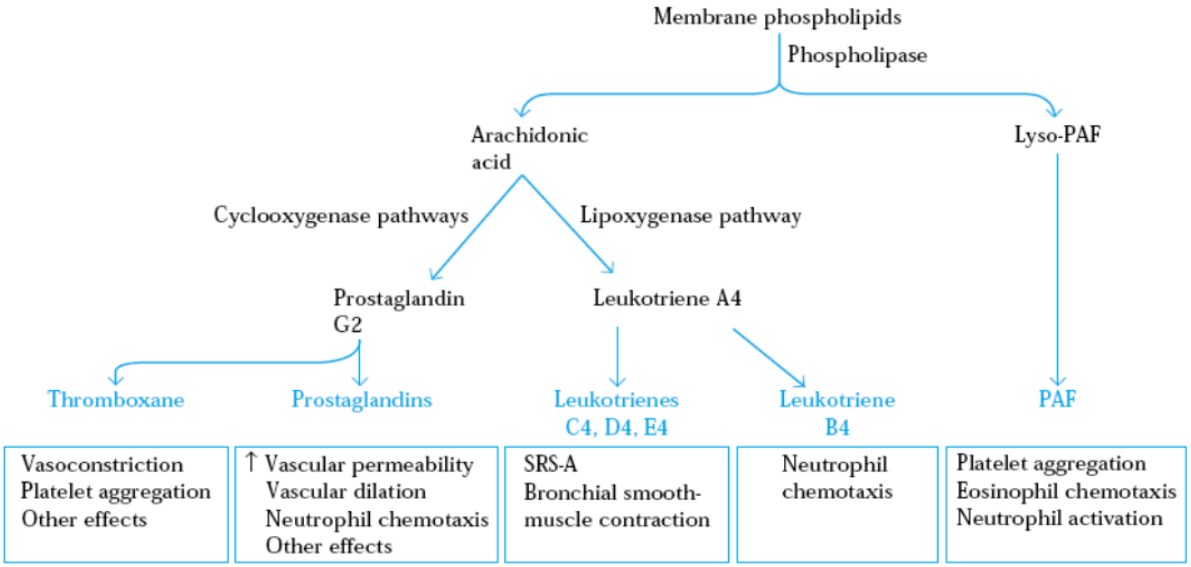
The Fibrinolytic System Yields Plasmin- Generated Mediators of Inflammation

Removal of the fibrin clot from the injured tissue is achieved by the fibrinolytic system. The end product of this pathway is the enzyme **plasmin,** which is formed by the conversion of plasminogen. Plasmin, a potent proteolytic enzyme, breaks down fibrin clots into degradation products that are chemotactic for neutrophils. Plasmin also contributes to the inflammatory response by activating the classical complement pathway.

The Complement System Produces Anaphylatoxins

Activation of the complement system by both classical and alternative pathways results in the formation of a number of complement split products that serve as important mediators of inflammation. Binding of the **anaphylatoxins** (C3a, C4a, and C5a) to receptors on the membrane of tissue mast cells induces degranulation with release of histamine and other pharmacologically active mediators. These mediators induce smooth-muscle contraction and increase vascular permeability. C3a, C5a, and C5b67 act together to induce monocytes and neutrophils to adhere to vascular endothelial cells, extravasate through the endothelial lining of the capillary, and migrate toward the site of complement activation in the tissues. Activation of the complement system thus results in influxes of fluid that carry antibody and phagocytic cells to the site of antigen entry.

Some Lipids Act as Inflammatory Mediators



Following membrane perturbations, phospholipids in the membrane of several cell types (e.g.,macrophages,monocytes, neutrophils, and mast cells) are degraded into arachidonic acid and lyso–platelet-activating factor (see Figure). The latter is subsequently converted into platelet-activating factor (PAF), which causes platelet activation and has many inflammatory effects,

including eosinophil chemotaxis and the activation and degranulation of neutrophils and eosinophils. Metabolism of arachidonic acid by the cyclooxygenase pathway produces **prostaglandins** and **thromboxanes.**

Different prostaglandins are produced by different cells: monocytes and macrophages produce large quantities of PGE2 and PGF2; neutrophils produce moderate amounts of PGE2; mast cells produce PGD2. Prostaglandins have diverse physiological effects, including increased vascular permeability, increased vascular dilation, and induction of neutrophil chemotaxis. The thromboxanes cause platelet aggregation and constriction of blood vessels. Arachidonic acid is also metabolized by the lipoxygenase pathway to yield the four **leukotrienes:**LTB4, LTC4, LTD4 and LTE4. Three of these (LTC4, LTD4, and LTE4) together make up what was formerly called **slow-reacting substance of anaphylaxis (SRS-A);** these mediators induce smooth-muscle contraction. LTB4 is a potent chemoattractant of neutrophils. The leukotrienes are produced by a variety of cells, including monocytes, macrophages, and mast cells.

Some Cytokines Are Important Inflammatory Mediators

Effect	IL-1	TNF-α	IL-6
Endogenous pyrogen fever	+	+	+
Synthesis of acute-phase proteins by liver	+	+	+
Increased vascular permeability	+	+	+
Increased adhesion molecules on vascular endothelium	+	+	–
Fibroblast proliferation	+	+	–
Platelet production	+	–	+
Chemokine induction (e.g., IL-8)	+	+	–
Induction of IL-6	+	+	–
T-cell activation	+	+	+
B-cell activation	+	+	+
Increased immunoglobulin synthesis	–	–	+

A number of cytokines play a significant role in the development of an acute or chronic inflammatory response. IL-1, IL-6, TNF-α, IL-12, and many chemokines exhibit redundant and pleiotropic effects that together contribute to the inflammatory response. Some of the effects mediated by IL-1, IL-6, and TNF-α are listed in the Table. In addition, IFN-α contributes to the inflammatory response, acting later in the acute response and contributing in a major way to chronic

inflammation by attracting and activating macrophages. IL-12 induces the differentiation of the proinflammatory T_H1 subset.

The Inflammatory Process

Inflammation is a physiologic response to a variety of stimuli such as infections and tissue injury. In general, an acute inflammatory response has a rapid onset and lasts a short while. Acute inflammation is generally accompanied by a systemic reaction known as the acute-phase response, which is characterized by a rapid alteration in the levels of several plasma proteins. In some diseases persistent immune activation can result in chronic inflammation, which often has pathologic consequences.

Neutrophils Play an Early and Important Role in Inflammation

In the early stages of an inflammatory response, the predominant cell type infiltrating the tissue is the neutrophil. Neutrophil infiltration into the tissue peaks within the first 6 h of an inflammatory response, with production of neutrophils in the bone marrow increasing to meet this need. A normal adult produces more than 10^{10} neutrophils per day, but during a period of acute inflammation, neutrophil production may increase as much as tenfold.

The neutrophils leave the bone marrow and circulate within the blood. In response to mediators of acute inflammation, vascular endothelial cells increase their expression of E- and P-selectin. Thrombin and histamine induce increased expression of P-selectin; cytokines such as IL-1 or TNF-_ induce increased expression of E-selectin. The circulating neutrophils express mucins such as PSGL-1 or the tetrasaccharides sialyl Lewisa and sialyl Lewisx, which bind to E- and P-selectin.

As described earlier, this binding mediates the attachment or tethering of neutrophils to the vascular endothelium, allowing the cells to roll in the direction of the blood flow. During this time, chemokines such as IL-8 or other chemoattractants act upon the neutrophils, triggering a G-protein–mediated activating signal that leads to a conformational change in the integrin adhesion molecules, resulting in neutrophil adhesion and subsequent transendothelial migration
(see Figure 15-3).

Once in tissues, the activated neutrophils also express increased levels of receptors for chemoattractants and consequently exhibit **chemotaxis,** migrating up a gradient of the chemoattractant. Among the inflammatory mediators that are chemotactic for neutrophils are several chemokines, complement split products (C3a, C5a, and C5b67), fibrinopeptides, prostaglandins, and leukotrienes. In addition,molecules released by microorganisms, such as formyl

methionyl peptides, are also chemotactic for neutrophils. Activated neutrophils express increased levels of Fc receptors for antibody and receptors for complement, enabling these cells to bind more effectively to antibody- or complement-coated pathogens, thus increasing phagocytosis.

The activating signal also stimulates metabolic pathways to a respiratory burst, which produces **reactive oxygen intermediates** and **reactive nitrogen intermediates** (see Chapter 2). Release of some of these reactive intermediates and the release of mediators from neutrophil primary and secondary granules (proteases, phospholipases, elastases, and collagenases) play an important role in killing various pathogens. These substances also contribute to the tissue damage that can result from an inflammatory response. The accumulation of dead cells and microorganisms, together with accumulated fluid and various proteins,makes up what is known as pus.

Inflammatory Responses May Be Localized or Systemic

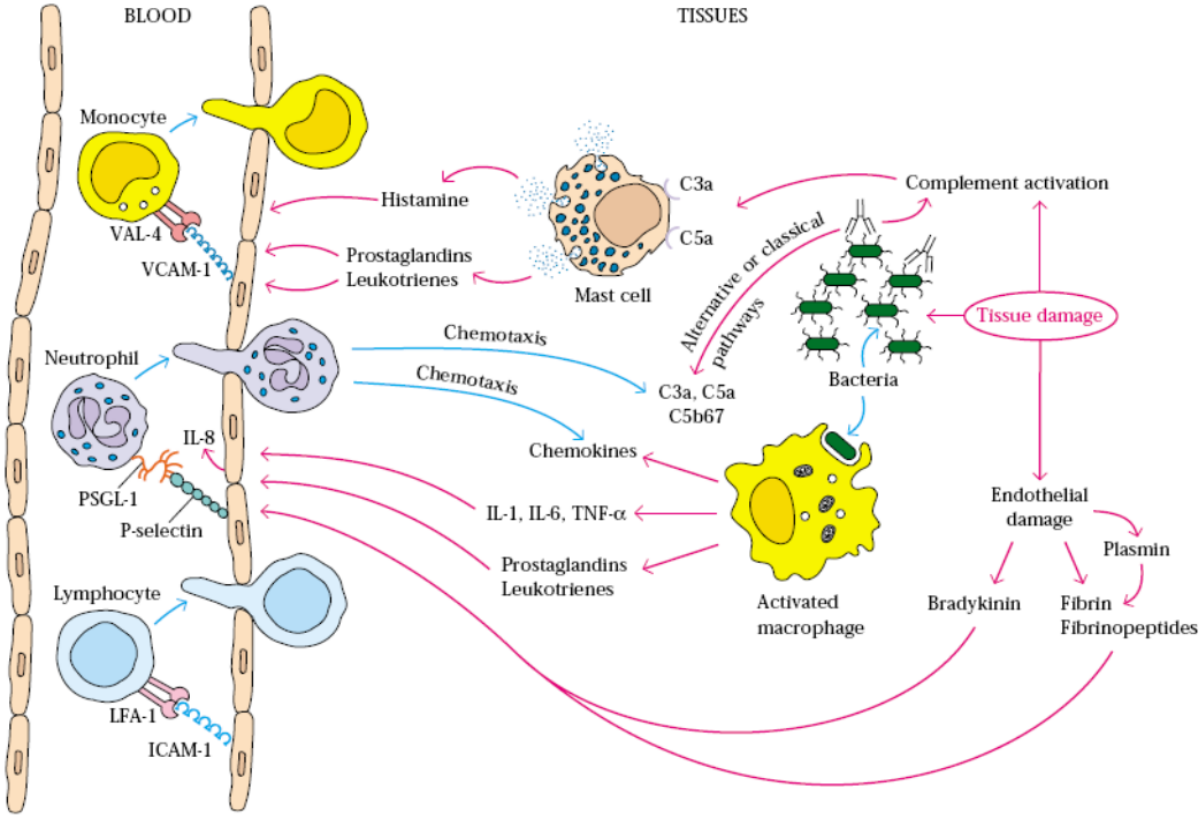
Infection or tissue injury induces a complex cascade of nonspecific events, known as the inflammatory response that provides early protection by restricting the tissue damage to the site of infection or tissue injury. The acute inflammatory response involves both localized and systemic responses.

Localized Inflammatory Response

The hallmarks of a localized acute inflammatory response, first described almost 2000 years ago, are swelling (*tumor*), redness (*rubor*), heat (*calor*), pain (*dolor*), and loss of function.Within minutes after tissue injury, there is an increase in vascular diameter (vasodilation), resulting in an increase in the volume of blood in the area and a reduction in the flow of blood. The increased blood volume heats the tissue and causes it to redden. Vascular permeability also increases, leading to leakage of fluid from the blood vessels, particularly at postcapillary venules. This results in an accumulation of fluid **(edema)** in the tissue and, in some instances, extravasation of leukocytes, contribut ing to the swelling and redness in the area.When fluid exudes from the bloodstream, the kinin, clotting, and fibrinolytic systems are activated.

Many of the vascular changes that occur early in a local response are due to the direct effects of plasma enzyme mediators such as bradykinin and fibrinopeptides, which induce vasodilation and increased vascular permeability. Some of the vascular changes are due to the indirect effects of the complement anaphylatoxins (C3a, C4a, and C5a), which induce local mast-cell degranulation with release of histamine. Histamine is a potent mediator of inflammation, causing vasodilation and smooth-muscle contraction. The prostaglandins can also contribute to the vasodilation and increased vascular permeability associated with the acute inflammatory response.

Within a few hours of the onset of these vascular changes, neutrophils adhere to the endothelial cells, and migrate out of the blood into the tissue spaces (see Figure).



Overview of the cells and mediators involved in a local acute inflammatory response

These neutrophils phagocytose invading pathogens and release mediators that contribute to the inflammatory response. Among the mediators are the macrophage inflammatory proteins (MIP-1α and MIP-1β), chemokines that attract macrophages to the site of inflammation. Macrophages arrive about 5–6 hours after an inflammatory response begins. These macrophages are activated cells that exhibit increased phagocytosis and increased release of mediators and cytokines that contribute to the inflammatory response.

Activated tissue macrophages secrete three cytokines (IL-1, IL-6, and TNF-α) that induce many of the localized and systemic changes observed in the acute inflammatory response. All three cytokines act locally, inducing coagulation and an increase in vascular permeability. Both TNF-α and IL-1 induce increased expression of adhesion molecules on vascular endothelial cells. For instance, TNF-α stimulates expression of E-selectin, an endothelial adhesion molecule that selectively binds adhesion molecules on neutrophils. IL-1 induces increased expression of ICAM-1 and VCAM-1, which bind to integrins on lymphocytes and monocytes. Circulating neutrophils,

monocytes, and lymphocytes recognize these adhesion molecules on the walls of blood vessels, adhere, and then move through the vessel wall into the tissue spaces. IL-1 and TNF-α also act on macrophages and endothelial cells to induce production of the chemokines that contribute to the influx of neutrophils by increasing their adhesion to vascular endothelial cells and by acting as potent chemotactic factors.

In addition, IFN-γ and TNF-α activate macrophages and neutrophils, promoting increased phagocytic activity and increased release of lytic enzymes into the tissue spaces. A local acute inflammatory response can occur without the overt involvement of the immune system. Often, however, cytokines released at the site of inflammation facilitate both the adherence of immune-system cells to vascular endothelial cells and their migration through the vessel wall into the tissue spaces. The result is an influx of lymphocytes, neutrophils, monocytes, eosinophils, basophils, and mast cells to the site of tissue damage, where these cells participate in clearance of the antigen and healing of the tissue. The duration and intensity of the local acute inflammatory response must be carefully regulated to control tissue damage and facilitate the tissue-repair mechanisms that are necessary for healing. TGF-β has been shown to play an important role in limiting the inflammatory response. It also promotes accumulation and proliferation of fibroblasts and the deposition of an extracellular matrix that is required for proper tissue repair. Clearly, the processes of leukocyte adhesion are of great importance in the inflammatory response. A failure of proper leukocyte adhesion can result in disease, as exemplified by leukocyte-adhesion deficiency.

Systemic Acute-Phase Response

The local inflammatory response is accompanied by a systemic response known as the **acute-phase response** (see Figure). This response is marked by the induction of fever, increased synthesis of hormones such as ACTH and hydrocortisone, increased production of white blood cells (leukocytosis), and production of a large number of **acute-phase proteins** in the liver. The increase in body temperature inhibits the growth of a number of pathogens and appears to enhance the immune response to the pathogen. C-reactive protein is a prototype acute-phase protein whose serum level increases 1000-fold during an acute-phase response. It is composed of five identical polypeptides held together by noncovalent interactions. C-reactive protein binds to a wide variety of microorganisms and activates complement, resulting in deposition of the opsonin C3b on the surface of microorganisms. Phagocytic cells, which express C3b receptors, can then readily phagocytose the C3b-coated microorganisms.

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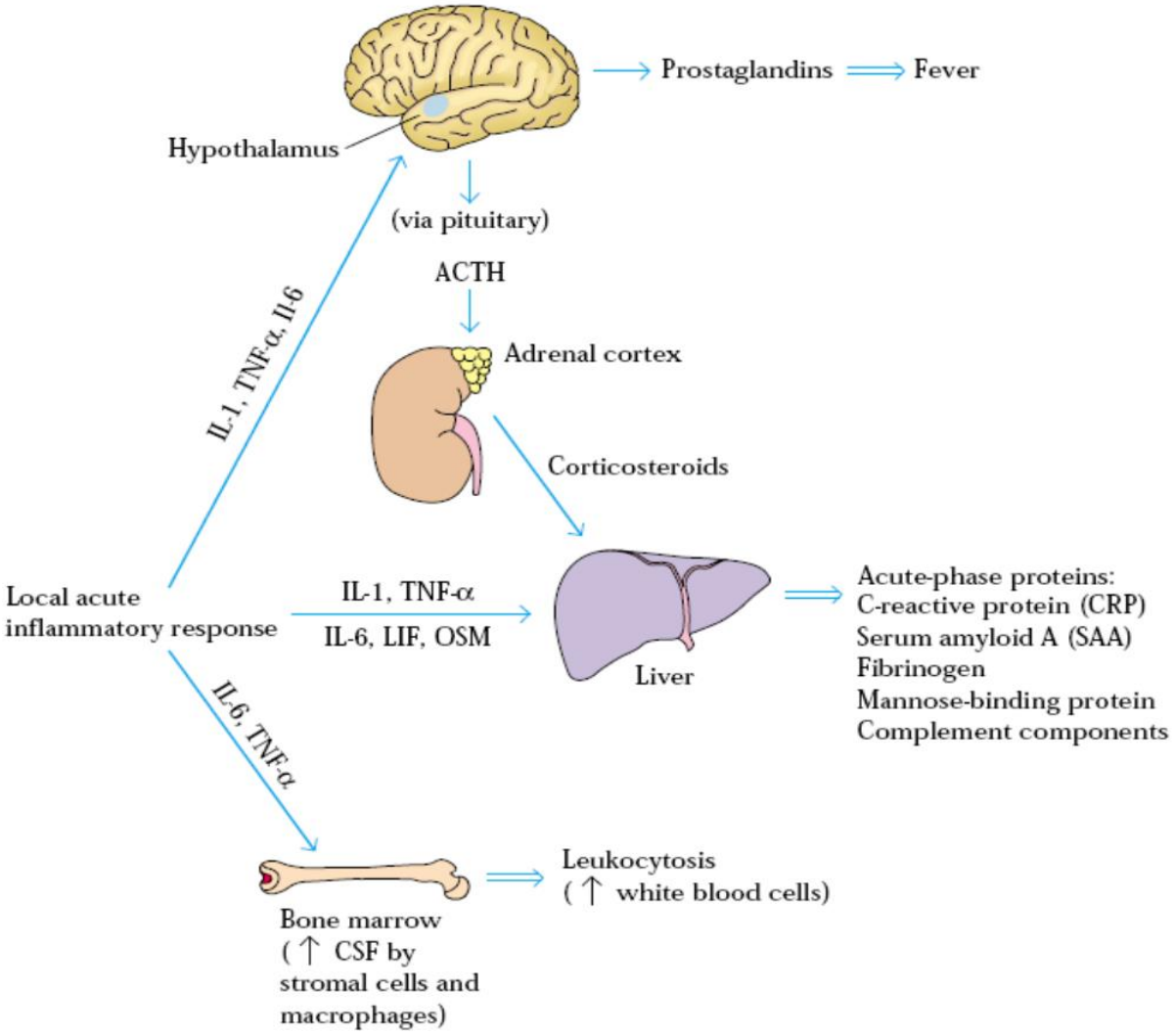
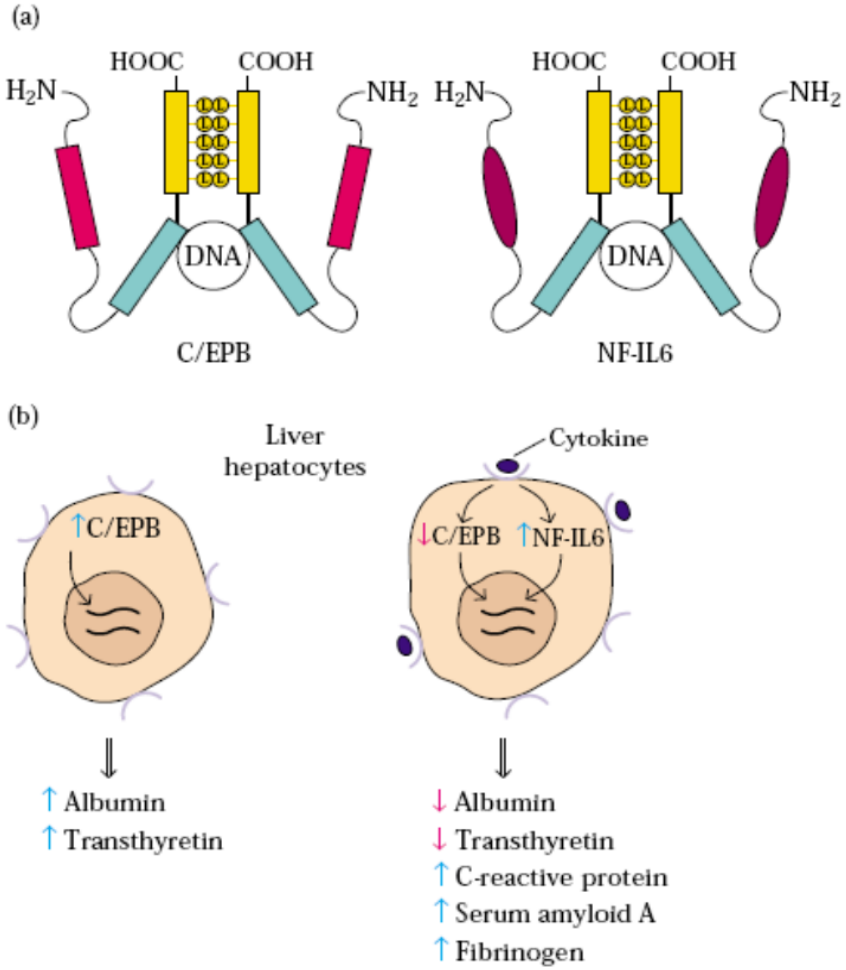
Many systemic acute-phase effects are due to the combined action of IL-1, TNF-α and IL-6 (see Figure). Each of these cytokines acts on the hypothalamus to induce a fever response.Within 12–24 h of the onset of an acute-phase inflammatory response, increased levels of IL-1, TNF-α and IL-6 (as well as leukemia inhibitory factor (LIF) and oncostatin M (OSM)) induce production of acute-phase proteins by hepatocytes. TNF-α also acts on vascular endothelial cells and macrophages to induce secretion of colony-stimulating factors (M-CSF,G-CSF, and GM-CSF). These CSFs stimulate hematopoiesis, resulting in transient increases in the number of white blood cells needed to fight the infection.



The redundancy in the ability of at least five cytokines (TNF-α, IL-1, IL-6, LIF, and OSM) to induce production of acute-phase proteins by the liver results from the induction of a common transcription factor,NF-IL6, after each of these cytokines interacts with its receptor. Amino-acid sequencing of cloned NF-IL6 revealed that it has a high degree of sequence identity with C/EBP, a liver-specific transcription factor (Figure a). Both NF-IL6 and C/EBP contain a leucine-zipper

domain and a basic DNA-binding domain, and both proteins bind to the same nucleotide sequence in the promoter or enhancer of the genes encoding various liver proteins. C/EBP, which stimulates production of albumin and transthyretin, is expressed constitutively by hepatocytes.

As an inflammatory response develops and the cytokines interact with their respective receptors on liver hepatocytes, expression of NF-IL6 increases and that of C/EBP decreases (Figure b). The inverse relationship between these two transcription factors accounts for the observation that serum levels of proteins such as albumin and transthyretin decline while those of acute-phase proteins increase during an inflammatory response.



Chronic Inflammation Develops When Antigen Persists

Some microorganisms are able to evade clearance by the immune system, for example by possessing cell-wall components that enable them to resist phagocytosis. Such organisms often induce a chronic inflammatory response, resulting in significant tissue damage. Chronic inflammation also occurs in a number of autoimmune diseases in which selfantigens continually

activate T cells. Finally, chronic inflammation also contributes to the tissue damage and wasting associated with many types of cancer.

The accumulation and activation of macrophages is the hallmark of chronic inflammation. Cytokines released by the chronically activated macrophages also stimulate fibroblast proliferation and collagen production. A type of scar tissue develops at sites of chronic inflammation by a process called **fibrosis,** a wound-healing reaction that can interfere with normal tissue function. Chronic inflammation may also lead to formation of a **granuloma,** a tumor-like mass consisting of a central area of activated macrophages surrounded by activated lymphocytes. The center of the granuloma often contains multinucleated giant cells formed by the fusion of activated macrophages. These giant cells typically are surrounded by large modified macrophages that resemble epithelial cells and therefore are called epithelioid cells.

Anti-Inflammatory Agents

Although development of an effective inflammatory response can play an important role in the body's defense, the response can sometimes be detrimental. Allergies, autoimmune diseases, microbial infections, transplants, and burns may initiate a chronic inflammatory response.Various therapeutic approaches are available for reducing long-term inflammatory responses and thus the complications associated with them.

Antibody Therapies Reduce Leukocyte Extravasation

Because leukocyte extravasation is an integral part of the inflammatory response, one approach for reducing inflammation is to impede this process. Theoretically, one way to reduce leukocyte extravasation is to block the activity of various adhesion molecules with antibodies. In animal models, for example, antibodies to the integrin LFA-1 have been used to reduce neutrophil buildup in inflammatory tissue. Antibodies to ICAM-1 have also been used, with some success, in preventing the tissue necrosis associated with burns and in reducing the likelihood of kidney-graft rejection in animal models. The results with antibodies specific for these adhesions have been so encouraging that a combination of antibodies (anti-ICAM-1 and anti-LFA-1) was used in clinical trials on human kidney-transplant patients. A combination of two anti-adhesins had to be used because failure to block both LFA-1 and ICAM-1 results in rejection.

Corticosteroids Are Powerful Anti-Inflammatory Drugs

The corticosteroids, which are cholesterol derivatives, include prednisone, prednisolone, and methylprednisolone.These potent anti-inflammatory agents exert various effects that result in a

reduction in the numbers and activity of immune-system cells. They are regularly used in anti-inflammatory therapy.

Corticosteroid treatment causes a decrease in the number of circulating lymphocytes as the result either of steroid induced lysis of lymphocytes (lympholysis) or of alterations in lymphocyte-circulation patterns. Some species (e.g., hamster, mouse, rat, and rabbit) are particularly sensitive to corticosteroid-induced lympholysis. In these animals, corticosteroid treatment at dosages as low as 10–7 M causes such widespread lympholysis that the weight of the thymus is reduced by 90%; the spleen and lymph nodes also shrink visibly. Immature thymocytes in these species appear to be particularly sensitive to corticosteroid-mediated killing. In rodents, corticosteroids induce programmed cell death of immature thymocytes, whereas mature thymocytes are resistant to this activity. Within 2h following in vitro incubation with corticosteroids, immature thymocytes begin to show the characteristic morphology of apoptosis, and 90% of the chromatin is degraded into the characteristic nucleosome ladder by 24 h after treatment. The steps involved in the induction of apoptosis by corticosteroids remain to be determined. In humans, guinea pigs, and monkeys, corticosteroids do not induce apoptosis but instead affect lymphocyte-circulation patterns, causing a decrease in thymic weight and a marked decrease in the number of circulating lymphocytes.

Like other steroid hormones, the corticosteroids are lipophilic and thus can cross the plasma membrane and bind to receptors in the cytosol. The resulting receptor-hormone complexes are transported to the nucleus, where they bind to specific regulatory DNA sequences, regulating transcription up or down. The corticosteroids have been shown to induce increased transcription of the NF-κB inhibitor (I-κB). Binding of this inhibitor to NF-κB in the cytosol prevents the translocation of NF-κB into the nucleus and consequently prevents NF-κB activation of a number of genes, including genes involved in T-cell activation and cytokine production. Corticosteroids also reduce both the phagocytic and killing ability of macrophages and neutrophils, and this effect may contribute to their anti-inflammatory action. In addition, they reduce chemotaxis, so that fewer inflammatory cells are attracted to the site of T_H-cell activation. In the presence of corticosteroids, expression of class II MHC molecules and IL-1 production by macrophages is dramatically reduced; such reductions would be expected to lead to corresponding reductions in T_H-cell activation. Finally, corticosteroids also stabilize the lysosomal membranes of participating leukocytes, so that decreased levels of lysosomal enzymes are released at the site of inflammation.

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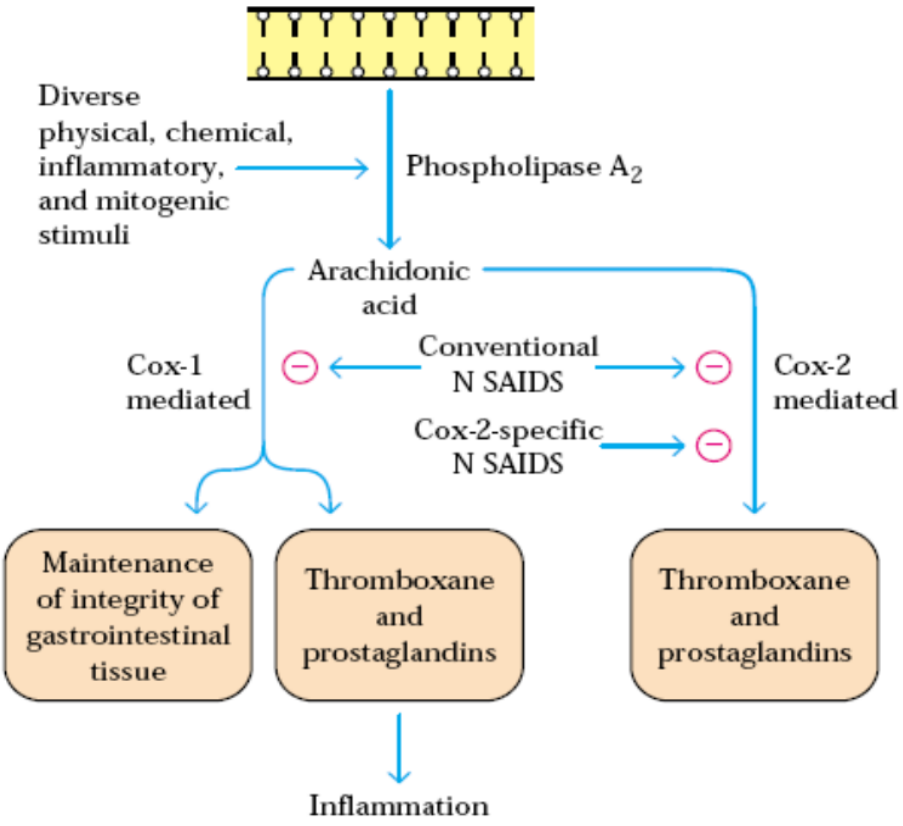
NSAIDs Combat Pain and Inflammation

Since the time of Hippocrates, extracts of willow bark have been used for relief of pain. The active ingredient, salicylate, which is found in aspirin, is just one of many nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs are the most frequently used medication for treating pain and inflammation. Clinically, NSAIDs have been shown to be effective for treatment of many acute and chronic inflammatory reactions. The major mechanism by which these drugs exert anti-inflammatory effects is by inhibiting the cyclooxygenase pathway that produces prostaglandins and thromboxanes from arachidonic acid. The reduction in prostaglandin production limits the increase in vascular permeability and neutrophil chemotaxis in the inflammatory response. As shown in Figure, the cyclooxygenase pathway is mediated by two enzymes, cyclooxygenase 1 and cyclooxygenase 2 (Cox-1 & Cox-2).

Although NSAIDs such as aspirin, Tylenol, ibuprofen, Naproxen, and others are routinely prescribed for the treatment of ailments as diverse as arthritis, sprains, tissue injury, and back pain, the duration of their use is limited by gastrointestinal side effects that include unease and abdominal pain and in more serious cases bleeding or perforation of the stomach or upper GI tract. Investigation of the mechanism of NSAIDs has provided a basis for the beneficial and deleterious effects of many NSAIDS.



Studies have shown that, although most NSAIDs inhibit both Cox-1 and Cox-2, it is the inhibition of Cox-2 that is responsible for the anti-inflammatory effects of NSAIDs. On the other hand, inhibition of Cox-1 by these agents causes damage to the GI tract but does not have significant anti-inflammatory benefits. This realization led to the design and development of a new generation of NSAIDs that specifically inhibit Cox-2 but have little effect on Cox-1 activity. The action of these highly targeted drugs is shown in Figure.