

## Review Article

*Advances in Immunology*IAN R. MACKAY, M.D., AND FRED S. ROSEN, M.D.,  
*Editors***T-CELL FUNCTION AND MIGRATION****Two Sides of the Same Coin**ULRICH H. VON ANDRIAN, M.D., PH.D.,  
AND CHARLES R. MACKAY, PH.D.

**S**INCE the pioneering work of Gowans and colleagues in the 1960s,<sup>1,2</sup> much progress has been made in understanding the pivotal role of cell migration in immunity. We now have considerable knowledge of the way in which specialized leukocytes are channeled to distinct target tissues in immune responses and inflammation (Fig. 1). This review will concentrate on the migration of T cells, which are at the heart of most adaptive immune responses.

Since T cells respond to pathogens only on direct contact with pathogen-derived antigen, they must migrate to sites where antigen is found. The T-cell receptor recognizes a peptide or lipid antigen bound to a major histocompatibility complex (MHC) or CD1, respectively, on the surface of another cell.<sup>4,5</sup> However, antigens occur in countless shapes and forms; theoretically, there are billions of ways to form an octapeptide, the minimal length of peptide antigens held in the MHC binding groove. Our immune system copes with this diversity by generating a large army of combat-ready T cells, each with a unique T-cell receptor.

The repertoire of T cells that have never encountered antigen, referred to as naive T cells, in adults consists of 25 million to 100 million distinct clones.<sup>6,7</sup> However, the number of cells whose T-cell receptors recognize any individual antigen is very limited (sev-

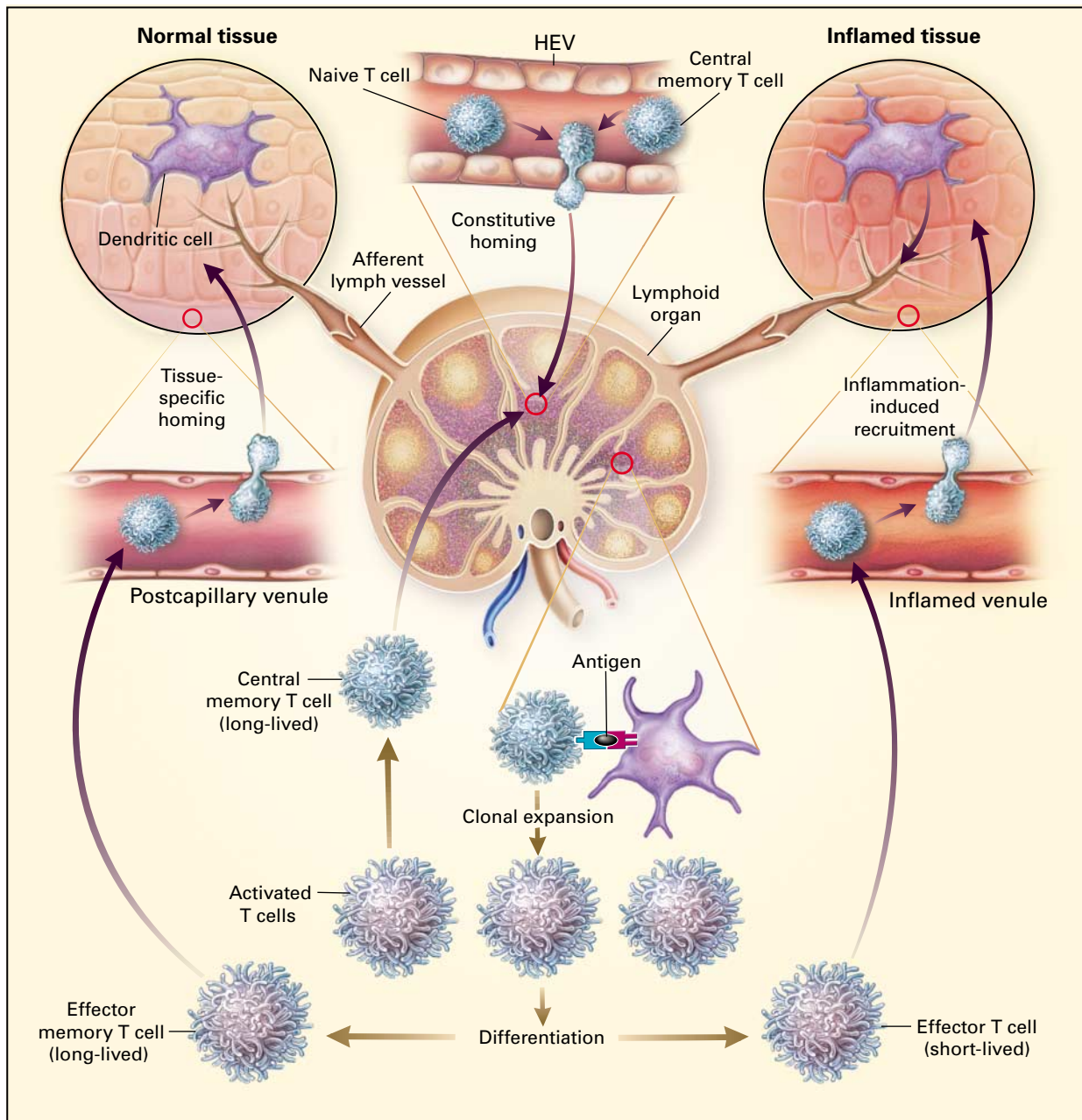
eral thousand at most). This poses a dilemma, which we can illustrate as follows. Visualize a balloon 150 m in diameter (about twice the vertical length of the Breitling Orbiter 3, which recently circumnavigated the globe). Its volume relative to Earth's roughly corresponds to that of a resting lymphocyte (approximately 125 femtoliters) in an adult (approximately 75 liters). Imagine now that a few thousand of these balloons must detect within hours tiny structures that arise suddenly anywhere within our planet and are recognizable only by direct contact. Clearly, an intricate guidance system must be at work to accomplish this feat.

In this article we will address the following questions: What enables T cells to find a rare foreign antigen rapidly in the body? How do some T cells proceed to eliminate pathogens, whereas others find pathogen-specific B cells, which need the help of T cells for efficient antibody responses? How do pathogens such as human immunodeficiency virus type 1 (HIV-1) exploit or subvert this seek-and-destroy system? What are the consequences of pathologically inefficient, excessive, or misguided migration of T cells? How can we exploit this knowledge for therapeutic or diagnostic purposes?

**THE CAREER OF A T CELL**

Initially, naive T cells must determine whether antigen is present and whether it poses a threat to the body. This information is provided by dendritic cells in secondary lymphoid organs, which collect and trap antigen produced elsewhere.<sup>8</sup> Naive T cells migrate preferentially to these lymphoid tissues, a process referred to as homing.<sup>9</sup> An encounter with an antigen induces the proliferation of T-cell clones, yielding approximately 1000 times more descendants with identical antigenic specificity. Eventually, these activated lymphocytes acquire effector functions and home to sites of inflammation, where they interact with antigen-bearing parenchymal cells and leukocytes such as eosinophils, mast cells, and basophils (in allergic reactions induced by type 2 helper T [Th2] cells) or macrophages and neutrophils (in inflammatory reactions mediated by type 1 helper T [Th1] cells). Other effector cells orchestrate humoral responses by contacting activated B cells in lymphoid organs. Most effector cells die after antigen is cleared, but a few antigen-experienced memory cells remain for long-term protection. Different subgroups of memory cells stand guard in lymphoid organs and patrol peripheral tissues to mount rapid responses whenever the antigen returns.<sup>3</sup>

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**Figure 1.** Migratory Routes of T Cells.

Naive T cells home continuously from the blood to lymph nodes and other secondary lymphoid tissues. Homing to lymph nodes occurs in high endothelial venules (HEV), which express molecules for the constitutive recruitment of lymphocytes. Lymph fluid percolates through the lymph nodes; the fluid is channeled to them from peripheral tissues, where dendritic cells collect antigenic material. In inflamed tissues, dendritic cells are mobilized to carry antigen to lymph nodes, where they stimulate antigen-specific T cells. On stimulation, T cells proliferate by clonal expansion and differentiate into effector cells, which express receptors that enable them to migrate to sites of inflammation. Although most effector cells are short-lived, a few antigen-experienced cells survive for a long time. These memory cells are subdivided into two populations on the basis of their migratory ability<sup>2</sup>: the so-called effector memory cells migrate to peripheral tissues, whereas central memory cells express a repertoire of homing molecules similar to that of naive T cells and migrate preferentially to lymphoid organs. The traffic signals that direct effector and memory cells to peripheral tissues are organ-specific (for example, molecules required for migration to the skin differ from those in the gut). They are modulated by inflammatory mediators, and they are distinct for different subgroups of T cells (for example, type 1 and type 2 helper T cells respond to different chemoattractants).

**TABLE 1. SELECTINS, INTEGRINS, AND THEIR LIGANDS.\***

ADHESION MOLECULE (ALTERNATIVE NAME)	DISTRIBUTION	REGULATION OF FUNCTION AND EXPRESSION
<b>Selectins</b>		
L-selectin (CD62L)	All leukocytes except effector and memory subgroups	All selectins are constitutively active Rapidly shed on activation
E-selectin (CD62E)	Endothelial cells	Expression induced by TNF- $\alpha$ , interleukin-1, and endotoxin
P-selectin (CD26P)	Endothelial cells, platelets	Stored intracellularly in resting cells; rapid surface translocation on activation by histamine, thrombin, or superoxide
<b>Selectin ligands</b>		
Sialyl-Lewis <sup>x</sup> (sCD15)	Myeloid cells, some memory (Th1) cells, high endothelial venules, other types of cells	Most relevant selectin ligands are sialylated, $\alpha$ 1,3-fucosylated O-glycans Expression in leukocytes depends on fucosyltransferase-VII
P-selectin glycoprotein ligand 1	All leukocytes	Glycosylation or tyrosine sulfation or both are essential for selectin binding
Peripheral-node addressin	High endothelial cells in lymph nodes and sites of chronic inflammation	Sulfated, sialyl-Lewis <sup>x</sup> -like sugar that is presented by 4 endothelial sialomucins: CD34, podocalixin, GlyCAM-1, and sgp200
Cutaneous lymphocyte antigen	Skin-homing T cells, dendritic cells, granulocytes, skin-tropic lymphomas	Unique glycoform of P-selectin glycoprotein ligand 1 on skin-homing memory T cells
<b><math>\beta_2</math> Integrins</b>		
$\alpha_1\beta_2$ (LFA-1, CD11aCD18)	All leukocytes	High-affinity binding is activation-dependent Enhanced expression on effector and memory cells
$\alpha_M\beta_2$ (Mac-1, CD11bCD18)	Myeloid cells, some activated T cells	Rapid up-regulation on activated myeloid cells
$\alpha_X\beta_2$ (p150,95, CD11cCD18)	Dendritic cells	Constitutive expression
$\alpha_D\beta_2$ (CD11dCD18)	Monocytes, macrophages, eosinophils	High levels of expression on foam cells in intimal plaques
<b><math>\alpha_4</math> Integrins</b>		
$\alpha_4\beta_1$ (VLA-4)	Most leukocytes except neutrophils	Function is regulated by activation signals Enhanced expression on effector and memory cells
$\alpha_4\beta_7$	Lymphocytes, natural killer cells, mast cells, basophils, monocytes	Enhanced expression on gut-homing effector and memory cells
<b>Immunoglobulin superfamily</b>		
ICAM-1 (CD54)	Most types of cells	Up-regulated by lipopolysaccharide and inflammatory cytokines
ICAM-2 (CD102)	Endothelial cells, platelets	Constitutive expression; no change in level of expression during inflammation
VCAM-1 (CD106)	Endothelial cells, bone marrow stroma, follicular dendritic cells, osteoblasts, mesothelium	Absent on most resting endothelial cells; induced by cytokines
Mucosal addressin-cell adhesion molecule 1	High endothelial venules in gut-associated lymphoid tissues and sites of chronic inflammation, lamina propria, spleen	Constitutive expression in high endothelial venules; induced in insulinitis, thymic hyperplasia, some forms of arthritis

**MICROVASCULAR DETERMINANTS OF T-CELL RECRUITMENT**

Specialized microvessels control the migration of T cells from blood into tissues. In most microvascular beds (except the spleen, lungs, and liver), post-capillary venules, but not arterioles or capillaries, interact efficiently with leukocytes, thus minimizing the effects of leukocyte adhesion on gas exchange in capillaries and on tissue perfusion, which is regulated by the arteriolar diameter.<sup>10</sup> Intravascular leukocytes are subjected to extreme physical conditions. Flowing

blood quickly dislodges cells that touch the vessel wall, because it exerts a shear stress of up to approximately 50 dyn per square centimeter. As an example, the jet d'eau fountain in Lake Geneva spouts 500 liters of water per second with a mean velocity of 200 km per hour, reaching a height of 140 m. Assuming that a cross-section of the water column is circular, the wall shear stress at the nozzle equals approximately 41.5 dyn per square centimeter.

Such extreme fluid dynamics require T cells to use adhesion receptors, which form stable bonds with

TABLE 1. CONTINUED.

ADHESION MOLECULE (ALTERNATIVE NAME)	LIGANDS AND COUNTERRECEPTORS	ROLE IN T-CELL MIGRATION
<b>Selectins</b>	All selectins bind sialyl-Lewis <sup>x</sup> -like sugars	
L-selectin (CD62L)	Peripheral-node addressin, P-selectin glycoprotein ligand 1, mucosal addressin-cell adhesion molecule type 1, E-selectin, others	Homing to lymph nodes and Peyer's patches
E-selectin (CD62E)	P-selectin glycoprotein ligand 1, ESL-1, cutaneous lymphocyte antigen, sialyl-Lewis <sup>x</sup> , glycoproteins and glycolipids	Homing of memory and effector cells to skin and sites of inflammation
P-selectin (CD26P)	P-selectin glycoprotein ligand 1, CD24, peripheral-node addressin	Homing of memory or effector (Th1) cells to sites of inflammation; platelet-mediated interaction with venules that express peripheral-node addressin
<b>Selectin ligands</b>		
Sialyl-Lewis <sup>x</sup> (sCD15)	All selectins	Function depends on presentation molecule
P-selectin glycoprotein ligand 1	Essential ligand for P-selectin; also binds L- and E-selectin	Homing of memory and effector (Th1) cells to inflamed tissue; binding to activated platelets
Peripheral-node addressin	L-selectin and P-selectin on activated platelets	Homing of naive T cells and central memory cells to lymph nodes
Cutaneous lymphocyte antigen	E-selectin	Homing of memory and effector cells to inflamed skin
<b><math>\beta_2</math> Integrins</b>		
$\alpha_1\beta_2$ (LFA-1, CD11aCD18)	ICAM-1, 2, 3, 4 and 5	Homing of all lymphocytes to lymph nodes, Peyer's patches, and most sites of inflammation; adhesion to antigen-presenting cells
$\alpha_M\beta_2$ (Mac-1, CD11bCD18)	ICAM-1, factor X, fibrinogen, C3b	Unknown
$\alpha_X\beta_2$ (p150,95, CD11cCD18)	Fibrinogen, C3b	Unknown
$\alpha_D\beta_2$ (CD11dCD18)	VCAM-1, ICAM-1 and 3	Unknown
<b><math>\alpha_4</math> Integrins</b>		
$\alpha_4\beta_1$ (VLA-4)	VCAM-1, fibronectin, $\alpha_4$ integrin	Homing of memory and effector cells to inflamed tissues, especially lung
$\alpha_4\beta_7$	Mucosal addressin-cell adhesion molecule 1, fibronectin, weak binding to VCAM-1	Homing of all lymphocytes to gut and associated lymphoid tissues
<b>Immunoglobulin superfamily</b>		
ICAM-1 (CD54)	$\alpha_1\beta_2$ integrin, $\alpha_M\beta_2$ integrin, fibrinogen	Critical endothelial ligand for $\beta_2$ integrins
ICAM-2 (CD102)	$\alpha_1\beta_2$ integrin	Unknown
VCAM-1 (CD106)	$\alpha_4\beta_1$ , $\alpha_4\beta_7$ , and $\alpha_D\beta_2$ integrin	Homing of memory and effector cells to inflamed tissue
Mucosal addressin-cell adhesion molecule 1	$\alpha_4\beta_7$ integrin, L-selectin	Homing of all lymphocytes to gut and associated lymphoid tissues

\*Integrins are named according to the composition of their constituent  $\alpha$  and  $\beta$  protein chains, which are each identified by a number or letter (e.g.,  $\alpha_4\beta_1$  and  $\alpha_D\beta_2$ ). Some integrins are frequently referred to by alternative names such as leukocyte function-associated antigen 1 (LFA-1) in the case of  $\alpha_1\beta_2$  integrin; the alternative names are given in parentheses. TNF- $\alpha$  denotes tumor necrosis factor  $\alpha$ , ESL-1 E-selectin ligand-1, Th1 type 1 helper T cells, GlyCAM-1 glycosylation-dependent cell-adhesion molecule 1, sgp200 sialylated glycoprotein of 200 kd, Mac-1 macrophage antigen 1, p150,95 protein with 150-kd and 95-kd subunits, ICAM intercellular adhesion molecule, VCAM-1 vascular-cell adhesion molecule 1, and VLA-4 very late antigen-4.

counterreceptors in the vascular wall (Table 1).<sup>11,12</sup> Not only are adhesion receptors mechanical anchors, but also many function as tissue-specific recognition molecules. For example, the specialized endothelial cells that line the high endothelial venules in lymph nodes and Peyer's patches constitutively express so-called addressins, which support the homing of naive lymphocytes, whereas endothelial cells elsewhere permit little or no leukocyte binding unless they are exposed to inflammatory mediators. Thus, we distinguish two venular beds: those in lymphoid organs that

recruit lymphocytes as a daily routine, and those that solicit the entry of leukocytes only when faced with danger signals.

#### ADHESION MOLECULES

Leukocytes must engage several sequential adhesion pathways to leave the circulation (Fig. 2). Initially, tethers are formed by adhesion receptors that are specialized to engage rapidly and with high tensile strength. The most important initiators of adhesion are the three selectins expressed on leukocytes

(L-selectin), endothelial cells (P- and E-selectin), and activated platelets (P-selectin).<sup>14</sup> All selectins bind oligosaccharides related to sialyl-Lewis<sup>x</sup>. The most relevant selectin-binding sugars are components of sialomucin-like glycoproteins.<sup>15</sup>

Selectin-mediated bonds are too impermanent to arrest cells at the vessel wall. As the flowing blood exerts pressure, adhesion bonds dissociate at the cell's upstream end and new bonds form downstream. This results in a rolling motion that is much slower than that of free-flowing cells. To stop rolling, cells must engage additional (secondary) receptors.<sup>16,17</sup> All secondary adhesion molecules belong to the integrin family, specifically leukocyte function–associated antigen type 1 ([LFA-1], also referred to as CD11aCD18 and  $\alpha_1\beta_2$  integrin) and the two  $\alpha_4$  integrins,  $\alpha_4\beta_1$  (also referred to as very late antigen 4, or VLA-4) and  $\alpha_4\beta_7$ . The  $\alpha_4$  integrins can also mediate tethering and rolling, albeit less efficiently than selectins.<sup>18,19</sup>

### CHEMOKINES

Whereas selectins are constitutively active, integrins must be activated to mediate adhesion. Rolling T cells activate integrins when they receive signals from chemokines on endothelial surfaces.<sup>20,21</sup> Chemokines are secreted polypeptides that bind to specific surface receptors, which transmit signals through G proteins (Table 2). Like adhesion molecules, chemokine receptors can be up-regulated or lost as cells differentiate, allowing leukocytes to coordinate their migratory routes with their immunologic function.

Some chemokines trigger intravascular adhesion,<sup>23</sup> whereas others direct the migration of leukocytes into and within the extravascular space. After a cell secretes

them, chemokines bind to heparin-like glycosaminoglycans on cell surfaces and in the extracellular matrix; leukocytes can track down these immobilized chemokines (a process called haptotaxis), which may persist at high concentrations in tissues longer than do freely diffusible chemoattractants. Since lymphocytes must be positioned correctly to interact with other cells, the pattern of chemokine receptors and the type and distribution of chemokines in tissues critically influence immune responses.<sup>20,21,24,25</sup>

More than 50 chemokines and 18 chemokine receptors have been identified.<sup>20,22</sup> Such a large number may be needed to ensure recruitment of inflammatory cells even if individual pathways are disabled by genetic defects or pathogens.<sup>26</sup> Also, the large number of chemokines may direct different types of cells to the anatomically distinct microenvironments that they need to function properly. For example, neutrophils sequentially use different chemoattractant receptors to follow various chemotactic gradients, a process termed “multistep navigation.”<sup>27</sup>

Chemokines are divided into four subfamilies on the basis of the position of a pair of cysteine residues. In the CXC ( $\alpha$  chemokine) subfamily, two cysteines (C) are separated by another amino acid (X). The various subtypes of their receptors are referred to by a number (e.g., CXCR1 and CXCR2). The second subfamily is the CC ( $\beta$ ) chemokines, which have two adjacent cysteines and receptors called CCRs. Each of the other two subfamilies, CX<sub>3</sub>C and XC, has a single receptor (CX<sub>3</sub>CR1 and XCR1, respectively).

Many of the chemokines were identified simultaneously by several groups and thus have been given up to four different names. A more systematic clas-

**Figure 2 (facing page).** Essential Molecular Players in the Multistep Adhesion Cascade.

The four distinct steps in adhesion that leukocytes must undergo to accumulate in a blood vessel are shown at the top of the diagram. Also shown are the predominant molecular determinants of each step with respect to leukocytes (middle of the diagram) and endothelial cells (bottom of the diagram). The arrows in the middle portion indicate the various interactions possible between molecules.

Leukocytes in the bloodstream (the graduated set of arrows at the top of the diagram symbolize the laminar flow profile in blood vessels, where the velocity of blood is fastest in the center and approaches zero at the vessel wall) become tethered to endothelial cells and roll slowly downstream. Tethering is greatly facilitated by leukocyte receptors that occur at high density on the tips of microvillous surface protrusions (L-selectin, P-selectin glycoprotein ligand 1 [PSGL-1], and  $\alpha_4$  integrins), whereas subsequent rolling is not influenced by the topography of adhesion receptors.<sup>13</sup> The most efficient tethering molecules are L-selectin and P-selectin. L-selectin recognizes sulfated sialyl-Lewis<sup>x</sup> (sLe<sup>x</sup>)-like sugars, called peripheral-node addressin (PNAd), in high endothelial venules. L-selectin also interacts with other ligands on inflamed endothelial cells (not shown) and with PSGL-1 on adherent leukocytes (indicated by the dashed arrow). The binding of PSGL-1 to L-selectin and P-selectin requires an sLe<sup>x</sup>-like sugar to be close to an N-terminal motif containing three tyrosines (Y) that must be sulfated. E-selectin can also interact with PSGL-1, but it does not require tyrosine sulfation. E-selectin also recognizes other sLe<sup>x</sup>-bearing glycoconjugates. E-selectin and the  $\alpha_4$  integrins can tether some leukocytes, but their predominant function is to reduce the velocity of rolling.

Rolling leukocytes respond to chemoattractants on endothelial cells because they express specific receptors with seven transmembrane domains (7 TMR), which transmit intracellular signals through G proteins. The most prominent chemoattractants that bind to 7 TMR are listed in the figure; some of these molecules, such as chemokines, are presented on the endothelial surface; others are secreted or diffuse freely into the vessel lumen. The activating signal induces rapid activation of  $\beta_2$  integrins,  $\alpha_4$  integrins, or both, which bind to members of the endothelial immunoglobulin superfamily. The  $\alpha_4$  integrins can mediate activation-independent rolling interactions as well as arrest rolling leukocytes. However, the latter function requires the activation of  $\alpha_4$  integrins (illustrated by the more open conformation of the integrin heterodimer, as compared with that of  $\alpha_4$  integrins that mediate rolling). C5a denotes activated C5, PAF platelet-activating factor, LTB<sub>4</sub> leukotriene B<sub>4</sub>, MAdCAM-1 mucosal addressin-cell adhesion molecule type 1, VCAM-1 vascular-cell adhesion molecule 1, ICAM-1 intercellular adhesion molecule 1, and ICAM-2 intercellular adhesion molecule 2.

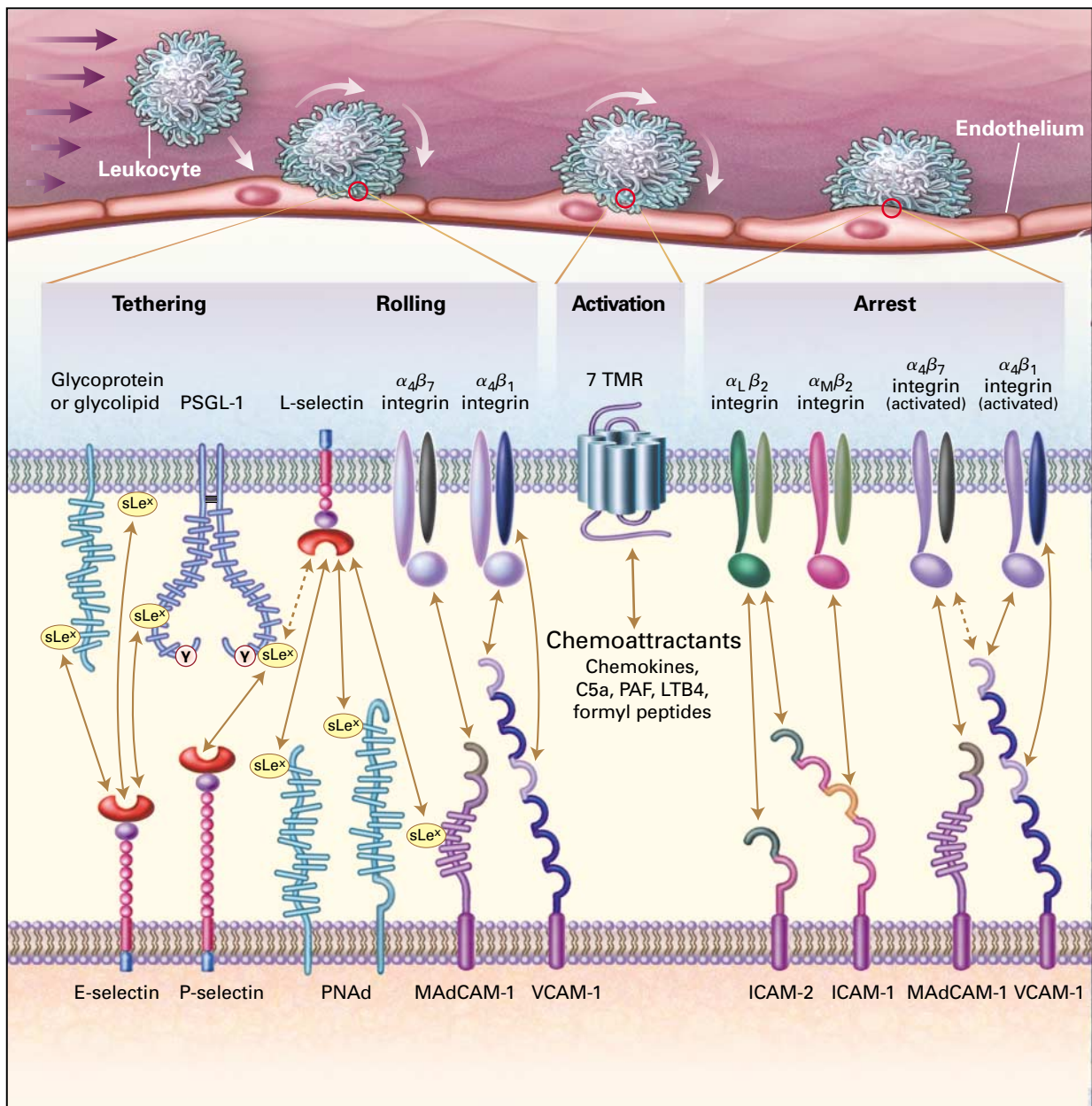
sification was recently proposed in which chemokines are designated according to their subfamily, followed by the letter L (for ligand) and a number corresponding to that of their respective gene.<sup>22</sup>

Chemokines are also classified as inflammatory or lymphoid. Inflammatory chemokines primarily attract neutrophils, monocytes, and other innate immune cells. Their major sources are activated endothelial cells, epithelial cells, and leukocytes, but virtually any cell can generate chemokines when stimulated by lipopolysaccharides (endotoxin) or inflammatory cytokines. Lymphoid chemokines are primarily pro-

duced in lymphoid tissues. They maintain the constitutive activity and compartmentalization of leukocytes in these organs.<sup>20,21,28</sup>

**MULTISTEP ADHESION CASCADES**

As we have seen, homing of leukocytes involves at least three consecutive steps: tethering and rolling mediated by primary adhesion molecules, exposure to a chemotactic stimulus provided by chemokines and G-protein-coupled receptors, and arrest mediated by activated integrins. Each of these steps is necessary for lymphocytes to enter lymphoid tissues (except the



**TABLE 2. ROLE OF CHEMOKINE RECEPTORS AND THEIR LIGANDS IN THE MIGRATION OF T CELLS.\***

BIOLOGIC ACTIVITY†	CHEMOKINE RECEPTORS	PREDOMINANT CHEMOKINE AGONISTS‡
Migration of naive T cells to lymph nodes and Peyer's patches	CCR7	SLC (also called TCA-4, 6C-kine, exodus-2), ELC (also called MIP-3β)
Migration of naive T cells within lymphoid tissues	CXCR4	SDF-1α
Migration of memory T cells to lymphoid tissues	CCR7	SLC (also called TCA-4, 6C-kine, exodus-2), ELC (also called MIP-3β)
Migration of memory T cells to the skin	CCR4	TARC, MDC-1
Migration of memory T cells to the gut	CCR9	TECK
Migration of memory T cells to sites of inflammation	CCR2 CCR5	MCP-1, 3, and 4 RANTES, MIP-1α and 1β
Migration of effector T cells (Th1)	CCR2 CCR5 CXCR3	MCP-1, 3, and 4 RANTES, MIP-1α and 1β IP-10, Mig, I-TAC
Migration of effector T cells (Th2)	CCR3 CCR4 CCR8 CXCR4	Eotaxin-1, 2, and 3; RANTES; MCP-2, 3, and 4; HCC-2 TARC, MDC-1 I-309 SDF-1α
Migration of B cells	CCR7 CXCR4 CXCR5	SLC (also called TCA-4, 6C-kine, exodus-2), ELC (also called MIP-3β) SDF-1α BLC (also called BCA-1)
Migration of dendritic cells to lymphoid tissues	CCR7	SLC (also called TCA-4, 6C-kine, exodus-2), ELC (also called MIP-3β)
Migration of dendritic cells to normal skin	CCR6	MIP-3α (also called LARC, exodus-1)
Migration of dendritic cells to sites of inflammation	CCR1 CCR2 CCR5 CXCR1	RANTES; MIP-1α; MCP-3; HCC-1, 2, and 4; MPIF-1 MCP-1, 3, and 4 RANTES, MIP-1α and 1β Interleukin-8, GCP-2
Recruitment of monocytes	CCR1 CCR2 CCR5 CCR8 CXCR1 CX <sub>3</sub> CR1	RANTES; MIP-1α; MCP-3; HCC-1, 2, and 4; MPIF-1 MCP-1, 3, and 4 RANTES, MIP-1α and 1β I-309 Interleukin-8, GCP-2 Fraktalkine (also called neurotactin)
Recruitment of neutrophils	CXCR1 CXCR2	Interleukin-8, GCP-2 Interleukin-8, Groα, β, and γ; Nap-2; GCP-2; ENA-78
Recruitment of eosinophils	CCR3	Eotaxin-1, 2 and 3; RANTES; MCP-2, 3, and 4; HCC-2
Migration of hematopoietic progenitor cells and B-cell development	CXCR4	SDF-1α
Function unknown	XCR1 CCX CKR GPR-2 D6	Lymphotactin, SCM-1β SLC (also called TCA-4, 6C-kine, exodus-2), ELC (also called MIP-3β), TECK CTACK (also called ILC) Multiple CC chemokines

\*CCR denotes receptor for CC chemokine, CXCR receptor for CXC chemokine, SLC secondary lymphoid-tissue chemokine, TCA-4 thymus-derived chemotactic agent 4, ELC Epstein-Barr virus-induced gene 1 ligand chemokine, MIP macrophage inflammatory protein, SDF-1α stroma-derived factor 1α, TARC thymus- and activation-regulated chemokine, MDC-1 macrophage-derived chemokine 1, TECK thymus-expressed chemokine, MCP monocyte chemotactic protein, RANTES regulated on activation normal T cell expressed and secreted, Th1 type 1 helper T cells, Th2 type 2 helper T cells, IP-10 inducible protein of 10 kd, Mig monokine induced by interferon-γ, I-TAC interferon-inducible T cell alpha chemoattractant, HCC human CC chemokine, BLC B-lymphocyte chemoattractant, BCA-1 B-cell-attracting chemokine 1, LARC liver- and activation-regulated chemokine, MPIF-1 myeloid progenitor inhibitory factor 1, GCP-2 granulocyte chemotactic protein 2, Gro growth-related activity, Nap-2 neutrophil-activating protein 2, ENA-78 epithelial-cell-derived neutrophil attractant 78, SCM-1β single C motif 1β, GPR-2 G-protein-coupled receptor 2, CTACK cutaneous T cell-attracting chemokine, and ILC interleukin-11 receptor alpha-locus chemokine.

†The physiologic relevance of several chemokine receptors varies. For instance, CCR9 functions in the homing of prothymocytes to the thymus and in the migration of T cells to the gut. CXCR4 is widely expressed and appears to have multiple roles.

‡The most common names that are currently in use for human chemokines are given here, with frequently used alternative names shown in parentheses. Recently, a more systematic classification for chemokines has been proposed that is based on the nomenclature for the corresponding chemokine genes.<sup>22</sup>

spleen) and for the accumulation of leukocytes at sites of inflammation.<sup>29-39</sup> In leukocyte adhesion deficiency syndrome, a genetic defect either in  $\beta_2$  integrins (type 1) or in fucosylated selectin ligands (type 2), neutrophils cannot stop or roll, respectively; this syndrome is characterized by marked leukocytosis and frequent soft-tissue infections.<sup>40,41</sup> The pronounced lymphocytosis in patients with *Bordetella pertussis* infection<sup>42</sup> is probably caused by pertussis toxin, which inactivates the signaling of G proteins and blocks chemokine-mediated activation of integrins. Hence, lymphocytes cannot stop rolling, and they remain in the circulation.<sup>43,44</sup>

The large number of leukocyte-adhesion receptors, endothelial counterreceptors, chemokines, and chemokine receptors means that there are hundreds of possible three-step combinations. These have been likened to the area codes in U.S. telephone numbers.<sup>11,45</sup> Indeed, several multistep combinations occur only in specialized tissues, where only a distinct subgroup of blood-borne cells can participate in every step.<sup>9,11,38,39,46</sup> Endothelial adhesion molecules with a dominant role in tissue-specific migration are often called "vascular addressins"; their counterreceptors on lymphocytes are called "homing receptors."<sup>9,11</sup>

#### HOMING OF NAIVE T CELLS TO LYMPHOID TISSUES

The best understood adhesion cascades mediate homing of naive T cells to lymph nodes and Peyer's patches.<sup>38,39,44,47</sup> Circulating lymphocytes gain access to both organs in specialized high endothelial venules.<sup>2,48</sup> A characteristic feature of high endothelial venules in lymph nodes is the peripheral-node addressin, whereas high endothelial venules in Peyer's patches express mucosal addressin-cell adhesion molecule 1 (Fig. 3). L-selectin binds both these addressins, but it maintains rolling only on peripheral-node addressin in lymph nodes, whereas in Peyer's patches, binding of  $\alpha_4\beta_7$  integrin to mucosal addressin-cell adhesion molecule 1 is also required.<sup>44,47</sup> Cells expressing high levels of  $\alpha_4\beta_7$  integrin (such as gut-homing effector cells) attach directly to mucosal addressin-cell adhesion molecule 1, whereas naive T cells first engage L-selectin.<sup>47,51</sup>

Some high endothelial venules in mesenteric lymph nodes express only peripheral-node addressin or mucosal addressin-cell adhesion molecule 1, whereas others express both addressins. These tissue-specific differences explain why a genetic deficiency in L-selectin or fucosyltransferase VII (an enzyme required for the synthesis of selectin ligands, including peripheral-node addressin) severely impairs the homing of lymphocytes to peripheral lymph nodes, whereas it has only a moderate effect on homing to mesenteric lymph nodes and little effect on homing to Peyer's patches.<sup>32,52</sup> Conversely, loss of  $\beta_7$  integrins abrogates hom-

ing to Peyer's patches and attenuates homing to mesenteric lymph nodes but does not affect migration to peripheral lymph nodes.<sup>34</sup>

The spleen lacks high endothelial venules, and no adhesion pathway appears to be essential for homing to that organ. However, chemokines are needed for lymphocytes and dendritic cells to navigate within the spleen; genetic defects in the chemokine receptor CXCR5 or CCR7 severely disrupt the normal splenic architecture.<sup>37,53</sup>

#### MIGRATION OF DENDRITIC CELLS

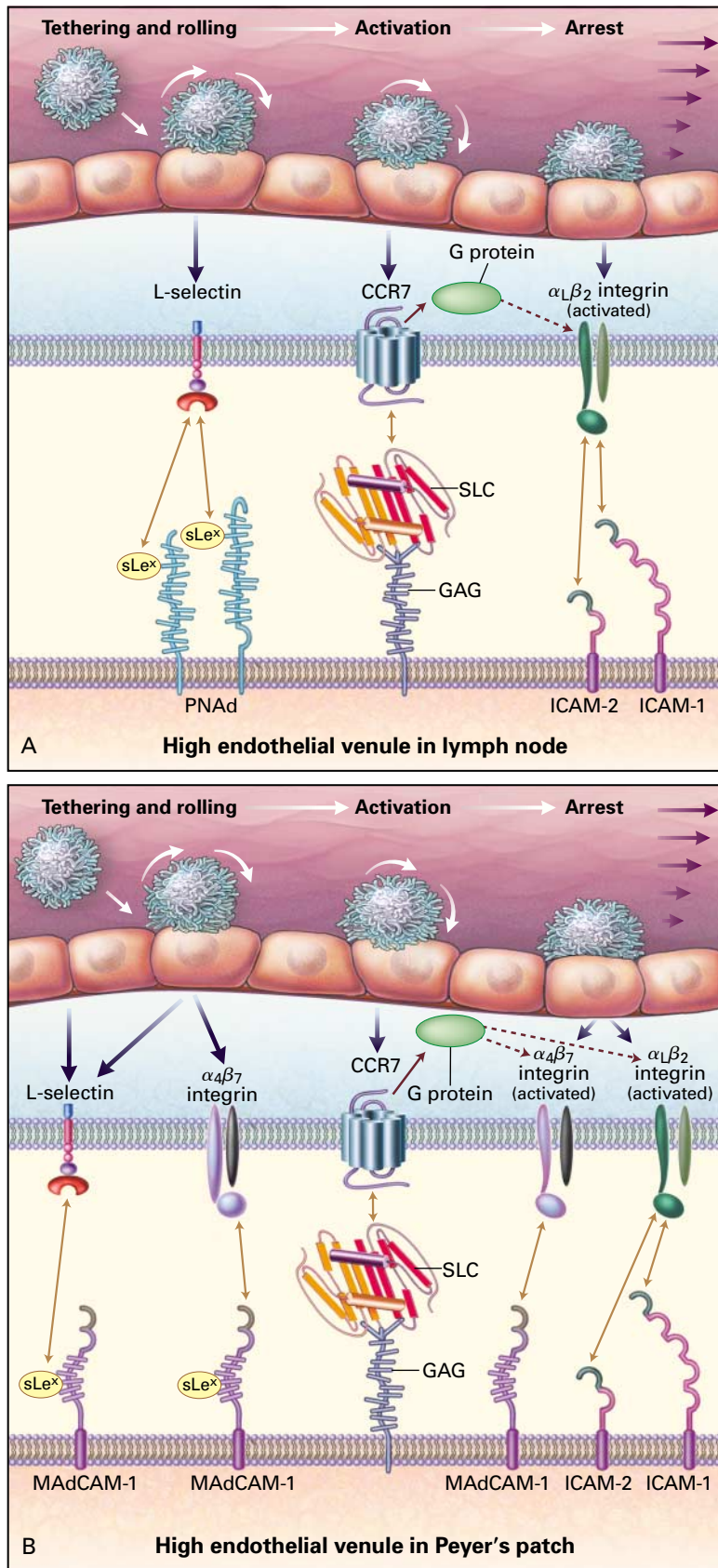
Homing to any lymphoid organs remains inconsequential unless lymphocytes encounter antigen in the appropriate context. Dendritic cells are critical in this regard.<sup>8</sup> Subgroups of monocytes are thought to home to tissues and differentiate into so-called immature dendritic cells. Both types of cells express receptors for inflammatory chemokines and other chemoattractants that are released during infections. This ability enables them to enter and migrate through inflamed tissues.<sup>54,55</sup> A subgroup of dendritic cells in skin, the Langerhans' cells, also express CCR6, which may promote their migration to normal skin, where there is a CCR6 agonist called macrophage inflammatory protein 3 $\alpha$ .<sup>56</sup>

Immature dendritic cells patrol tissues and engulf microorganisms, dead cells, and cellular debris. On exposure to inflammatory stimuli, they travel to local lymph nodes through afferent lymph vessels, undergo further maturation, lose their receptors for inflammatory chemokines, and up-regulate the expression of receptors for lymphoid chemokines.<sup>57</sup> These changes allow maturing dendritic cells to find their way to the T-cell area of lymph nodes. While in transit, dendritic cells also ready their apparatus for antigen presentation and begin to produce chemokines that make them attractive to T cells awaiting their arrival in lymph nodes.

#### ACTIVATION OF T CELLS AND COOPERATION BETWEEN T CELLS AND B CELLS

Antigenic stimulation in lymph nodes causes antigen-primed T and B cells to move in a synchronous fashion toward each other, meeting at the edges of B-cell follicles.<sup>58</sup> These movements are orchestrated by dynamic changes in the expression of chemokine receptors. When CD4+ T cells are stimulated by antigen, they up-regulate the expression of two receptors (CXCR5 and CCR4) for chemokines produced in B-cell follicles; Th2 cells lose the ability to express CCR7 because chemokines that stimulate this receptor might otherwise keep them in the T-cell area. Conversely, antigen-stimulated B cells become responsive to macrophage inflammatory protein 3 $\beta$ , which drives them toward the T-cell area.<sup>21,28,59</sup>





## MIGRATION OF EFFECTOR T CELLS

During primary responses, T cells differentiate into effector cells in lymphoid organs (Fig. 1). They must immediately home to peripheral tissues that contain pathogens, which elicit local inflammation by stimulating innate immune cells. Thus, effector cells up-regulate the expression of receptors for inflammation-induced endothelial adhesion molecules and inflammatory chemokines.<sup>9</sup> However, different pathogens elicit different effector responses mediated by either Th1 or Th2 cells. Since these two subgroups can suppress each other, they may require physical separation for maximal responses. Indeed, Th1 and Th2 cells express distinct receptors and obey different traffic signals.<sup>24</sup>

Distinctive chemokine receptors on Th1 cells include CCR5 and CXCR3,<sup>60,61</sup> which bind inflammatory chemokines. In patients with rheumatoid arthritis and multiple sclerosis (two Th1-related diseases), virtually all infiltrating T cells express CCR5 and CXCR3.<sup>62</sup> People with a homozygous mutation that disrupts the *CCR5* gene<sup>63</sup> may also be less susceptible to some inflammatory disorders, including rheumatoid arthritis.<sup>64</sup> Adhesion molecules also have a role; Th1 cells express selectin ligands abundantly. P- and E-selectin, which occur on inflamed endothelium, and their ligand, P-selectin glycoprotein ligand 1, are critical for the migration of Th1 cells to inflamed skin<sup>65,66</sup> and peritoneum.<sup>67</sup> The expression of fucosyltransferase VII is necessary for cells to synthesize selectin ligands.<sup>52</sup> This enzyme is induced by interleukin-12, which drives the differentiation of Th1 cells, whereas exposure of T cells to the Th2-governed cytokine interleukin-4 decreases the expression of selectin ligands.<sup>68,69</sup>

A characteristic chemokine receptor on Th2 cells is CCR3, the eotaxin receptor.<sup>70</sup> Eotaxin is involved in

the recruitment of eosinophils into hyperreactive airways and is prominent in mucosal tissues where allergic and antiparasitic responses are occurring.<sup>71</sup> The production of eotaxin is stimulated by cytokines secreted by Th2 cells such as interleukin-4 and interleukin-13. It is absent from Th1-mediated lesions.<sup>72</sup> CCR3 is also expressed on eosinophils, basophils, and mast cells. This pattern of expression presumably allows these allergy-related leukocytes to colocalize and interact at sites of eotaxin production.<sup>73</sup> Other chemoattractant receptors are also preferentially (but not exclusively) expressed on Th2 cells, including CCR4, CCR8, and CXCR4.<sup>74</sup>

The traffic signals that direct CD8+ effector cells to inflamed tissues have not been studied as extensively as those for the CD4+ subgroup, but they appear to be similar.<sup>67,75</sup> When stimulated by antigen, cytotoxic T cells secrete inflammatory chemokines.<sup>76</sup> Through this mechanism, CD8+ T cells are thought to increase the recruitment of neutrophils, monocytes, and Th1 cells.<sup>77</sup>

## HOMING TO NONLYMPHOID TISSUES

Antigen-experienced T cells often display tissue specificity that may improve their chances of reencountering antigen. T cells that have been exposed to cutaneous pathogens in skin-draining lymph nodes migrate preferentially to the skin, whereas effector cells that arise in Peyer's patches in response to enteroviral infections are most useful in the gut.<sup>9</sup> Indeed, lymphocytes express different homing receptors after stimulation by the same antigen, depending on whether it is given orally or parenterally.<sup>78</sup> The best-understood tissue-selective homing pathways are in the skin and intestine,<sup>9,46</sup> but there may be other selective migration streams, such as to the lungs, joints, and central nervous system.<sup>79</sup>

**Figure 3 (facing page).** Homing Cascades That Direct Naive T Cells to Lymph Nodes (Panel A) and Peyer's Patches (Panel B).

In Panel A, the tethering and rolling of lymphocytes in high endothelial venules of lymph nodes are mediated by the binding of L-selectin to peripheral-node addressin (PNAd), a group of endothelial sialomucins — CD34, podocalyxin, glycosylation-dependent cell-adhesion molecule 1 (GlyCAM-1), and sialylated glycoprotein of 200 kd (sgp200) — all of which include a sulfated sialyl-Lewis<sup>x</sup> (sLe<sup>x</sup>)—like motif. High endothelial venules synthesize a large amount of secondary lymphoid-tissue chemokine (SLC), which is presented on the luminal surface, presumably as a result of noncovalent binding to a glycosaminoglycan (GAG).<sup>38,49</sup> Exposure of chemokine receptor 7 (CCR7) on rolling T cells to secondary lymphoid-tissue chemokine precipitates a signaling cascade that is initiated by the dissociation of heterotrimeric G proteins ( $\alpha$ ,  $\beta$ , and  $\gamma$ ). Activation-induced release of the  $\alpha$  subunit of the G protein enables the  $\beta$ - $\gamma$  complex to initiate biochemical events that activate the  $\beta_2$  integrin  $\alpha_L\beta_2$ .<sup>44</sup> Activated  $\alpha_L\beta_2$  integrin binds with high affinity to intercellular adhesion molecule 1 (ICAM-1) and intercellular adhesion molecule 2 (ICAM-2), thus stopping the cell from rolling.

In Panel B, L-selectin is also responsible for most tethering events in Peyer's patches. High endothelial venules in these organs express mucosal addressin-cell adhesion molecule 1 (MAdCAM-1), which possesses two distal immunoglobulin domains and a membrane-proximal mucin domain that contains a binding site for L-selectin.<sup>50</sup> Once the T cell is tethered,  $\alpha_4\beta_7$  integrin must interact with mucosal addressin-cell adhesion molecule 1 in order to initiate efficient rolling.<sup>47</sup> Analogous to peripheral lymph nodes, chemotactic stimulation of rolling T cells in Peyer's patches relies on the pathway involving CC chemokine receptor 7,<sup>39</sup> which activates  $\alpha_4\beta_7$  integrin and  $\alpha_L\beta_2$  integrin.  $\alpha_L\beta_2$  Integrin must be activated for naive T cells to home to Peyer's patches, whereas gut-homing lymphoblasts or effector cells, which express large numbers of  $\alpha_4\beta_7$  integrin on their surface, can home to venules with high levels of expression of mucosal addressin-cell adhesion molecule 1 (such as inflamed gut) without contributions from other adhesion pathways.<sup>47</sup> Homing of B cells in high endothelial venules of lymph nodes and Peyer's patches involves the same adhesion molecules but requires an unknown chemoattractant that is distinct from secondary lymphoid-tissue chemokine.

### IMMUNE SURVEILLANCE BY MEMORY T CELLS

Memory T cells can be divided into CCR7+ and CCR7- subgroups.<sup>3</sup> Most CCR7+ T cells express L-selectin, suggesting that they home to lymph nodes. Conversely, CCR7- T cells do not express L-selectin but do express homing receptors for peripheral nonlymphoid tissues and display characteristic features of effector T cells on stimulation.<sup>3</sup> In contrast, CCR7+ T cells serve as precursors of the CCR7- subgroup. They have been referred to as "central memory cells," and the CCR7- population has been referred to as "effector memory cells."<sup>3</sup> It is likely that both subgroups share the burden of providing immunologic memory. Central memory cells stand guard in lymphoid tissues, strategically positioned to orchestrate a rapid and vigorous immune response should an antigen return, whereas effector memory cells patrol peripheral organs. From there, they may also reach local lymph nodes through afferent lymph vessels.<sup>80</sup> Although this job-sharing concept of memory subgroups is intriguing, several aspects have yet to be proved experimentally.

### VIRAL ASSAULT ON HOMING MOLECULES

One of the earliest studies of the interplay of viruses with homing molecules showed that rhinoviruses, which cause the common cold, bind to intercellular adhesion molecule 1 when they infect mucosal epithelial cells.<sup>81</sup> Recently, much attention has been devoted to viral interactions with the chemokine system. Dramatic examples are CCR5 and CXCR4, which are essential coreceptors for the entry of HIV-1 into cells.<sup>82-87</sup> These two receptors are expressed on reciprocal populations of CD4+ T cells: CCR5+ Th1 effector cells migrate through peripheral tissues, whereas CXCR4+ naive T cells recirculate through lymphoid organs.<sup>88</sup> Macrophage-tropic strains of HIV-1, which require CCR5 to enter CD4+ T cells, are predominantly transmitted through sexual intercourse or contact with blood. T-cell-tropic viral strains, which use CXCR4, arise during later stages of the infection.<sup>89,90</sup>

Macrophage-tropic HIV-1 probably evolved because CCR5 is abundant on CD4+ T cells at the most common sites of viral transmission, the mucosa of the intestinal and genital tracts. Infected cells that return to the bloodstream serve inadvertently as "Trojan horses," spreading the virus to lymphoid organs and throughout the body. Moreover, by concentrating its initial attack on CCR5+ T cells, HIV-1 targets Th1 cells, which are necessary for antiviral responses. Once the Th1 reservoir is exhausted, the virus is free to redirect its attack toward CXCR4+ naive T cells. Inhibitors of HIV-1-binding to CCR5 and CXCR4 hold promise for treating or preventing HIV-1 infections. This notion is supported by the finding that people carrying a defective CCR5 gene (CCR5 $\Delta$ 32) have

increased protection against infection with macrophage-tropic HIV-1 and, once infected, have a slower rate of disease progression.<sup>63</sup>

Many other viruses subvert immune responses by inhibiting the recruitment of leukocytes.<sup>77</sup> Herpesviruses and poxviruses contain several genes for chemokine and chemokine-receptor-like proteins.<sup>91</sup> The best-studied viral chemokines are viral macrophage inflammatory protein (vMIP) I and II encoded by the Kaposi's sarcoma-associated human herpesvirus 8.<sup>92,93</sup> Viral macrophage inflammatory protein II binds numerous CC and CXC chemokine receptors. It antagonizes Th1-associated receptors and stimulates Th2 receptors. Thus, the virus stunts antiviral Th1 responses, especially in HIV-1-infected patients, in whom Th1 responses by CCR5+ T cells are weakened. Other viral proteins compete with chemokine receptors. For example, the poxvirus-derived viral CC chemokine inhibitor binds virtually all known CC chemokines with high affinity.<sup>91</sup> Drugs that inhibit or mimic these viral molecules could be of potential therapeutic benefit.

### CLINICAL APPLICATIONS

Since chemokine receptors and adhesion molecules are promising targets for new antiinflammatory therapies,<sup>12,46,77,90,94-103</sup> the development of antagonists is among the most actively pursued areas in pharmaceutical research (Table 3). Several landmark studies are worth mentioning. Two studies reported the profound effect of antagonists of Th2 chemokines and  $\alpha_4\beta_1$  integrins in animal models of asthma.<sup>104,105</sup> Antibodies to  $\alpha_4$  integrins also block the development of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis.<sup>106</sup> Numerous antibodies, recombinant soluble adhesion molecules, receptor-blocking mutant chemokines, and small molecules are being evaluated as treatments for asthma, multiple sclerosis, inflammatory bowel disease, arthritis, and psoriasis, and some should work. Small molecules are the drug of choice for commercial development, and there are already several potent small-molecule antagonists of chemokine receptors.<sup>107,108</sup> Interactions involving integrins or selectins are more difficult to inhibit with small molecules, but there have been successes with antagonists of interactions between  $\alpha_4\beta_1$  integrin and vascular-cell adhesion molecule 1 and between LFA-1 and intercellular adhesion molecule 1.<sup>105,109</sup>

These advances should enable clinicians to choose between highly selective treatments. For example, by modulating essential elements of the homing of naive T cells or dendritic cells, clinicians might prevent, attenuate, or enhance immune responses to new antigens, such as allografts or vaccines. Because this treatment would not interfere with the responses of memory T cells, it should not be globally immunosuppressive. Similarly, new drugs that inhibit organ-

TABLE 3. CLINICALLY RELEVANT ADHESION PATHWAYS AND CHEMOKINE OR CHEMOKINE RECEPTORS.\*

TARGET PATHWAY	ROLE IN T-CELL MIGRATION	POTENTIAL CLINICAL APPLICATIONS	COMMENTS
<b>Adhesion pathways</b>			
Interactions between $\alpha_4\beta_1$ integrin and VCAM-1	Homing to numerous types of inflamed tissues and Th2-mediated lesions	Asthma, multiple sclerosis, vasculitis	Critical for embryogenesis; inhibitors may alter hematopoiesis
Interactions between $\alpha_4\beta_7$ integrin and MAdCAM-1	Homing to nonpulmonary mucosal tissues	Inflammatory bowel disease	Efficacy of antagonists not determined in humans
Interactions between $\alpha_M\beta_2$ integrin and ICAM-1	Homing to inflamed tissues?	Ischemia, reperfusion injury	Role in T-cell migration uncertain
Interactions between $\alpha_1\beta_2$ integrin and ICAM-1	Homing to most inflamed tissues and sites of T-cell interactions with antigen-presenting cells	Numerous	May lead to LAD type 1-like immunosuppression
Interactions between selectins and PSGL-1	Homing to sites of acute inflammation and Th1-mediated lesions, especially in the skin	Numerous	Efficacy of antagonists in humans not demonstrated
Fucosyltransferase-VII	Homing to acutely inflamed tissues and Th1-mediated lesions	Numerous	Requires intracellular inhibitors; none have been described so far
<b>Chemokine pathways</b>			
Interactions between interleukin-8 and CXCR1,2	Homing to some types of inflamed tissues, especially skin	Psoriasis	Efficacy of antagonists not determined in humans
Interactions between MCP-1 and CCR2	Homing to numerous types of inflamed tissues	Rheumatoid arthritis	Efficacy of treatment not determined in humans
Interactions between cotaxin and CCR3	Homing to Th2-mediated lesions	Asthma, allergies	Mild phenotype in knockout mice suggests redundancy of Th2-mediated chemokine pathways
Interactions between MDC, TARC, and CCR4	Homing to skin and Th2-mediated lesions	Asthma, psoriasis, atopic dermatitis	Importance not established in vivo
Interactions between MIP-1 $\alpha$ , RANTES, and CCR5	Homing to Th1-mediated lesions	Rheumatoid arthritis; HIV infection	Possible redundancy of Th1-mediated chemokine pathways
Interactions between TECK and CCR9	Homing to intestine	Inflammatory bowel disease	Importance not established in vivo
Interactions between IP-10 and CXCR3	Homing to Th1-mediated lesions	Rheumatoid arthritis	Possible redundancy of Th1-mediated chemokine pathways
Interactions between SDF-1 $\alpha$ and CXCR4	Homing to or migration within numerous types of tissues	HIV infection	Critical for embryogenesis; inhibitors may alter hematopoiesis

\*Most of these molecular pathways have been targeted with either small-molecule antagonists or blocking monoclonal antibodies and are currently being evaluated in clinical trials for various indications. In addition, numerous experiments in animals have validated these pathways for various diseases. VCAM-1 denotes vascular-cell adhesion molecule 1, Th1 type 1 helper T cells, Th2 type 2 helper T cells, MAdCAM-1 mucosal addressin-cell adhesion molecule 1, ICAM-1 intercellular adhesion molecule 1, LAD leukocyte adhesion deficiency syndrome, PSGL-1 P-selectin glycoprotein ligand 1, CXCR receptor for CXC chemokine, MCP monocyte chemoattractant protein, CCR receptor for CC chemokine, MDC macrophage-derived chemokine, TARC thymus- and activation-regulated chemokine, MIP-1 $\alpha$  macrophage inflammatory protein 1 $\alpha$ , RANTES regulated on activation normal T cell expressed and secreted, TECK thymus-expressed chemokine, IP-10 inducible protein of 10 kd, SDF-1 $\alpha$  stroma-derived factor 1 $\alpha$ , and HIV human immunodeficiency virus.

specific homing cascades of populations of pathogenic leukocytes would permit the use of tissue-selective antiinflammatory interventions. Conversely, clinical trials are under way of infusions of viral-specific cytotoxic T cells to patients with the acquired immunodeficiency syndrome or other viral infections.<sup>110,111</sup> These cells must be able to home to any tissue where virus-infected cells may linger. Similarly, patients could be immunized against pathogens and even advanced tumors by an injection of antigen-loaded dendritic cells, which must find their way into secondary lymphoid organs.<sup>112-114</sup> Modifications that direct injected cells to sites that are not on their regular itinerary might boost their therapeutic usefulness.

#### FUTURE DIRECTIONS

Many questions remain. What adhesion molecules are employed during the migration of leukocytes within tissues? How do leukocytes decide which signal to

follow when faced with different chemoattractants? How do they decide when to stay put and when to leave? What orchestrates the transportation, presentation, and neutralization of chemokines? Are there negative modulators of migration, such as antiadhesins or repellents, in addition to chemoattractants and adhesion molecules?

One antiadhesive molecule was found in CD43, which attenuates the homing of T cells.<sup>115</sup> Recently, it was also shown that low concentrations of stroma-derived factor 1 $\alpha$  attract T cells, whereas high concentrations repel some subgroups.<sup>116</sup> The identification and further characterization of such negative regulators could open yet another avenue for therapeutic intervention. Eventually, we must translate the wealth of new data on migratory molecules into an understanding of their physiologic and pathologic relevance in humans. This task will require new experimental and diagnostic tools, such as antibodies, re-

combinant proteins, small-molecule inhibitors, screening assays, and ultimately, carefully planned and executed clinical trials.

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