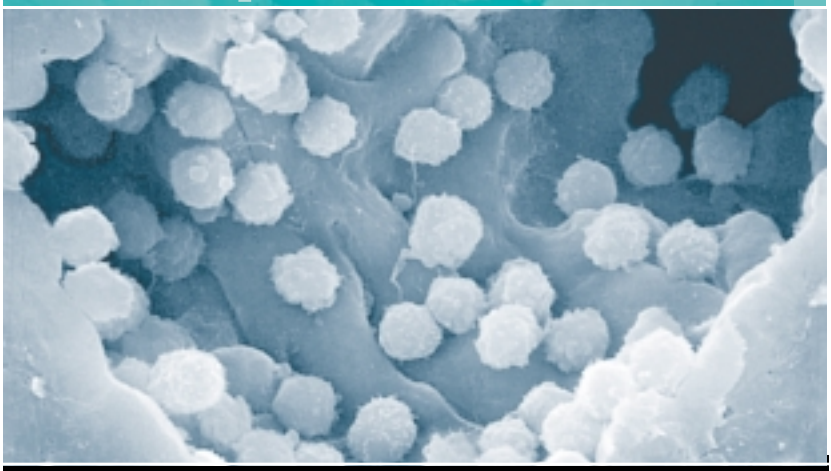
chapter 15

Leukocyte Migration and Inflammation



Lymphocytes Attached to the Surface of a High-Endothelial Venule

Many types of leukocytes 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. This chapter covers the molecules and processes that play a role in leukocyte migration, various molecules that mediate inflammation, and the characteristic physiologic changes that accompany inflammatory responses.

Lymphocyte Recirculation

Lymphocytes are capable of a remarkable level of recirculation, continually moving through the blood and lymph to the various lymphoid organs (Figure 15-1). 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 extralymphoid 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 extralymphoid 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 10^5 lymphocytes recognizes 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

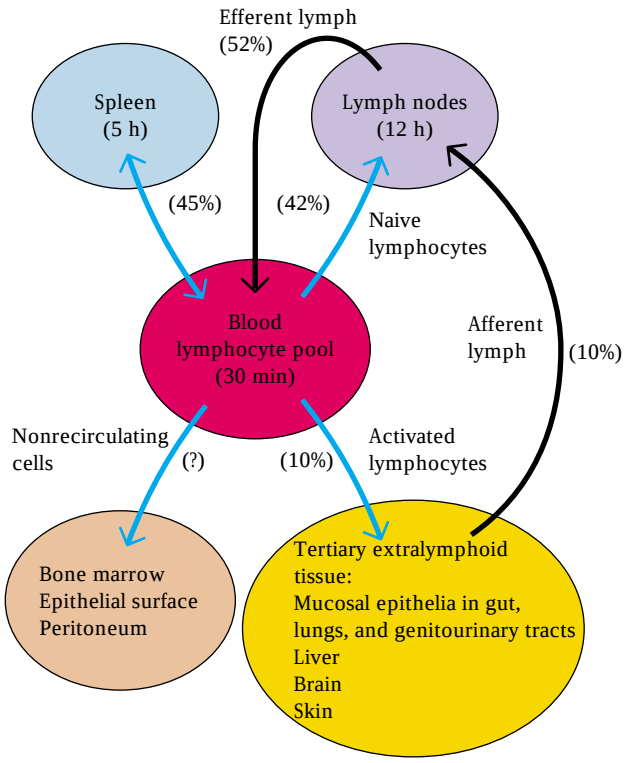


FIGURE 15-1 Lymphocyte recirculation routes. The percentage of the lymphocyte pool that circulates to various sites and the average transit times in the major sites are indicated. Lymphocytes migrate from the blood into lymph nodes through specialized areas in postcapillary venules called high-endothelial venules (HEVs). Although most lymphocytes circulate, some sites appear to contain lymphocytes that do not. *[Adapted from A. Ager, 1994,* Trends Cell Biol. ***4:****326.]*

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.

A number of endothelial and leukocyte CAMs have been cloned and characterized, providing new details about the extravasation process. Most of these CAMs belong to four families of proteins: the selectin family, the mucin-like family, the integrin family, and the immunoglobulin (Ig) superfamily (Figure 15-2).

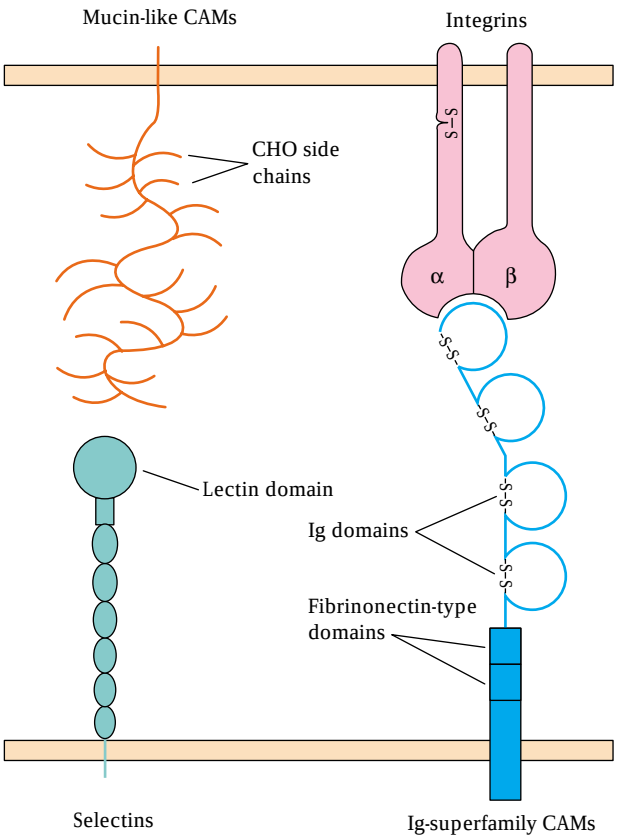
SELECTINS The **selectin** family of membrane glycoproteins has a distal lectin-like domain that enables these molecules to bind to specific carbohydrate groups. Selectins interact primarily with sialylated carbohydrate moieties, which are often linked to mucin-like molecules. The selectin family includes 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.

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 described later in this chapter (see the Clinical Focus). 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.

(a) General structure of CAM families



(b) Selected CAMs belonging to each family

Mucin-like CAMs:	Selectins:
GlyCAM-1	L-selectin
CD34	P-selectin
PSGL-1	E-selectin
MAdCAM-1	
Ig-superfamily CAMs:	Integrins:
ICAM-1, -2, -3	α4β1 (VLA-4, LPAM-2)
VCAM-1	α4β7 (LPAM-1)
LFA-2 (CD2)	α6β1 (VLA-6)
LFA-3 (CD58)	αLβ2 (LFA-1)
MAdCAM-1	αMβ2 (Mac-1)
	αXβ2 (CR4, p150/95)

FIGURE 15-2 Schematic diagrams depicting the general structures of the four families of cell-adhesion molecules (a) and a list of representative molecules in each family (b). The lectin domain in selectins interacts primarily with carbohydrate (CHO) moieties on mucin-like molecules. Both component chains in integrin molecules contribute to the binding site, which interacts with an Ig domain in CAMs belonging to the Ig superfamily. MAdCAM-1 contains both mucin-like and Ig-like domains and can bind to both selectins and integrins.

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 15-3a). In the first step, neutrophils attach loosely to the endothelium by a low-affinity selectin-carbohydrate 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 mucin-like cell-adhesion molecules on the neutrophil membrane or with a sialylated lactosaminoglycan called sialyl Lewis^x (Figure 15-3b). 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 neu-

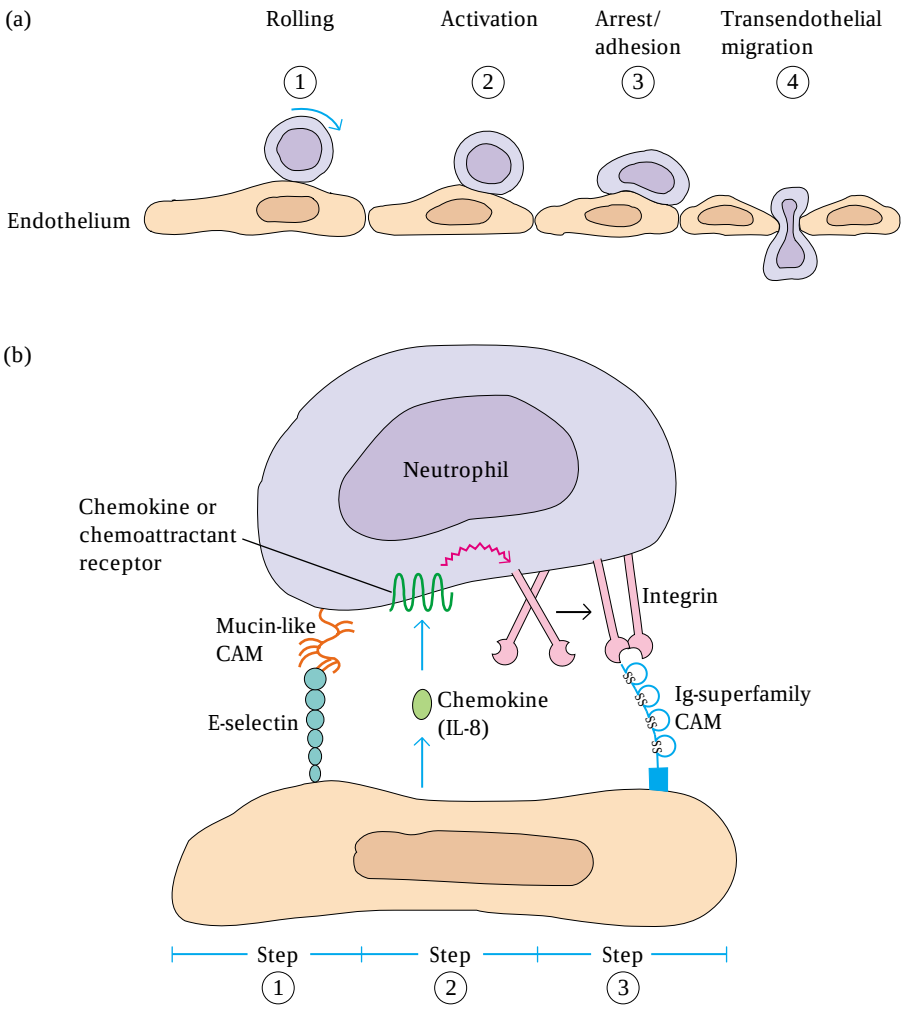


FIGURE 15-3 (a) The four sequential but overlapping steps in neutrophil extravasation. (b) Cell-adhesion molecules and chemokines involved in the first three steps of neutrophil extravasation. Initial rolling is mediated by binding of E-selectin molecules on the vascular endothelium to sialylated carbohydrate moieties on mucin-like CAMs. A chemokine such as IL-8 then binds to a G-protein–linked receptor on the neutrophil, triggering an activating signal. This signal induces a conformational change in the integrin molecules, enabling them to adhere firmly to Ig-superfamily molecules on the endothelium.

trophil 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. As with neutrophils, extravasation of lymphocytes involves interactions among a number of cell-adhesion molecules (Table 15-1). The overall process is similar to what happens during neutrophil extravasation and comprises the same four stages of contact and rolling, activation, arrest and adhesion, and, finally, transendothelial migration.

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** (Figure 15-4a, b). 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. When frozen sections of lymph nodes, Peyer's patches, or tonsils are incubated with lymphocytes and washed to remove unbound cells, over 85% of the

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Leukocyte Extravasation

TABLE 15-1 Some interactions between cell-adhesion molecules implicated in leukocyte extravasation*

Receptor on cells	Expression	Ligands on endothelium	Step involving interaction†	Main function
CLA or ESL-1	Effector T cells	E-selectin	Tethering/rolling	Homing to skin and migration into inflamed tissue
L-selectin	All leukocytes	GlyCAM-1, CD34, MAdCAM-1	Tethering/rolling	Lymphocyte recirculation via HEVs to peripheral lymph nodes and migration into inflamed tertiary sites
LFA-1 ($\alpha L\beta 2$)	Leukocyte subsets	ICAM-1, 2, 3	Adhesion/arrest	General role in lymphocyte extravasation via HEVs and leukocyte migration into inflamed tissue
LPAM-1 ($\alpha 4\beta 7$)	Effector T cells, monocytes	MAdCAM-1, VCAM-1	Rolling/adhesion	Homing of T cells to gut via mucosal HEV; migration into inflamed tissue
Mac-1 ($\alpha M\beta 2$)	Monocytes	VCAM-1	—	Monocyte migration into inflamed tissue
PSGL-1	Neutrophils	E- and P-selectin	Tethering/rolling	Neutrophil migration into inflamed tissue
VLA-4 ($\alpha 4\beta 1$)	Neutrophils, T cells, monocytes	VCAM-1 MAdCAM-1, fibronectin	Rolling/adhesion	General role in leukocyte migration into inflamed tissue
VLA-6 ($\alpha 6\beta 1$)	T cells	Laminin	—	Homing of progenitor T cells to thymus; possible role in T-cell homing to nonmucosal sites

*Most endothelial and leukocyte CAMs belong to four groups of proteins as shown in Figure 15-2. In general, molecules in the integrin family bind to Ig-superfamily CAMs, and molecules in the selectin family bind to mucin-like CAMs. Members of the selectin and mucin-like families can be expressed on both leukocytes and endothelial cells, whereas integrins are expressed only on leukocytes, and Ig-superfamily CAMs are expressed only on endothelium.

†See Figures 15-3a and 15-7 for an illustration of steps in the extravasation process.

bound cells are found adhering to HEVs, even though HEVs account for only 1%–2% of the total area of the frozen section (Figure 15-4c).

It has been estimated that as many as 1.4×10^4 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. The role of antigenic activation of lymphocytes in the maintenance of HEVs has been demonstrated by surgically blocking the afferent lymphatic vasculature to a node, so that antigen entry to the node is blocked. Within a short period of time, the HEVs show impaired function and eventually revert to a more flattened morphology.

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 mucin-like 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 combi-

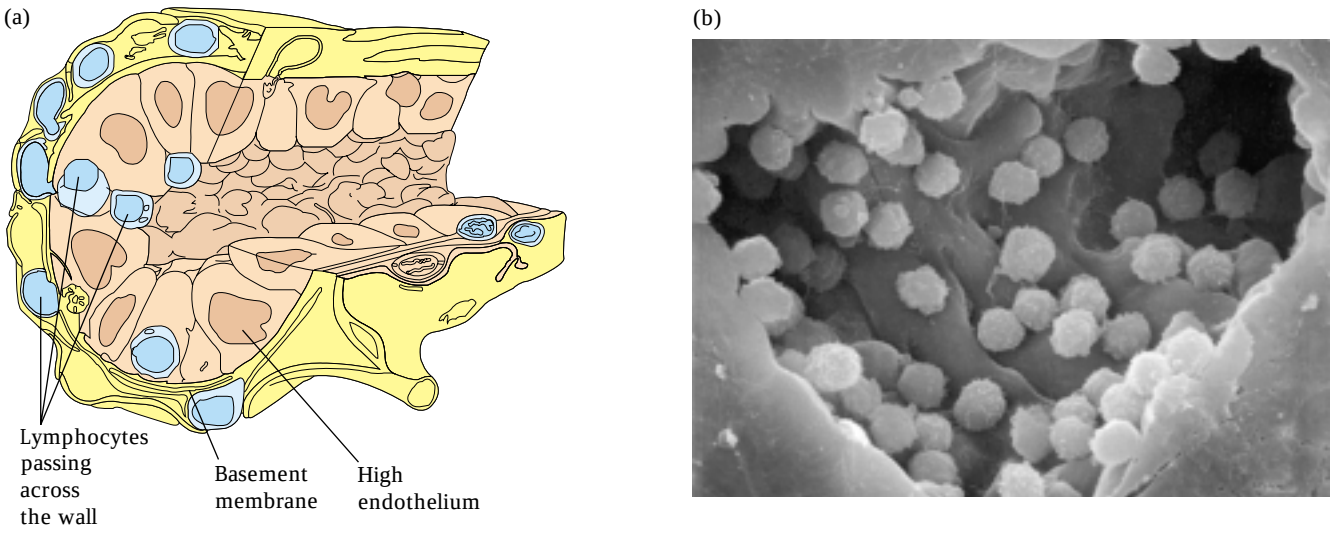

FIGURE 15-4 (a) Schematic cross-sectional diagram of a lymph-node postcapillary venule with high endothelium. Lymphocytes are shown in various stages of attachment to the HEV and in migration across the wall into the cortex of the node. (b) Scanning electron micrograph showing numerous lymphocytes bound to the surface of a high-endothelial venule. (c) Micrograph of frozen sections of lymphoid tissue. Some 85% of the lymphocytes (darkly stained) are bound to HEVs (in cross section), which comprise only 1%–2% of the total area of the tissue section. *[Part (a) adapted from A. O. Anderson and N. D. Anderson, 1981, in* Cellular Functions in Immunity and Inflammation, *J. J. Oppenheim et al. (eds.), Elsevier, North-Holland; part (b) from S. D. Rosen and L. M. Stoolman, 1987,* Vertebrate Lectins, *Van Nostrand Reinhold; part (c) from S. D. Rosen, 1989,* Curr. Opin. Cell Biol. ***1**:913.]*

nations of adhesion molecules and chemokines; receptors that direct the circulation of various populations of lymphocytes to particular lymphoid and inflammatory tissues are 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 (see Table 15-1). As is described later, in the section on chemokines, these molecules play a major role in determining the heterogeneity of lymphocyte circulation patterns.

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 15-5a). 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

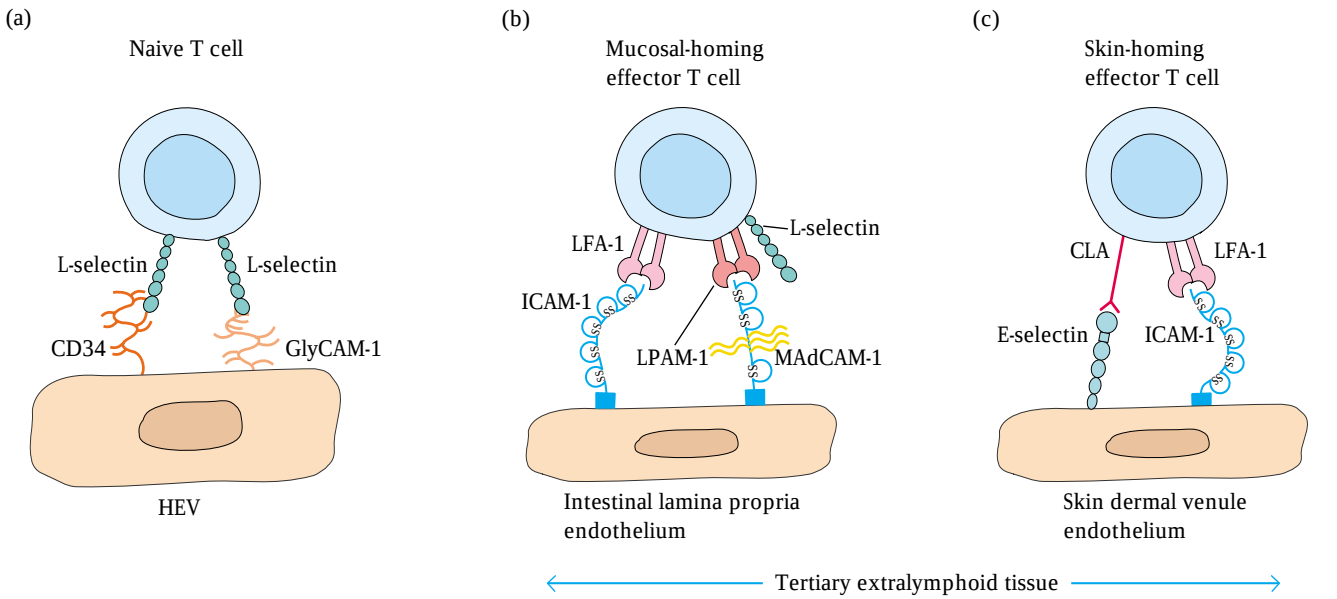

FIGURE 15-5 Examples of homing receptors and vascular addressins involved in selective trafficking of naive and effector T cells. (a) Naive T cells tend to home to secondary lymphoid tissues through their HEV regions. The initial interaction involves the homing receptor L-selectin and mucin-like cell-adhesion molecules such as CD34 or GlyCAM-1 expressed on HEV cells. (b, c) Various subsets of effector T cells express high levels of particular homing receptors that allow them to home to endothelium in particular tertiary extralymphoid tissues. The initial interactions in homing of effector T cells to mucosal and skin sites are illustrated.

region of the secondary lymphoid tissue. During this phase, called the shut-down phase, the antigen-specific lymphocytes cannot be detected in the circulation (Figure 15-6). 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.

Effector and Memory Lymphocytes Adopt Different Trafficking Patterns

The trafficking patterns of effector and memory lymphocytes differ from those of naive lymphocytes. Effector cells tend to home to regions of infection by recognizing inflamed vascular endothelium and chemoattractant molecules that are generated during the inflammatory response. Memory lymphocytes, on the other hand, home selectively to the type of tissue in which they first encountered antigen. Presumably this ensures that a particular memory cell will return to the tissue where it is most likely to reencounter a subsequent threat by the antigen it recognizes.

Effector and memory cells express increased levels of certain cell-adhesion molecules, such as LFA-1, that interact with ligands present on tertiary extralymphoid tissue (such as skin and mucosal epithelia) and at sites of inflammation, allowing effector and memory cells to enter these sites. Naive cells lack corresponding cell-adhesion molecules and do not home to these sites. Inflamed endothelium expresses a number of adhesion molecules, including E- and P-selectin and the Ig-superfamily molecules VCAM-1 and ICAM-1, that bind to the receptors expressed at high levels on memory and effector cells.

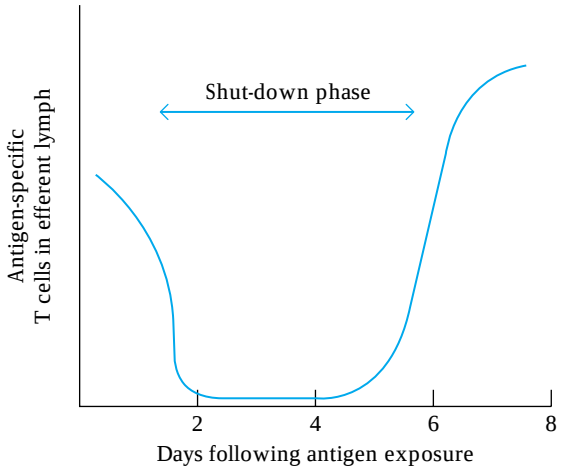

FIGURE 15-6 T-cell activation in the paracortical region of a lymph node results in the brief loss of lymphocyte recirculation. During this shut-down phase, antigen-specific T cells cannot be detected leaving the node in the efferent lymph.

Unlike naive lymphocytes, subsets of the memory and effector populations exhibit tissue-selective homing behavior. Such tissue specificity is imparted not by a single adhesion receptor but by different combinations of adhesion molecules. For example, a mucosal homing subset of memory/effector cells has high levels of the integrins LPAM-1 ($\beta\alpha4\beta7$) and LFA-1 (αLb2), which bind to MAdCAM and various ICAMs on intestinal lamina propria venules (see Figure 15-5b). However, these cells avoid direction to secondary lymphoid tissues because they have low levels of the L-selectin that would facilitate their entry into secondary lymphoid tissue. A second subset of memory/effector cells displays preferential homing to the skin. This subset also expresses low levels of L-selectin but displays high levels of cutaneous lymphocyte antigen (CLA) and LFA-1, which bind to E-selectin and ICAMs on dermal venules of the skin (see Figure 15-5c). Although effector and memory cells that express reduced levels of L-selectin do not tend to home through HEVs into peripheral lymph nodes, they can enter peripheral lymph nodes through the afferent lymphatic vessels.

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. Figure 15-7 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.

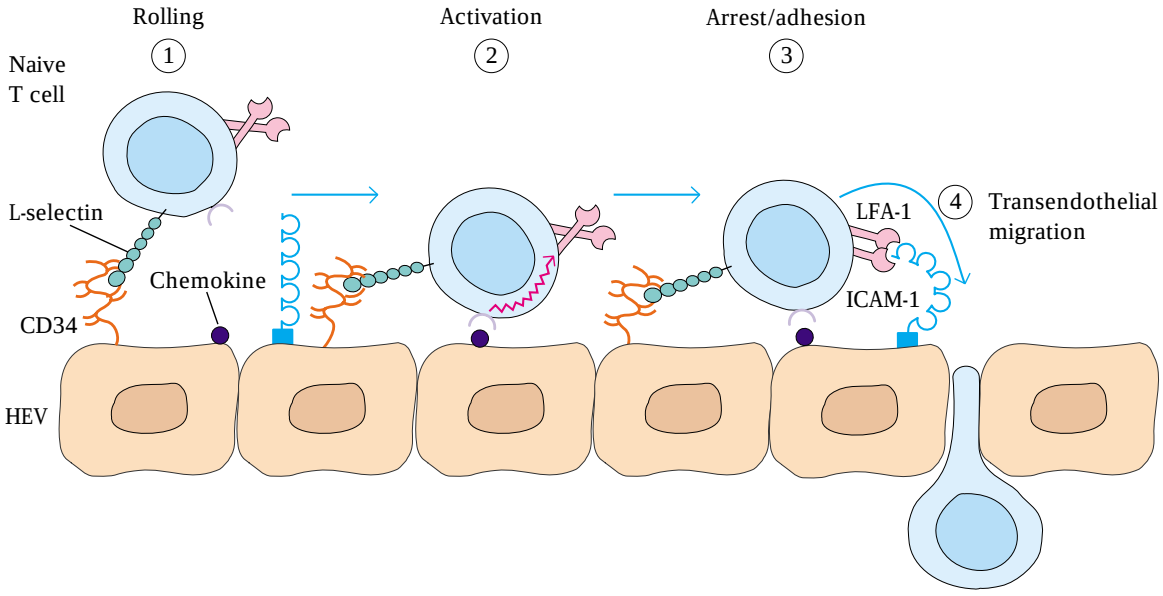

FIGURE 15-7 Steps in extravasation of a naive T cell through a high-endothelial venule into a lymph node. Extravasation of lymphocytes includes the same basic steps as neutrophil extravasation but some of the cell-adhesion molecules differ. Activation of the integrin LFA-1, induced by chemokine binding to the lymphocyte, leads to firm adhesion followed by migration between the endothelial cells into the tissue.

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. Mice that lack either the chemokine CXCL12 (also called SDF-1) or its receptor (see Table 15-2) show major defects in the development of the brain and the heart. Members of the chemokine family have also been shown to play regulatory roles in the development of blood vessels (angiogenesis), and wound healing.

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 (Table 15-2).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 ($K_a > 10^9$) and high specificity. However, as Table 15-2 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 signal-transduction process that generate such potent second messengers as cAMP, IP_3, Ca^{2+}, and activated small G pro-

TABLE 15-2 Human chemokines and their receptors*

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

*This table lists most known chemokine receptors but not all chemokines. The full names for a number of the chemokines abbreviated in the table are as follows: ELC (Ebl1 ligand chemokine); ENA-78 (epithelial-cell-derived neutrophil-activating protein); GCP-2 (granulocyte chemotactic protein 2); Gro-α, β, γ (growth-related oncogene α, β, γ); MCP-1, 2, 3, or 4 (monocyte chemoattractant protein 1, 2, 3, or 4); Mig (monokine induced by interferon γ); MIP-1α, 1β, or 5 (macrophage inflammatory protein 1α, 1β, or 5); NAP-2 (netrophil-activating protein 2); RANTES (*r*egulated upon *a*ctivation, *n*ormal *T*-cell *e*xpresssed and *s*ecreted); TARC (thymus- and activation-regulated chemokine.)

SOURCE: Adapted from Nelson and Krensky, 1998, *Curr. Opin. Immunol.* ***10***:265, and Baggiolini, 1998, *Nature* ***392***:565.

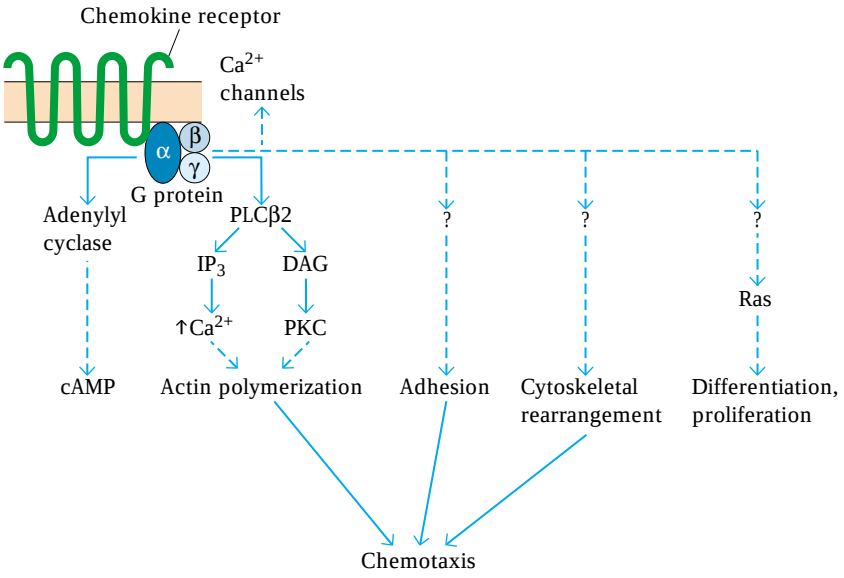

FIGURE 15-8 Chemokines signal through receptors coupled with heterotrimeric large G proteins. Binding of a chemokine to its receptor activates many signal-transduction pathways, resulting in a variety of modifications in the physiology of the target cell. If the signal-transduction pathway is not known or incompletely worked out, dashed lines and question marks are used here to represent probable pathways. *[Adapted from Premack et al., 1996,* Nature Medicine ***2:****1174.]*

teins (Figure 15-8). 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.

Chemokine-Receptor Profiles Mediate Leukocyte Activity

Among major populations of human leukocytes, neutrophils express CXCR1, -2, and -4; eosinophils have CCR1 and CCR3 (Figure 15-9). 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

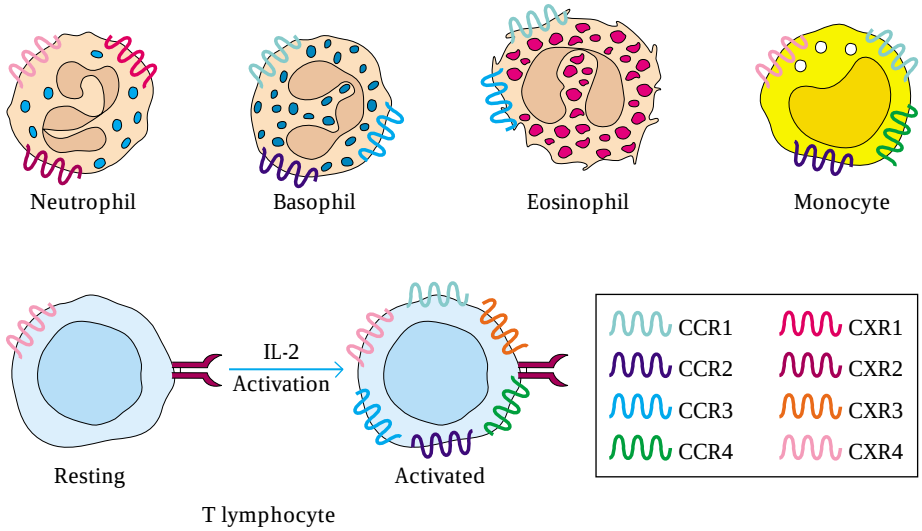

FIGURE 15-9 Patterns of expression of some principal chemokine receptors on different classes of human leukocytes. So far the greatest variety of chemokine receptors has been observed on activated T lymphocytes. *[Adapted from M. Baggiolini, 1998,* Nature ***392:****565.]*

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 enhance 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.

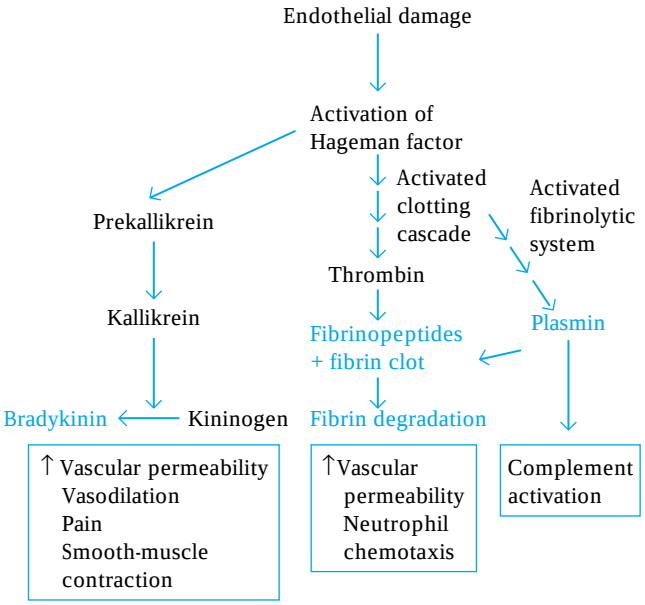

FIGURE 15-10 Tissue damage induces formation of plasma enzyme mediators by the kinin system, the clotting system, and the fibrinolytic system. These mediators cause vascular changes, among the earliest signs of inflammation, and various other effects. Plasmin not only degrades fibrin clots but also activates the classical complement pathway.

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 15-10). 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 (see Chapter 13). 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 (Figure 15-11). 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

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 Table 15-3. In addition, IFN-γ 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 role of several of these inflammatory cytokines in the development of acute and chronic inflammation will be described more fully in the next section.

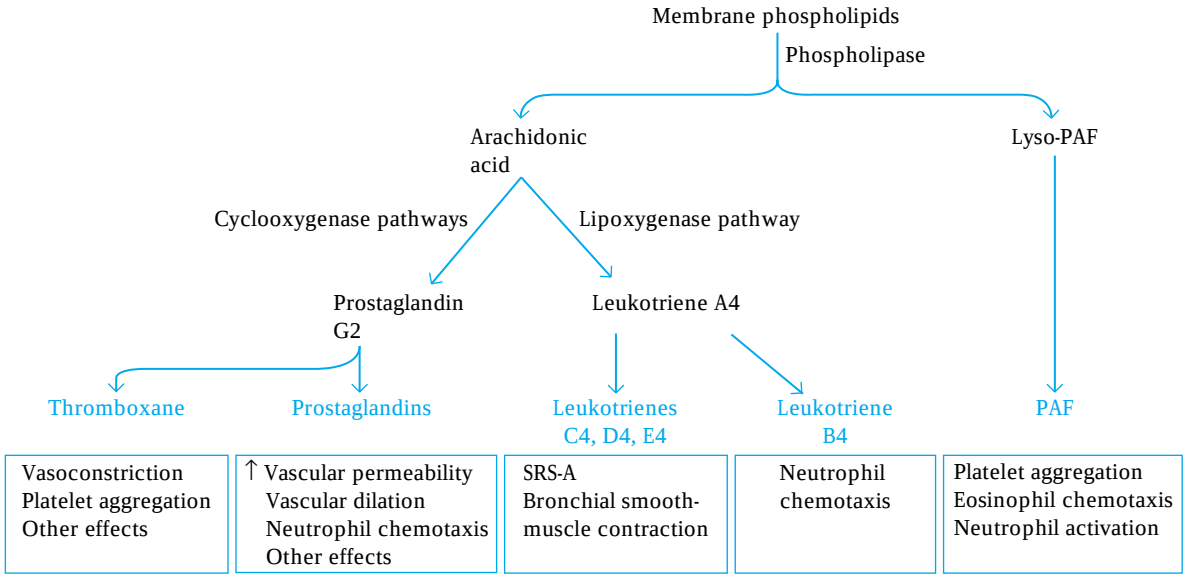

FIGURE 15-11 The breakdown of membrane phospholipids generates important mediators of inflammation, including thromboxane, prostaglandins, leukotrienes, and platelet-activating factor (PAF).

TABLE 15-3 Redundant and pleiotropic effects of IL-1, TNF-α, and IL-6

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	–	–	+

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-α induce increased expression of E-selectin. The circulating neutrophils express mucins such as PSGL-1 or the tetrasaccharides sialyl Lewis^a and sialyl Lewis^x, 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-

VISUALIZING CONCEPTS

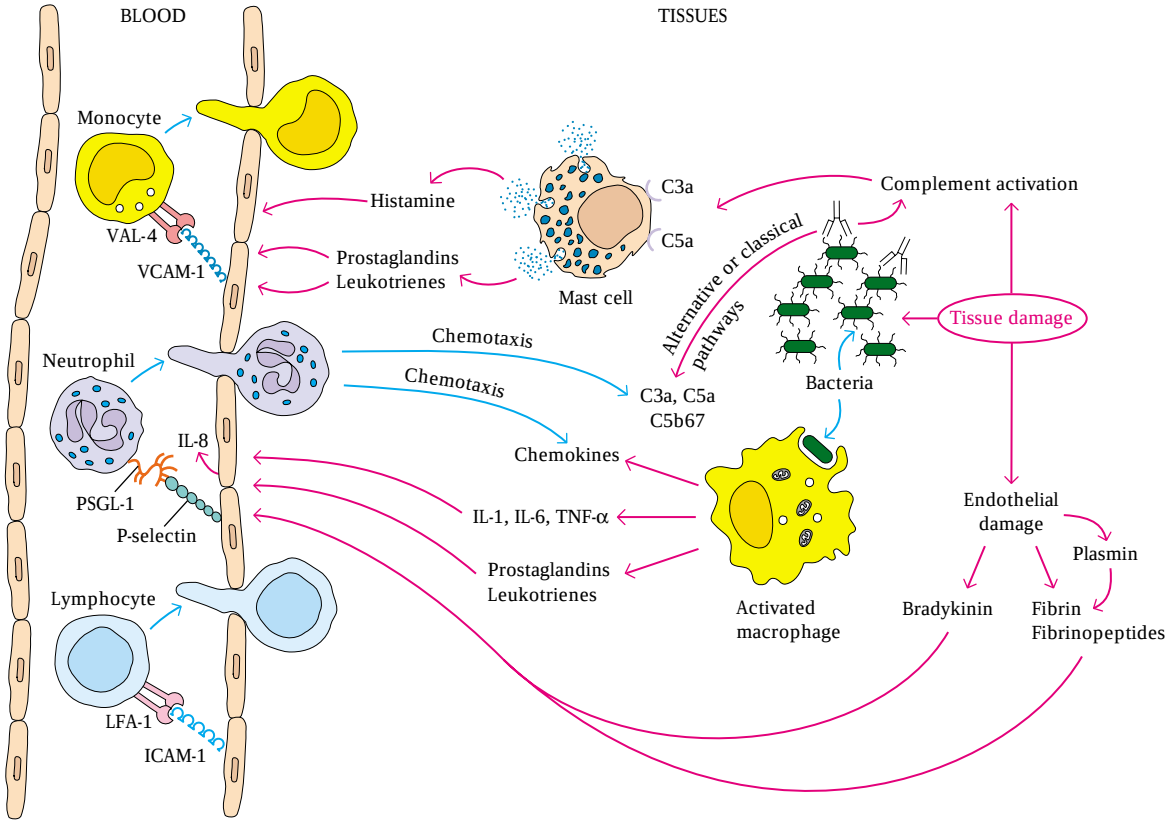

FIGURE 15-12 Overview of the cells and mediators involved in a local acute inflammatory response. Tissue damage leads to the formation of complement products that act as opsonins, anaphylatoxins, and chemotactic agents. Bradykinin and fibrinopeptides induced by endothelial damage mediate vascular changes. Neutrophils generally are the first leukocytes to migrate into the tissue, followed by monocytes and lymphocytes. Only some of the interactions involved in the extravasation of leukocytes are depicted.

ing to the swelling and redness in the area. When fluid exudes from the bloodstream, the kinin, clotting, and fibrinolytic systems are activated (see Figure 15-10). 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 (Figure 15-12). 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 (see Table 15-3). 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 (see Clinical Focus on page 358).

SYSTEMIC ACUTE-PHASE RESPONSE

The local inflammatory response is accompanied by a systemic response known as the **acute-phase response** (Figure 15-13). 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.

Many systemic acute-phase effects are due to the combined action of IL-1, TNF-α and IL-6 (see Figure 15-13). 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 15-14a). 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 15-14b). 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 self-antigens 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

VISUALIZING CONCEPTS

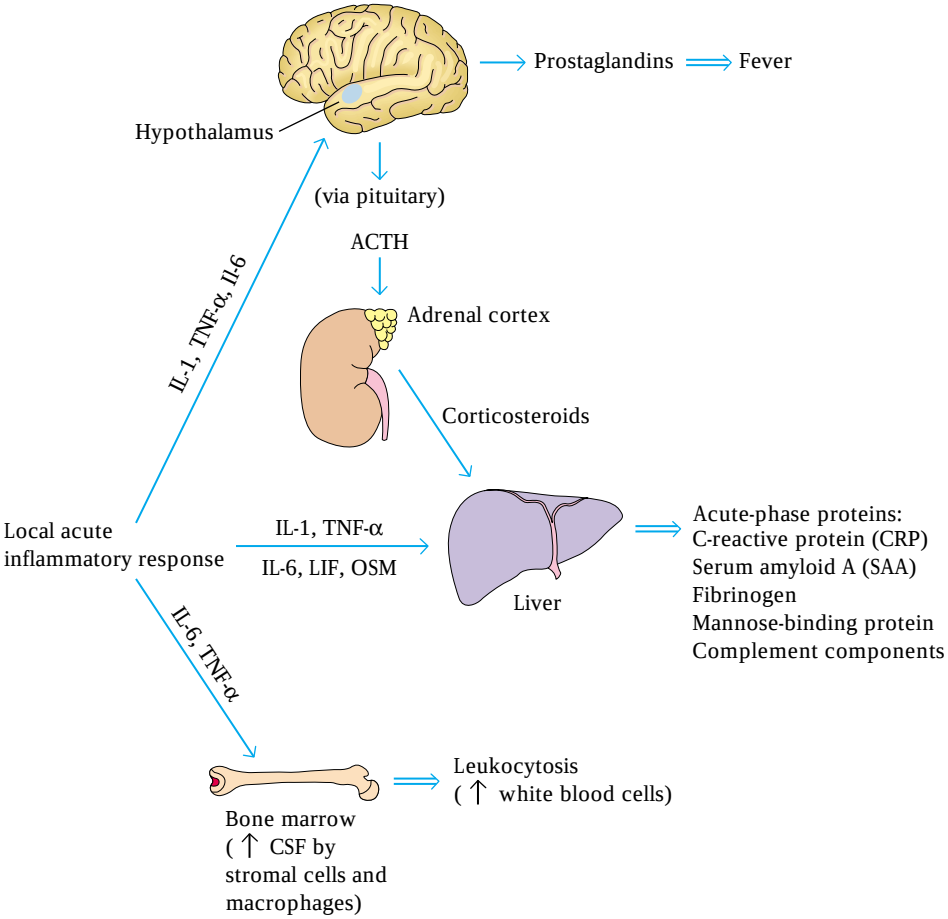

FIGURE 15-13 Overview of the organs and mediators involved in a systemic acute-phase response. IL-1, IL-6, and TNF-α, which are produced by activated macrophages at the site of inflammation, are particularly important in mediating acute-phase effects. LIF = leukemia inhibitory factor; OSM = oncostatin M.

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.

Roles of IFN-γ and TNF-α in Chronic Inflammation

Two cytokines in particular, IFN-γ and TNF-α, play a central role in the development of chronic inflammation. T_H1 cells, NK cells, and T_C cells release IFN-γ, while activated macrophages secrete TNF-α.

Members of the interferon family of glycoproteins (IFN-α and IFN-β) are released from virus-infected cells and confer antiviral protection on neighboring cells. Exactly which interferon is produced depends on the type of cell infected. IFN-α is produced by leukocytes, IFN-β, often called fibroblast

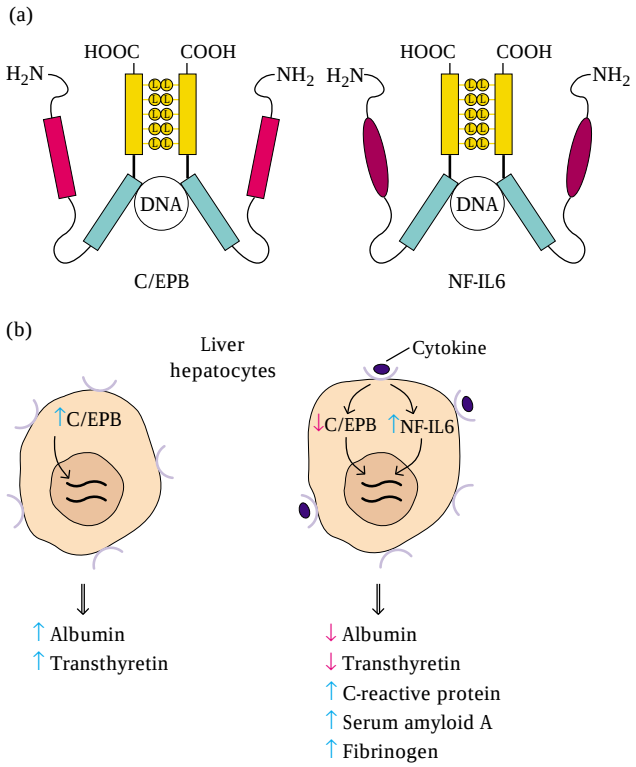

FIGURE 15-14 Comparison of the structure and function of C/EBP and NF-IL6. (a) Both transcription factors are dimeric proteins containing a leucine-zipper domain (light orange) and a basic DNA-binding domain (blue). (b) C/EBP is expressed constitutively in liver hepatocytes and promotes transcription of albumin and transthyretin genes. During an inflammatory response, binding of IL-1, IL-6, TNF-α, LIF, or OSM to receptors on liver hepatocytes induces production of NF-IL6, which promotes transcription of the genes encoding various acute-phase proteins. Concurrently, C/EBP levels decrease and the levels of albumin and transthyretin consequently decrease.

interferon, is made largely by fibroblasts. IFN-γ is produced exclusively by T cells and NK cells. However, IFN-γ has a number of pleiotropic activities that distinguish it from IFN-α and IFN-β and contribute to the inflammatory response (Figure 15-15). One of the most striking effects of IFN-γ is its ability to activate macrophages. Activated macrophages exhibit increased expression of class II MHC molecules, increased cytokine production, and increased microbicidal activity compared with nonactivated macrophages; thus, they are more effective in antigen presentation and killing of intracellular microbial pathogens. In a chronic inflammatory response, however, the large numbers of activated macrophages release various hydrolytic enzymes and reactive oxygen and nitrogen intermediates, which are responsible for much of the damage to surrounding tissue.

One of the principal cytokines secreted by activated macrophages is TNF-α. The activity of this cytokine was first observed around the turn of the century by the surgeon William Coley. He noted that when cancer patients developed certain bacterial infections, the tumors would become necrotic. In the hope of providing a cure for cancer, Coley began to inject cancer patients with supernatants derived from various bacterial cultures. These culture supernatants, called "Coley's toxins," did induce hemorrhagic necrosis in the tumors but had numerous undesirable side effects, making them unsuitable for cancer therapy. Decades later, the active component of Coley's toxin was shown to be a lipopolysaccharide (endotoxin) component of the bacterial cell wall. This endotoxin does not itself induce tumor necrosis but instead induces macrophages to produce TNF-α. This cytokine has a direct cytotoxic effect on tumor cells but not on normal cells (Figure 15-16a). Potential immunotherapeutic approaches using TNF-α for the treatment of cancer are examined in Chapter 22.

Several lines of evidence indicate that TNF-α also contributes to much of the tissue wasting that characterizes chronic inflammation. For example, mice carrying a TNF-α transgene become severely wasted (Figure 15-16b). In studies by A. Cerami and coworkers, rabbits were found to lose nearly half of their body mass within 2 months of being infected with trypanosomes. These workers subsequently discovered that a macrophage-derived factor was responsible for the profound wasting; they called the factor cachetin. Cloning of the genes for TNF-α and cachetin revealed that they were the same protein.

Activation of macrophages by IFN-α promotes increased transcription of the TNF-α gene and increases the stability of TNF-α mRNA. Both effects result in increased TNF-α production. TNF-α acts synergistically with IFN-γ to initiate a chronic inflammatory response. Both cytokines together induce much greater increases in ICAM-1, E-selectin, and class I MHC molecules than either cytokine alone. The increase in intercellular adhesion molecules facilitates the recruitment of large numbers of cells in a chronic inflammatory response.

CHRONIC INFLAMMATORY DISEASES

Recent studies suggest that regions of plump endothelial cells resembling HEVs appear along the vasculature in tertiary extralymphoid sites of chronic infection. These HEV-like regions, which appear to be sites of lymphocyte extravasation into the inflamed tissue, express several mucins (e.g., GlyCAM-1, MAdCAM-1, and CD34) that are often displayed on normal HEVs. Several cytokines, notably IFN-γ and TNF-α, that are associated with chronic inflammation may play a role in the induction of HEV-like regions along the vasculature.

These HEV-like regions have been observed in a number of chronic inflammatory diseases in humans, including

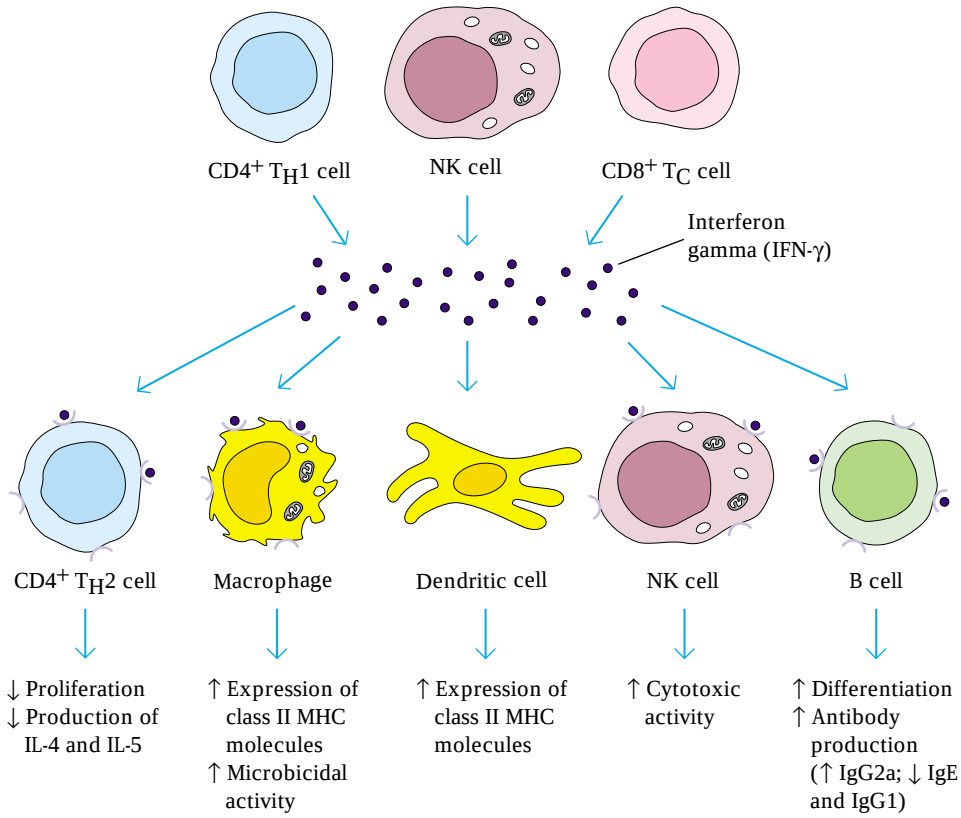

FIGURE 15-15 Summary of pleiotropic activity of interferon gamma (IFN-γ). The activation of macrophages induced by IFN-γ plays a critical role in chronic inflammation. This cytokine is secreted by T_H1 cells, NK cells, and T_C cells and acts on numerous cell types. *[Adapted from Research News, 1993,* Science ***259:****1693.]*

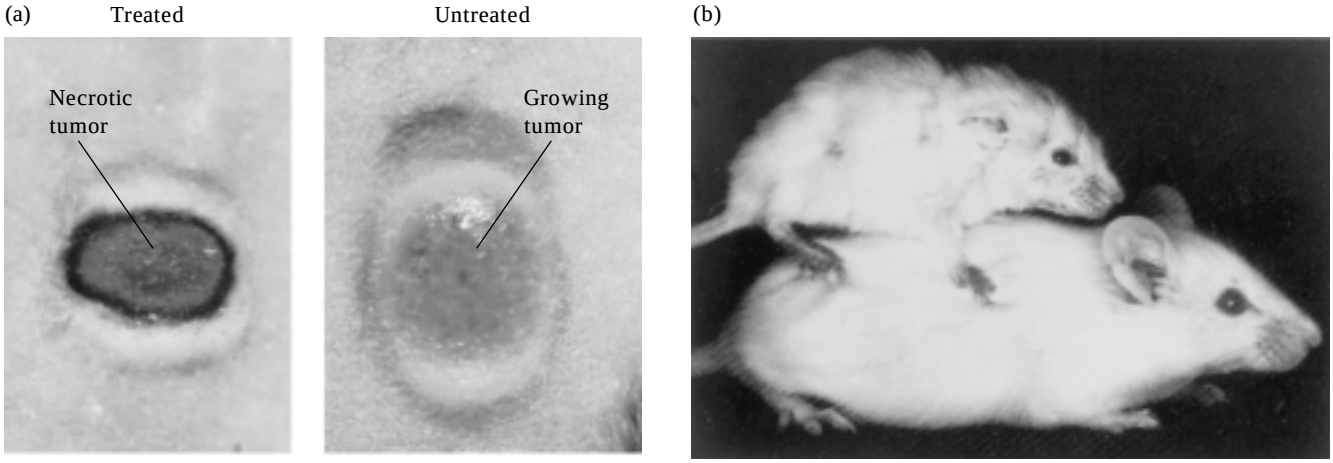

FIGURE 15-16 Biological activities of TNF-α. (a) A cancerous tumor in a mouse injected with endotoxin (*left*) shows hemorrhagic necrosis, unlike a tumor in an untreated mouse (*right*). Endotoxin induces the production of TNF-α, which then acts to destroy the tumor. (b) Transgenic mouse (*top*) bearing a TNF-α transgene becomes anorectic and severely wasted. Normal mouse is shown on the bottom. *[Part (a) from L. J. Old, 1988,* Sci. Am. ***258:****59; part (b) from B. Beutler, 1993,* Hosp. Prac. *(April 15):45.]*

CLINICAL FOCUS

Leukocyte-Adhesion Deficiency (LAD) in Humans and Cattle

The immune system uses inflammation to assemble the components of an effective response and focus these resources at the site of infection. Inflammation is complex, involving vasodilation, increased vascular permeability, exudation of plasma proteins, and a gathering of inflammatory cells. Chemoattractants are key elements in calling leukocytes to sites of inflammation. These include chemokines such as IL-8, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1 (MIP-1) and peptide fragments, such as C5a, generated during complement fixation. Chemoattractants signal passing leukocytes to adhere tightly to the vascular surface, and, using adhesive interactions for traction, these cells push their way between endothelial cells and gain entry into the surrounding tissue. Once in, they are guided by gradients of chemoattractants to the sites of the inflammatory responses and become participants in the process. The key players in the adhesive interactions that are central to adhesion and extravasation are heterodimeric integrin molecules on the surface of the migrating leukocytes. There are a number of integrins, among which are LFA-1 (composed of CD11a and CD18); Mac-1, also called CR3 (components: CD11b and CD18); and p150/95 or CR4 (components: CD11c and CD18). When leukocytes encounter the appropriate chemokine or other chemoattractant, their complement of membrane integrin molecules undergoes a conformational change that transforms them from a slightly adhesive to a highly adhesive state.

In 1979, a paper entitled "Delayed separation of the umbilical cord, widespread infections, and defective neutrophil mobility" appeared in *Lancet,* a British medical journal. This was the first in a series of reports that have appeared over the years describing patients afflicted with a rare autosomal recessive disease in which the first indication is quite often omphalitis, a swelling and reddening around the stalk of the umbilical cord. Although no more susceptible to virus infections than normal controls, those afflicted with this disorder suffer recurrent and often chronic bacterial infections, and sites where one would expect to find pus are instead pus-free. This observation is particularly striking because the patients are not deficient in granulocytes; in fact, they typically have greatly elevated numbers of granulocytes in the circulation. Detailed immunological workups of these patients showed that Ig levels were in the normal range and that they had nearly normal B-, T-, and NK-cell function. However, examination of leukocyte migration in response to tissue damage revealed the root cause of the disease in these patients.

One method of evaluating leukocyte migration involves gently scraping the

rheumatoid arthritis, Crohn's disease, ulcerative colitis, Graves' disease, Hashimoto's thyroiditis, and diabetes mellitus (Table 15-4). Development of this HEV-like vasculature is likely to facilitate a large-scale influx of leukocytes, contributing to chronic inflammation. These observations suggest that an effective approach for treating chronic inflammatory diseases may be to try to control the development of these HEV-like regions.

TABLE 15-4 Chronic inflammatory diseases associated with HEV-like vasculature

Disease	Affected organ	Plump endothelium	Mucin-like CAMs on endothelium*
Crohn's disease	Gut	+	+
Diabetes mellitus	Pancreas	+	+
Graves' disease	Thyroid	+	+
Hashimoto's thyroiditis	Thyroid	+	+
Rheumatoid arthritis	Synovium	+	+
Ulcerative colitis	Gut	+	+

*Includes GlyCAM-1, MAdCAM-1, and CD34.

SOURCE: Adapted from J. P. Girard and T. A. Springer, 1995, *Immunol. Today* **16**:449.

skin from a small area of the arm; the cell populations that move into the abraded area are then sampled by capturing some of those cells on a glass coverslip placed onto the wounded skin. A series of glass coverslips is sequentially placed, incubated, and removed over a period of several hours. Typically, each coverslip is left in place for two hours, and the procedure is repeated four times over an eight-hour period. Examination of the coverslips under a microscope reveals whether leukocytes have adhered to the coverslips. In normal individuals, the response of the immune system to tissue injury is to deliver leukocytes to the damaged area, and one finds these cells on the coverslips. However, in the patients described here, the coverslips were largely negative for leukocytes. Examination of white blood cells in these patients revealed an absence of CD18, an essential component of a number of integrins. A key element in the migration of leukocytes is integrin-mediated cell adhesion, and these patients suffer from an inability of their leukocytes to undergo adhesion-dependent migration into sites of inflammation. Hence, this syndrome has been named leukocyte-adhesion deficiency (LAD).

Bacterial infections in these patients can be treated with antibiotics, but they recur. Furthermore, there are antibiotic-resistant strains of many pathogenic bacteria, and LAD patients must live under this microbial Sword of Damocles, never knowing when the life-saving thread of antibiotics will fail. If a suitable bone-marrow donor can be found (almost always a close relative), however, there is a curative strategy. The LAD patient's hematopoietic system is destroyed, perhaps by treatment with cytotoxic chemicals, and then bone-marrow transplantation is performed. If successful, this procedure provides the patient with leukocytes that have normal levels of functional integrin and display the full range of migratory capacities.

This disease is not limited to humans. A strikingly similar version known as bovine leukocyte adhesion disease (BLAD) occurs in cattle. The cause of BLAD in these animals is identical to the cause of LAD in human patients—the lack of a functional integrin subunit. What is different in some dairy herds is the incidence of the disease; though rare in humans, it can occur at economically important frequencies in cattle. This is a consequence of the high degree of inbreeding that exists in populations of dairy cattle. Typically, dairy herds are sired by the artificial insemination of semen from very few bulls. As a consequence of this practice, by the 1980s, almost 1 in 20 dairy bulls could be traced back to a single Holstein bull who happened to be heterozygous for BLAD. Such a high frequency of this recessive trait in the sire population dramatically raised the frequency of this disease in dairy herds. During the early 1990s, in some countries, the incidence of the BLAD gene was as high as 10% in a number of dairy herds. The gene for bovine CD18 has been cloned, which has allowed the design of a PCR-based assay for the aberrant forms of this gene. It is now possible to routinely screen sires and recipients for the BLAD allele. As a result, bulls that are carriers of the BLAD gene have been identified and eliminated from the breeding pool. This has led to a dramatic reduction in the frequency of new BLAD cases as well as in the overall frequency of the BLAD allele in dairy-herd populations.

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 adhesins 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 2 h 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.

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 15-17, the cyclooxygenase pathway is mediated by two enzymes, cyclooxygenase 1 and cyclooxygenase 2 (Cox-1 & Cox-2).

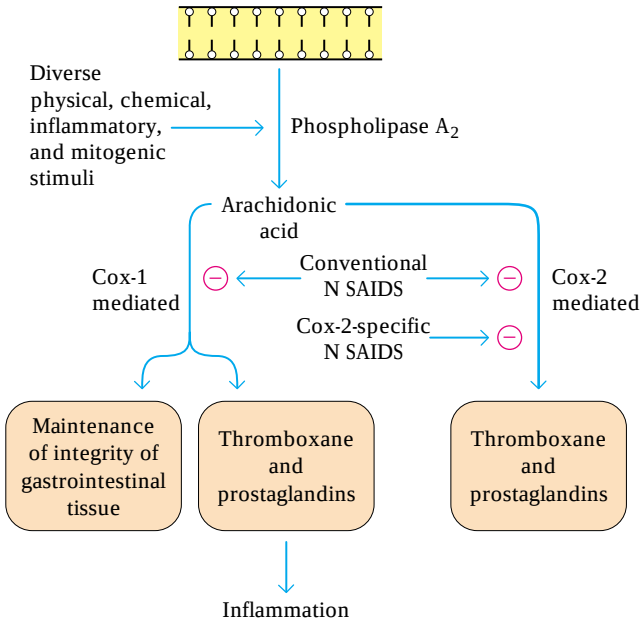

FIGURE 15-17 Inhibition of cyclooxygenase 1 and 2 by NSAIDs. A variety of agents trigger the release of arachidonic acid from the cell membrane by the action of phospholipase A_2. The subsequent action of Cox-1 and Cox-2 initiates the conversion of arachidonic acid to a variety of lipid mediators of inflammation and many other processes. Many NSAIDs inhibit both enzymatic pathways, but those with greater specificity for the Cox-2 arm produce antinflammatory effects with fewer side effects. *[Adapted from G. A. FitzGerald and C. N. Patrono, 2001,* New England Journal of Medicine ***345****:433.]*

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, inhibi-

tion of Cox-1 by thses 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 15-17.

SUMMARY

- Lymphocytes undergo constant recirculation between the blood, lymph, lymphoid organs, and tertiary extralymphoid tissues, increasing the chances that the small number of lymphocytes specific for a given antigen (about 1 in 10^5 cells) will actually encounter that antigen.
- Migration of leukocytes into inflamed tissue or into lymphoid organs requires interaction between cell-adhesion molecules (CAMs) on the vascular endothelium and those on the circulating cells.
- Most CAMs fall into one of four protein families: the selectins, the mucin-like family, integrins, or the Ig superfamily. Selectins and mucin-like CAMs interact with each other, and members of each family are expressed on both leukocytes and endothelial cells. Integrins, expressed on leukocytes, interact with Ig-superfamily CAMs, expressed on endothelial cells.
- Extravasation of both neutrophils and lymphocytes involves four steps: rolling, activation, arrest and adhesion, and transendothelial migration. Neutrophils are generally the first cell type to move from the bloodstream into inflammatory sites.
- Unlike neutrophils, various lymphocyte populations exhibit differential extravasation into various tissues. Homing receptors on lymphocytes interact with tissue-specific adhesion molecules, called vascular addressins, on high-endothelial venules (HEVs) in lymphoid organs and on the endothelium in tertiary extralymphoid tissues.
- Naive lymphocytes home to secondary lymphoid organs, extravasating across HEVs, whereas effector lymphocytes selectively home to inflamed vascular endothelium.
- Inflammation is a physiologic response to a variety of stimuli such as tissue injury and infection. An acute inflammatory response involves both localized and systemic effects. The localized response begins when tissue and endothelial damage induces formation of plasma enzyme mediators that lead to vasodilation and increased vascular permeability.
- Several types of mediators play a role in the inflammatory response. Chemokines act as chemoattractants and activating molecules during leukocyte extravasation. Plasma enzyme mediators include bradykinin and fibrinopeptides, which increase vascular permeability; plasmin is a proteolytic enzyme that degrades fibrin clots into chemotactic products and activates complement; and various complement products act as anaphylatoxins, opsonins, and chemotactic molecules for neutrophils and monocytes. Lipid inflammatory mediators include thromboxanes, prostaglandins, leukotrienes, and platelet-activating factor. Three cytokines, IL-1, IL-6, and TNF-α, mediate many of the local and systemic features of the acute inflammatory response
- Activation of tissue macrophages and degranulation of mast cells lead to release of numerous inflammatory mediators, some of which induce the acute-phase response, which includes fever, leukocytosis, and production of corticosteroids and acute-phase proteins.
- A chronic inflammatory response may accompany allergies, autoimmune diseases, microbial infections, transplants, and burns. Drug-based therapies employing corticosteroids and a variety of nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used medications for pain and inflammation.

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USEFUL WEB SITES

http://www.mdsystems.com

The Cytokine Mini-Reviews Section (Site Map/Reviews & Technical Notes) of the R&D Systems site contains extensive,

detailed, and well-illustrated reviews of many chemokines and chemokine receptors.

http://www.ncbi.nlm.nih.gov/Omim/

Online Mendelian Inheritance in Man is a catalog of human genes and genetic disorders. It contains pictures and references on many diseases, including LAD.

Study Questions

CLINICAL FOCUS QUESTION Why does a defect in CD18 result in an increased vulnerability to bacterial infection? Please address, as precisely as you can, the cell biology of cell migration.

1. Indicate whether each of the following statements is true or false. If you think a statement is false, explain why.

a. Chemokines are chemoattractants for lymphocytes but not other leukocytes.
b. Integrins are expressed on both leukocytes and endothelial cells.
c. Leukocyte extravasation involves multiple interactions between cell-adhesion molecules.
d. Most secondary lymphoid organs contain high-endothelial venules (HEVs).
e. Mucin-like CAMs interact with selectins.
f. An acute inflammatory response involves only localized effects in the region of tissue injury or infection.
g. MAdCAM-1 is an endothelial adhesion molecule that binds to L-selectin and to several integrins.
h. Granuloma formation is a common symptom of local inflammation.

2. Various inflammatory mediators induce expression of ICAMs on a wide variety of tissues. What effect might this induction have on the localization of immune cells?

3. Extravasation of neutrophils and of lymphocytes occurs by generally similar mechanisms, although some differences distinguish the two processes.

a. List in order the four basic steps in leukocyte extravasation.
b. At which sites are neutrophils most likely to extravasate? Why?
c. Different lymphocyte subpopulations migrate preferentially into different tissues, a process called homing (or trafficking). Discuss the roles of the three types of molecules that permit homing of lymphocytes.

4. Which three cytokines secreted by activated macrophages play a major role in mediating the localized and systemic effects associated with an acute inflammatory response?

5. An effective inflammatory response requires differentiation and proliferation of various nonlymphoid white blood cells. Explain how hematopoiesis in the bone marrow is induced by tissue injury or local infection.

6. For each pair of molecules listed below, indicate whether the molecules interact during the 1st, 2nd, 3rd, or 4th step in neutrophil extravasation at an inflammatory site. Use N to indicate any molecules that do not interact.

a. _____ Chemokine and L-selectin
b. _____ E-selectin and mucin-like CAM
c. _____ IL-8 and E-selectin
d. _____ Ig-superfamily CAM and integrin
e. _____ ICAM and chemokine
f. _____ Chemokine and G-protein–coupled receptor
g. _____ ICAM and integrin

7. Discuss the main effects of IFN-γ and TNF-α during a chronic inflammatory response.

8. Five cytokines (IL-1, IL-6, TNF-α, LIF, and OSM) induce production of C-reactive protein and other acute-phase proteins by hepatocytes. Briefly explain how these different cytokines can exert the same effect on hepatocytes.

9. For each inflammation-related term (a–h), select the descriptions listed below (1–11) that apply. Each description may be used once, more than once, or not at all; more than one description may apply to some terms.

Terms

a. _____ Tertiary extralymphoid tissue
b. _____ P- and E-selectin
c. _____ Prostaglandins
d. _____ Nonsteroidal anti-inflammatory drugs
e. _____ ICAM-1, -2, -3
f. _____ MAdCAM
g. _____ Bradykinin
h. _____ Inflamed endothelium

Descriptions

(1) Bind to sialylated carbohydrate moieties
(2) Inhibit cyclooxygenase pathway
(3) Induce expression of NF-κB inhibitor
(4) Has both Ig domains and mucin-like domains
(5) Region of vascular endothelium found in postcapillary venules
(6) Expressed by inflamed endothelium
(7) Exhibits HEV-like vasculature in chronic inflammation
(8) Belong to Ig-superfamily of CAMs
(9) Exhibits increased expression of CAMs
(10) Increase vascular permeability and induce fever
(11) Induce fever