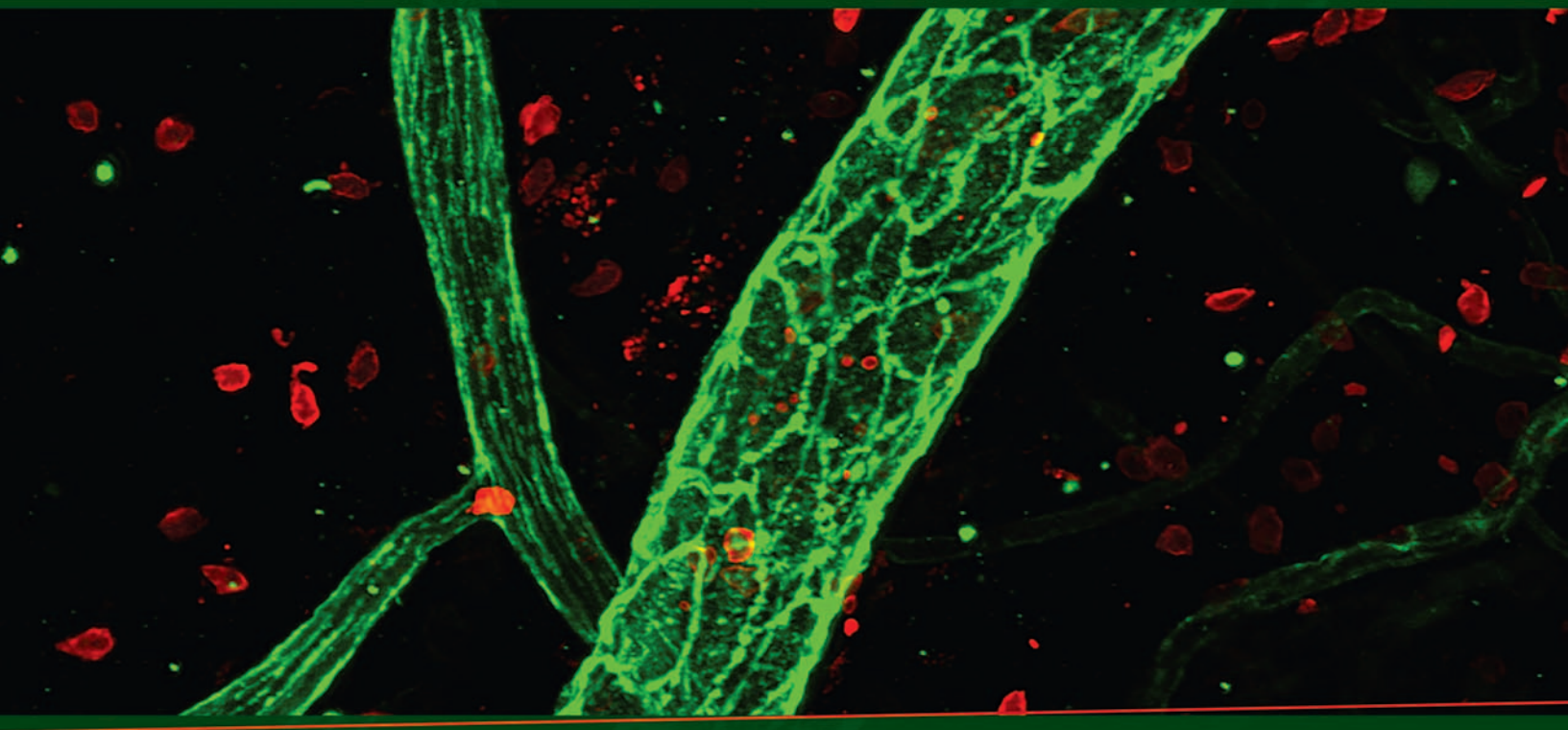
CELL MOVEMENT IN HEALTH AND DISEASE



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CHAPTER

11

Leukocyte movement during immune responses

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Introduction

Inflammation at several degrees can be triggered by infectious agents but also by endogenous molecules upon tissue damage. Most of the time, innate immune mechanisms control harmful agents; however, if these strategies succumb, the mounting of immune responses must take place to maintain homeostasis. First, an infected or damaged tissue recruits different leukocytes by producing pro-inflammatory mediators and chemokines. The first cells to be recruited to an injured tissue are the neutrophils which in humans represent the most abundant leukocyte in peripheral blood (PB). Later on, others such as monocytes and lymphocytes arrive at the injured tissue to exert function. Every blood cell lineage is endowed with specific mechanisms and molecules to achieve recruitment; however, some parallelisms among these leukocytes can also be made [1]. Migration relies on chemokine receptors, adhesion molecules, and the cytoskeleton connected to the outside portion of the leukocyte by adaptor proteins in the cytoplasm. The cytoskeleton is a biopolymer network composed of actin, microtubules, and intermediate filaments [2]. All these polymers work in conjunction to deform a leukocyte and any other cell to recognize and respond to a stimulus dynamically.

During an immune response, leukocyte recruitment occurs; however, mechanisms such as mature leukocyte intravasation from the bone marrow (BM) to PB also occur. Antigen-presenting cells (APCs) perform intravasation from inflamed tissues to lymphatic circulation to arrive at the lymph nodes (LNs). Moreover, homing to the LN by lymphoid cells occurs, and inside these organs, all these cells follow chemokine gradients to interact with each other. Strikingly, even platelets can exert migration; their interaction with other leukocytes is essential for proper movement. Finally, when the harmful agent disappears, the damaged tissue produces antiinflammatory mediators to turn off the immune response. Even at this stage, leukocyte movement is modulated to shut down the immune response. This chapter will review how leukocytes during immune responses traffic to different tissues as well as the general mechanisms these cells employ during these processes. After reading this chapter, the reader should be able to understand: (1) the general mechanisms leukocytes employ to exert movement; (2) the different steps of the leukocyte extravasation cascade; (3) that leukocyte movement in primary and secondary lymphoid organs (SLOs) is fundamental for responding to harmful agents; and (4) the general mechanisms to inhibit leukocyte movement during the resolution of inflammation.

Leukocyte movement. A general view

Leukocyte motility is an essential and highly dynamic phenomenon during immune responses. It implies that a leukocyte firmly adheres to activated endothelial cells (ECs), the extracellular matrix in a living organism, or proteins on a coverslip in vitro to spread and polarize. Even before a leukocyte firmly adheres, it must resist the shear stress generated by the blood flow inside a vessel or in vitro in particular controlled systems [3]. Leukocytes must interact through adhesion molecules such as glycoproteins and integrins with a substrate. The cytoskeleton generates motility-driving forces generating an equilibrium so that the leukocyte does not detach. The cytoskeleton is a polymer network composed of actin filaments, microtubules, and

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intermediate filaments, working in conjunction to achieve migration [2,4]. The cytoskeleton connects to the intracytoplasmic portion of the integrins through a diversity of adaptor proteins. Integrins are heterodimeric proteins conformed by α and β chains that change conformation once G-protein-coupled receptors (GPCRs) recognize chemokines on activated ECs during inflammation or under experimental in vivo and in vitro settings [3]. During these events, water and ion fluxes inside leukocytes also play a decisive role in polarization and movement. For example, upon the exertion of mechanical forces or chemokine recognition, calcium fluxes are critical to activating integrins, an event that relies on the cytoskeleton remodeling. All these mentioned events denote their interdependency to achieve effective leukocyte movement [4].

During migration through chemotactic gradients, a leukocyte enters repetitive cycles of deformation, which are comprehended first, by extending protrusions at the leading edge, followed by the adhesion of the formed protrusions to a substrate; then, the cytoplasm must contract to induce the detachment from the previous adhesion spots located at the rear (Fig. 11.1). The leading edge formation depends on actin polymerization and the guanosine triphosphate (GTP) hydrolases known as the GTPases, which catalyze GTP to guanosine diphosphate (GDP) and inorganic phosphate. Two important GTPases in forming the leading edge are Ras-related C3 botulinum toxin substrate 1 (Rac1) and cell division control protein 42 homolog (cdc42). Simultaneously, the uropod possesses high actomyosin contractility, poor adhesion, and counts on the action of the GTPase Ras homolog family member A (RhoA) [5]. GTPases are considered molecular switches that constantly cycle from on or off states (bound to GTP or GDP, respectively). In a classical view, GTPases act in a mutually exclusive manner at the front and the rear of a migrating leukocyte [6,7]. The coordinated action of these enzymes and the formation of these structures dictate the speed and the direction a leukocyte might follow [8,9].

Dimer pairs of globular actin monomers form actin filaments at the leading edge of a migrating leukocyte by actin polymerization. Tubulin protofilaments have a rigid rod-like structure and form the microtubules, which are the stiffest portion of the cytoskeleton. Lastly, intermediate filaments are the most flexible of the three cytoskeleton components. Depending on the leukocyte in question, its protein composition varies [2]. Upon recognizing a chemokine gradient, chemokine receptors induce signaling that includes actin polymerization, among other processes. Proteins such as the Wiskott–Aldrich Syndrome protein and the actin-related proteins 2/3 complex are fundamental to extend a protrusion. Simultaneously, cofilin performs actin depolymerization, an essential phenomenon that enables leukocytes to build up new actin filaments and protrusions. Actin filaments generate movement by interacting with myosins. These motor proteins are composed of a head,

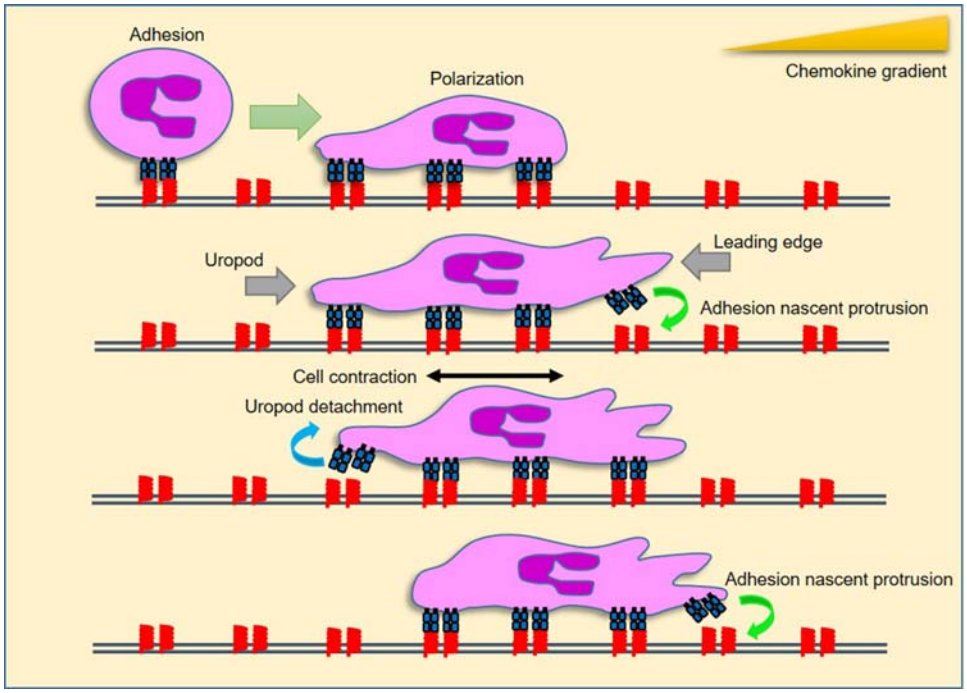

FIGURE 11.1 **Leukocyte movement through chemokine gradients.** Leukocyte movement implies that a cell must firmly adhere to a substrate to spread and polarize. Polarization induces the formation of a leading edge and a uropod. During the leading-edge formation, nascent protrusions adhere by employing integrins such as Mac-1 (represented in blue) that bind to ICAM-1, among other molecules. Then, the cell contracts its central portion inducing the detachment of the uropod. Finally, newly nascent protrusions form to continue this behavior in cycles.

neck, and tail region. The head domain is in charge of movement while the tail connects to protein cargo, other myosins, vesicles, or other actin filaments. Their interactions with actin filaments generate contractile forces and, in consequence, leukocyte movement [10]. Toward the uropod of a neutrophil, microtubules extend radially from the cellular centrosome and stabilize this structure together with the integrin Macrophage-1 antigen (Mac-1) [5,7]. Intermediate filaments give structural integrity to a leukocyte. During migration, a leukocyte can disassemble a new protrusion projected at the leading edge, a phenomenon called adhesion turnover [11]. Suppose a leukocyte prioritizes another chemokine gradient to follow or senses a not desired signal. In that case, the leukocyte moves away from that particular point by disassembling the nascent protrusions and polymerizing actin in another direction [2]. In this way, leukocytes can change their directionality to chase, for example, pathogens. The following sections will describe a general immune response highlighting the molecules that leukocytes employ to arrive at a desired damaged tissue.

The initial challenge: how does an immune response begin?

Humans and other organisms constantly expose to harmful agents such as bacteria, viruses, parasites, and fungi [12]. Most of the time, the innate immune mechanisms clear these agents within a tissue; however, when these strategies succumb, the cellular component of the innate immune system must be activated. Tissue-resident macrophages constantly search for pathogens and other signs of tissue damage to ingest and clear the noxious agent. These cells use pattern recognition receptors to recognize evolutionary-conserved molecules constitutive of infectious agents named pathogen-associated molecular patterns. The ligation of these receptors induces complement activation, the coagulation cascade, phagocytosis, pro-inflammatory signaling pathways, and apoptosis [13]. Every tissue in the body has different proportions of tissue-resident macrophages that can vary depending on the context an individual exposes. For example, during infection of the urinary tract tissue, macrophages induce the recruitment of circulating phagocytes by producing large amounts of macrophage inhibitory factor (MIF) and the chemokines C-X-C motif chemokine ligand (CXCL) 1 (CXCL1), CXCL2, and CXCL6. Then, recruited macrophages produce large amounts of tumor necrosis factor α (TNFα), which induces resident macrophages to secrete CXCL2 and, in turn, activate the neutrophil secretion of matrix metalloproteinase (MMP) 9 (MMP9) to degrade extracellular matrix, reach the infection site, and exert function [14,15].

The endothelium separates the bloodstream from tissues and regulates the exchange of solutes, fluids, and cells among these two tissues. The molecular interactions that maintain ECs in close contact are the tight and adherent junctions (TJ and AJ, respectively). Occludins and cloudins are part of TJ and bind to cortical actin through adaptor molecules such as zonula occludens. Homotypic binding of vascular endothelial-cadherin conforms to the AJ and connects to the cytoskeleton through adaptors of the catenin family. Pro-inflammatory agents such as TNFα induce actin remodeling to favor the formation of stress fibers to generate pulling forces that destabilize EC junctions [16]. Upon activation with TNFα and/or interleukin (IL)-1β (IL-1β), these cells express P- and E-selectin almost immediately after a challenge. The expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) can take a few hours until expressed. ECs are also in charge of presenting chemokines that drive leukocyte diapedesis [17]. Most actin-binding proteins participating in the EC cytoskeleton remodeling have been reviewed elsewhere [18,19]. TNFα and IL-1β activate pericytes to produce and present CXCL1, a critical chemokine that drives neutrophil migration in mice [20]. ECs upregulate toll-like receptors (TLRs) 2 and 4 (TLR2 and TLR4) expression upon TNFα or lipopolysaccharide (LPS) stimulation. These, in turn, induce ICAM-1 expression and MIF secretion to guide the recruited cells to the damaged tissue [21]. It is essential to highlight that leukocyte diapedesis occurs mainly in venous blood vessels, where the length, the type of endothelium, and blood flow velocity are optimal for their interactions [11]. Thus, resident macrophages begin producing inflammatory mediators to activate the endothelium and facilitate subsequent leukocyte recruitment within the damaged tissues.

Neutrophil and monocyte diapedesis

Once the endothelium expresses the selectins (P- and E-selectin) and adhesion molecules (ICAM-1 and VCAM-1) and presents chemokines (CXCL1 and CXCL2) on the lumen of a vessel, neutrophils and monocytes can interact with the inflamed endothelium to start the diapedesis cascade [1] (Table 11.1). It is worth noting that the classical diapedesis cascade is studied in vivo by intravital microscopy. However, the mechanisms employed for this process vary from one organ to another substantially [3,22]. Neutrophil and monocyte diapedesis comprehend a series of events beginning with their tethering and rolling over activated ECs (see also Chapter 10). These cells progressively decrease their velocity upon sensing optimal concentrations of chemokines presented by the endothelium (Fig. 11.2). Once this

TABLE 11.1 General overview of the receptors, adhesion molecules, and chemokines involved in leukocyte trafficking.

Leukocyte	Selectin ligands	Receptor	Chemokine	Integrins	General context
Neutrophils	PSGL1	CXCR2	CXCL1, CXCL2	LFA-1, Mac-1	TNFα inflamed cremaster; urinary tract infection
Monocytes				VLA-4, LFA-1, Mac-1	
Platelets	P-selectin, CD41	S1P1	S1P	$\alpha_{IIb}\beta_3$	Thrombus reorganization
HSPC		CXCR4	CXCL12	VLA-4	Homeostasis at BM niche
DC (tissue)				LFA-1, Mac-1	Inflammation; intravasation to the lymph
DC (lymph)	L-selectin	CCR7	CCL19, CCL21	LFA-1, Mac-1	Accessing the LN
$CD4^+$ T cell	PSGL1, L-selectin	CXCR4, CCR7, CCR9	CXCL12, CCL19, CCL21	LFA-1, VLA-4	CNS under inflammation
	E-selectin	CCR4	CCL17, CCL22	LFA-1	Skin homing
	L-selectin	CCR7	CCL19, CCL21		HEV at the LN
Tfh		CXCR5	CXCL13		Interfollicular region; LN
		S1P2	S1P		Migration to GC
$CD8^+$ T cell	L-selectin	CCR9	CCL25	α4β7	Gut homing
		CCR7	CCL21	LFA-1	HEV
		CCR5	CCL3, CCL4		APC interaction at the LN
		S1P1	S1P		Egress from the LN
B cell		CCR7	CCL19, CCL21		HEV
		CXCR5, CCR7 CXCR4 S1P2	CXCL13 BAFF CXCL12 S1P		Attraction to germinal centers at the LN Migration to GC
Plasma cell	PSGL-1	CXCR4	CXCL12	VLA-4, α4β7	BM homing
		CCR9 CCR10	CCL25 CCL28		Traffic to intestine, salivary glands, mammary glands
NK cell	L-selectin	CCR7,	CCL19, 21	α4β7	Homing at the LN
		CXCR4 CXCR1 CXCR2 S1P5	CXCL12 CXCL1 CXCL2 S1P		Recruitment to inflamed tissues
		CXCR3 CCR5	CXCL10 CCL5		Skin recruitment
		CCR2	CCL2		Infection
ILC 1		CCR7, CCR9	CCL19, CCL21, CCL25		Homing to SLO, Mesenteric LN
ILC 2				LFA-1	
ILC 3				LFA-1, VLA-4	Intestine

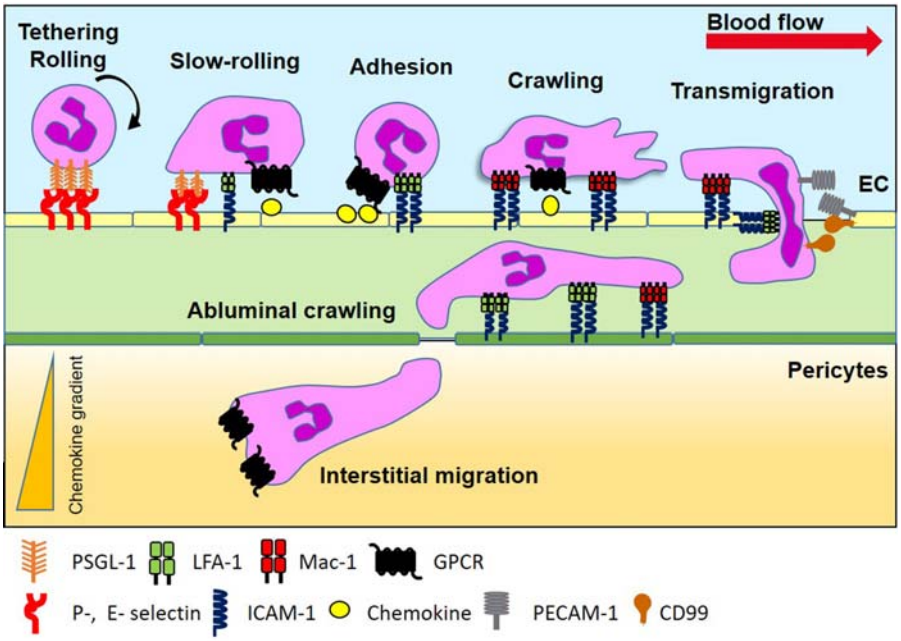

FIGURE 11.2 **The neutrophil extravasation cascade.** Neutrophil diapedesis begins with tethering and rolling, where PSGL1 recognizes selectins on activated ECs. Then slow-rolling depends on PSGL-1 and low levels of GPCR signaling. High levels of chemokines presented at the luminal portion of a vessel induce firm arrest in an LFA-1-dependent manner. Crawling depends on the Mac-1 integrin and serves to look for a proper spot for extravasation. Transmigration occurs in a paracellular (depicted in the figure) or transcellular manner. Then, abluminal crawling occurs by neutrophil interaction with EC and ICAM-1-expressing pericytes. Finally, the neutrophil trespasses the pericyte layer to perform interstitial migration to reach the site of infection or injury by following chemokine gradients to exert its effector function.

occurs, a myeloid cell firmly arrests on the endothelium to then spread, polarize, and crawl with the porpoise of searching for the right site for paracellular extravasation. Then, these cells perform abluminal crawling (in between ECs and the pericytes) to detach from pericytes to start interstitial migration following chemotactic gradients. These, in the end, bring leukocytes to their final destination to exert function [23]. Neutrophils can die in situ, but recent data have demonstrated these cells can prolong their lifespans according to the given stimulus and can even return to the bloodstream to home, for example, the BM, and instruct immune responses [24].

The initial interactions of the neutrophil diapedesis cascade imply that endothelial P- and E-selectin interact with P-selectin glycoprotein ligand-1 (PSGL-1) on the neutrophil to initiate rolling, which means the activation of a signaling pathway that leads to the acquisition of the intermediate-affinity conformation of lymphocyte function–associated antigen-1 (LFA-1). LFA-1 switches from a resting state with a bent ectodomain and closed headpiece ($E^- H^-$) to its intermediate-affinity conformation with an extended ectodomain but closed headpiece ($E^+ H^-$) [25,26] (Fig. 11.3). In essence, this conformation is essential for the neutrophil to slow down and perform slow-rolling and implies the recruitment of Talin-1 to the intracytoplasmic portion of the integrin. Suboptimal chemokine concentrations can also induce the partial activation of LFA-1. Therefore, it seems both PSGL-1 and GPCR signaling cooperate to reduce rolling velocity and enhance endothelial–neutrophil interaction [26,27]. The sensing of high chemokine concentrations such as CXCL1 in mice leads to Rac1 activation and the acquisition of the high-affinity conformation of LFA-1 ($E^+ H^+$), permitting full recognition of ICAM-1 and leukocyte arrest [26]. To adopt this conformation requires recruitment of the adaptors Talin-1 and kindlin-3 to the intracellular portion of LFA-1 [28]. Recently, the bent and open headpiece ($E^- H^+$) of LFA-1 has been described, which implies it binds to ICAM1 in cis (in the same neutrophil) to work as a regulatory mechanism during acute inflammation [29,30]. Monocytes achieve firm arrest through GPCR-dependent signaling but, unlike neutrophils, employing the integrin very late antigen-4 (VLA-4) [1]. Under artificial conditions in vivo, neutrophil arrest depends on LFA-1 activation upon CXCL1 stimulation, but after 5 min, the strengthening of adhesion relies on Mac-1 [31,32]. This integrin is also fundamental for neutrophil crawling in vivo as its blockage simply inhibits this type of movement [33]. In addition to Mac-1, monocytes also employ LFA-1 to crawl; however, this seems to depend on the severity of inflammation [34]. Crawling leads the neutrophils and monocytes to look for the proper spot for extravasation. During crawling, neutrophils and monocytes build an integrin- and chemokine-receptor-rich leading-edge parallel to continuous actin

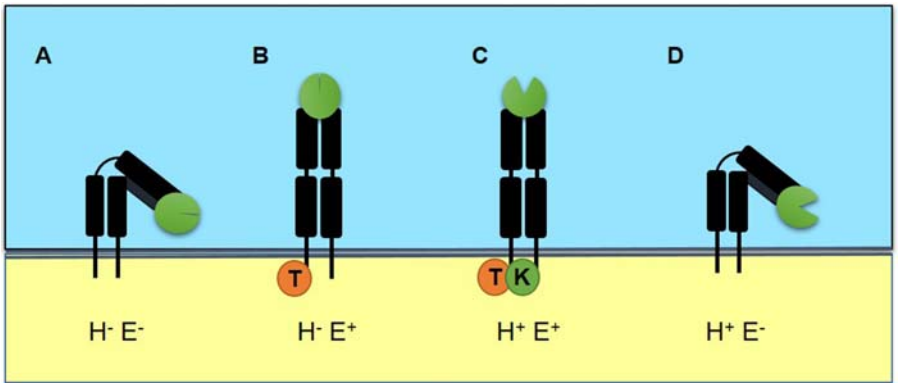

FIGURE 11.3 **LFA-1 conformations.** Under basal conditions, LFA-1 rests with a closed headpiece and a bent ectodomain (H^- E^-) (A). PSGL-1 and/or GPCR signaling induces the intermediate-affinity conformation of the integrin, which adopts the closed headpiece and extended ectodomain (H^- E^+). The acquisition of this conformation requires Talin-1 recruitment to the intracytoplasmic portion of the integrin (B). High concentrations of chemokines induce the high-affinity conformation of LFA-1; it acquires the open headpiece and full-extended ectodomain (H^+ E^+) in a Talin-1- and kindlin-3-dependent fashion (C). The open headpiece and bent ectodomain conformation (H^+ E^-) is adopted as a regulatory adhesion mechanism during extreme inflammatory conditions (D).

polymerization. At the same time, Mac-1 participates in stabilizing the uropod, as previously mentioned [7].

Neutrophils and monocytes transmigrate in between EC, a process known as paracellular transmigration. During this process, neutrophils produce neutrophil elastase and MMP to facilitate the formation of paracellular gaps for transmigration [28]. Deformation of the neutrophil nucleus is also a crucial step during transmigration. It requires the actin cytoskeleton remodeling in a process dependent on Myosin 1f that links it to the nuclear membrane [35]. During transmigration, LFA-1 and Mac-1 interact with the junctional adhesion molecules A and C (JAM-A and JAM-C) and, in turn, with platelet and endothelial cell adhesion molecule-1 (PECAM-1) and the single-chain type-1 glycoprotein (CD99). Neutrophils can produce CXCL2 to guide themselves through junctions to the abluminal portion of vessels [20]. Monocytes, in addition to PECAM-1 and CD99 homophilic interactions, employ DNAX Accessory Molecule-1 to recognize the Poliovirus Receptor on the endothelial surface [1]. Once the neutrophils and monocytes have transmigrated, they must perform abluminal crawling between the basement of the endothelium and the pericytes surrounding the vessel. Pericytes express ICAM-1 under inflammatory conditions and are crucial for integrin interactions during neutrophil and monocyte abluminal crawling to cross the pericyte layer and start following chemokine gradients. During chemotaxis, these leukocytes can follow a straightforward gradient; however, in vitro experiments have shown, these cells can also turn to follow another chemokine gradient, which means these cells can prioritize the external signals received during inflammation [36,37].

Platelets

Platelets are known to interact with neutrophils but also with lymphocytes, dendritic cells (DCs), monocytes, eosinophils, and basophils to promote their diapedesis [38,39]. This is conceivable because the blood flow maintains circulating platelets near the vessel wall promoting their interactions. Also, platelets scan for proteins expressed by the endothelium and extracellular matrix components. During an inflammatory challenge, activated platelets interact with the activated endothelium through the cluster of differentiation (CD) 40 ligand (CD40L)—CD40 axis, which on ECs induce ICAM-1 and VCAM-1 expression together with the endothelial secretion of C—C motif ligand (CCL) 2 (CCL2) and CXCL8. These interactions amplify the inflammatory response, promote leukocyte extravasation, and at the same time support vascular integrity [39]. Strikingly, activated platelets can also tether, roll, adhere, and spread. In an inflamed tissue, adhered platelets exert movement independent of the blood flow or migrating leukocytes. Plasma proteins, including fibrinogen, induce platelet spreading and a half moon-shape polarization in an actin-dependent manner. Collagen, in turn, causes the release of secondary platelet mediators such as adenosine diphosphate and thromboxane A2 that promote spreading and migration in an integrin $\alpha_{IIb}\beta_3$-dependent manner. This type of movement has a role during thrombus reorganization and consolidation. Importantly, and in line with neutrophils, platelets are also considered a first line of defense because they can bind and collect bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Listeria*

monocytogenes and because they promote neutrophil activation and extracellular traps release during an infection [8]. Moreover, activated platelets bind the activated endothelium and guide crawling neutrophils and monocytes to extravasation sites during inflammation; during this process, the PSGL-1–P-selectin axis is fundamental [40].

Even at SLO, activated platelets promote homing of T cells bypassing the initial L-selectin (CD62-L) interactions (see below) [41]. Platelets are also significant producers of CXCL4 (platelet factor 4), CCL5 (RANTES), and CXCL17 (neutrophil-activating peptide-7, NAP7), which also are involved in neutrophil recruitment and activation. For example, C-X-C motif chemokine receptor (CXCR) 2 (CXCR2) recognizes CXCL17 and also promotes neutrophil chemotaxis. As previously mentioned, during inflammation and infection, neutrophils employ PSGL-1, Mac-1, and LFA-1 to interact with activated platelets that express P-selectin, glycoprotein (GP)Ib (GPIb), $\alpha_{IIb}\beta_3$ (both binding Mac-1 on the neutrophil side), and ICAM-2. These interactions promote integrin activation and, therefore, adhesion in vivo [42]. Thus, platelets are an essential component of the immune system as they can trap pathogens, seal wounds, and interact with other leukocytes to promote their activation and migration.

Hematopoietic stem and progenitor cells during inflammation and mobilization

When pathogens trespass the cellular immunity, the local microenvironment must produce pro-inflammatory mediators such as granulocyte colony-stimulating factor (G-CSF), IL-1β, IL-6, IL-8, IL-12, TNFα, interferon γ (IFNγ), and chemokines such as CXCL1 and CXCL2. In addition, bacterial-derived products such as LPS can also be secreted by pathogens and circulate through the bloodstream; all of the factors mentioned above can instruct the cellular component of the BM microenvironment. According to a given stimulus, HSPC produces in an evolutionary-conserved fashion the required cells to combat the harmful agents within the BM niches. Factors such as granulocyte and macrophage colony-stimulating factor (GM-CSF), IL-6, IL-3, and G-CSF can mobilize immature cells from the BM; however, G-CSF might be the most studied one due to its clinical use in harvesting HSPCs for transplantation in the treatment of a variety of oncological conditions. During inflammation, stromal cells produce G-CSF upon LPS stimulation in a nuclear factor-kB-dependent manner [43]. Within the BM, high concentrations of G-CSF and TNFα reduce CXCL12 production by stromal cells, which induces HSPC mobilization [44,45]. Also, G-CSF can activate BM neutrophils to secrete proteases that cleave CXCR4, CXCL12, VCAM-1, and C-KIT, the receptor to stem cell factor. Neutrophils express CXCR4 and CXCR2, the receptor for CXCL1 and CXCL2. Neutrophil egress from the BM responds to the balance of the signaling among these two receptors. Under basal conditions, CXCR4 signaling dominates over the one from CXCR2 and retains neutrophils inside the BM through VLA-4 binding VCAM-1 on ECs and stromal cells. As neutrophils maturate, they reduce the expression of CXCR4 and VLA-4 to switch signaling dominance from CXCR4 to CXCR2 and to induce intravasation from the BM to PB [46]. In parallel, under emergency conditions, myelopoiesis implies expansion of the HSPC pool and mobilization of lymphocytes from the BM to the spleen in a TNFα and IL-1β-dependent manner [47–49]. Thus, pro-inflammatory cytokines and pathogen-derived products modulate hematopoiesis and induce HSPCs and neutrophil mobilization from the BM to PB by perturbing the CXCR4–CXCL12 and VLA-4–VCAM-1 interactions. It is also plausible that neutrophil-induced serine protease cleavage of CXCL12 also perturbs the signaling that maintains VLA-4 in its active conformation impeding HSPC and/or neutrophil interactions within the BM microenvironment.

Under normal circumstances, megakaryocytes (MKs) produce and pour between 5000 and 10,000 platelets into PB. This event implies the formation of transendothelial MK extensions that connect the vascular niche with PB, a phenomenon that relies on the chemotactic lipid sphingosine-1 phosphate (S1P)–S1P receptor (S1P1) axis. Interestingly, the deficiency of this receptor does not perturb the positioning and migration of the MK to the vascular niche [50]. The spleen and the lungs import MK from the BM, organs from where platelets can also be poured into PB [39]. It is estimated in vivo that MK from the lungs produces nearly half of total platelet production [51]. Insults such as the ones recognized by TLR2 can induce human HSPC to produce MK [52]. In mice, TLR3 stimulation leads to dramatic platelet depletion, which recovers 6 days after the challenge. Polyinosinic: polycytidylic acid (Poly I: C, a TLR3 ligand), LPS, and TNFα stimulation induce the generation of MK progenitors, which express the platelet glycoprotein IIb (CD41) and P-selectin [53]. Although these molecules are essential for platelet interaction with mature cells, it is unknown how the MKs can interact with mature cells within the vascular niche. While CD41 is involved in proplatelet formation [54] and the adhesion and activation of MKs [55], P-selectin is an adhesion molecule that also denotes activation but is involved in the release of α-granules [56]. During inflammation, IL-6 and IL-1β promote platelet production while CCL5, secreted by platelets upon activation, instructs MK in the BM toward platelet production.

Thus, MKs traffic from the BM to the lungs and spleen to produce platelets in situ. Expressing CD41 and P-selectin on MKs under inflammatory conditions could indicate that newly produced platelets can interact with leukocytes despite their immaturity.

DC trafficking to the LN

During an infection or tissue damage, leukocytes such as DCs or macrophages must travel to SLO to begin the process of antigen presentation (Fig. 11.4). As inflammation promotes vascular leakage and extravasation within an inflamed tissue, these cells can also intravasate from this site to access the lymph [57]. Under basal conditions, recirculating memory T cells are the most abundant leukocyte in lymphatic circulation, followed by DCs and B cells. Upon a given challenge, DCs acquire migratory properties by upregulating CXCR4 to recognize CXCL12 and gain entrance to lymphatic circulation [58]. Lymphatic ECs gather together by AJ and TJ. Under inflammatory conditions, DCs gain access to the lymph by adhering to PECAM-1, hyaluronic acid (CD44), and the lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1), which has a similar structure to CD44 [59,60]. Once DC reaches the lumen of the lymphatic vessel, it experiences very low flow rates; therefore, DCs crawl following a CCL21 gradient through its C−C motif chemokine receptor (CCR) 7 (CCR7) and employ LFA-1 to recognize ICAM-1 expressed by lymphatic EC. After these steps, the APC arrives at the LN carried by the crescent passive lymphatic flow where it interacts with ECs of the LN by recognizing ICAM-1 to transmigrate in an LFA-1- and Mac-1-dependent manner. After transmigration, the APC produces chemoattractant factors to lymphocytes to begin antigen presentation [57].

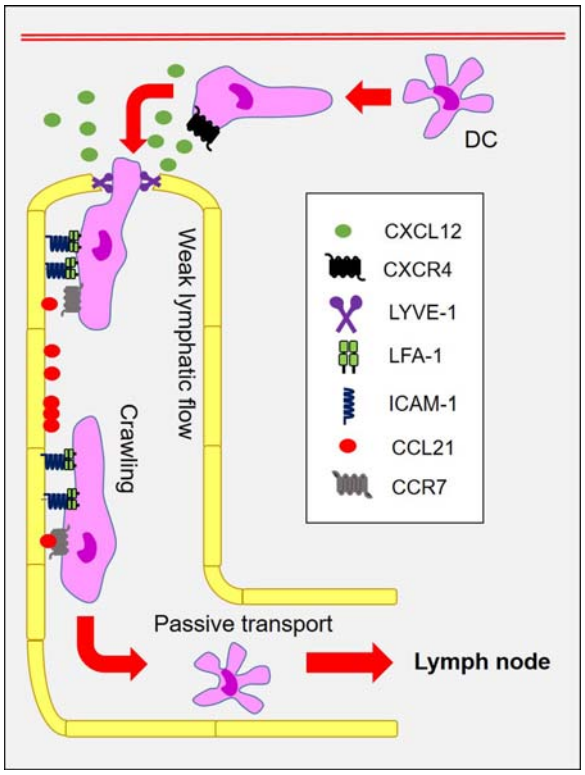

FIGURE 11.4 **Dendritic cell trafficking to the LN.** DC resides in tissues such as the skin. Upon tissue damage or infection, DC acquires migratory properties, which include CXCR4 expression. Following CXCL12 gradients, DC arrives at the lymphatic vessels, which express LIVE-1. DC intravasates and crawls employing CCR7 to recognize CCL21; these cells also employ LFA-1 to recognize ICAM-1 on the activated lymphatic endothelium. Then, the cell traffics through lymphatic circulation until it reaches the LN.

T- and B-cell trafficking to the LN

While B cells develop in the BM, they acquire maturity and specificity in SLO such as the spleen and peripheral LN. In the case of T cells, the BM exports hematopoietic progenitors to colonize the thymus in a PSGL-1-, CCR7-, and CCR9-dependent fashion [61,62]. These progenitors differentiate and expand within the thymus to finally produce naïve T cells that egress from it following the chemotactic factor S1P, found in PB at high levels [63]. B and T cells rely on L-selectin to interact with the high endothelial venules (HEVs) expressing the sialomucin CD34 and glycosylation-dependent cell adhesion molecule 1 (Gly-CAM-1) [64]. Stromal cells produce CCL19 and CCL21, deposited on the luminal side of the HEV (Fig. 11.5). Lymphocytes and DCs recognize these chemokines through CCR7. The recognition of these chemokines induces LFA-1 activation with the consequent T cell arrest.

T cells interact through the T cell receptor (TCR) with the major histocompatibility complex (MHC) expressed by APC and monitor for the presence of unknown peptides within the T cell area. If a T cell does not recognize an antigen, it leaves the LN and patrols other tissues. On the contrary, if it recognizes a strange peptide through its TCR, this signal leads to LFA-1 activation and ICAM-1 recognition on APC to promote their interaction [65]. S1P gradients also govern the trafficking of T cells within the LN. The recognition of an antigen induces the activation, proliferation, and downregulation of S1P1 on T cells to restrict their exit from the LN. Once the cell has gained functionality, it recovers S1P1 expression to follow S1P gradients and egress from SLO. It is worth noting that pro-inflammatory cytokines induce S1P production within an inflamed tissue and, therefore, can attract T cells to exert their function [66]. During their differentiation, T-follicular helper cells (Tfh) will upregulate CXCR5 and downregulate CCR7. This event

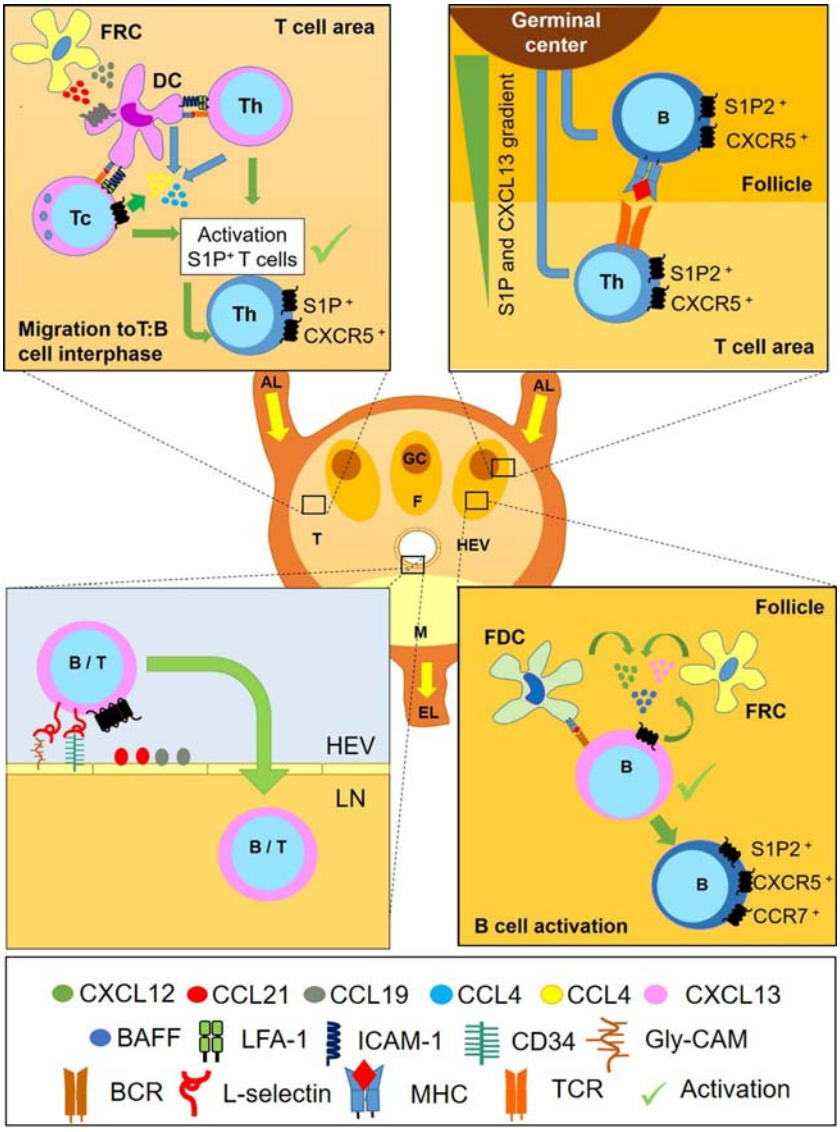

FIGURE 11.5 **Lymphocyte movement in the LN.** The LN is composed of specific areas where DCs and lymphocytes interact to mount an adaptative immune response. The lymphatic circulation irrigates the LN through the afferent lymph and drains the LN through the efferent lymph. Lymphocytes use high endothelial venules (HEVs) to home to the LN, composed of the T cell area (T), the follicle, and a germinal center (GC), which forms during immune responses (Center panel). Lymphocytes home to the LN through the HEVs and employ L-selectin to interact with CD34 and Gly-CAM. CCL19 and CCL21 are deposited on the vessel's lumen so that lymphocytes, through CCR7, recognize them (lower left panel). Follicular reticular cells (FRCs) produce CCL19 and CCL21 to attract DCs and T cells. DC interacts through MHC with Th cells, which employ the TCR. Both cells produce CCL3 and CCL4 to attract $CD8^+$ T cells to interact with DC. Once the Th cell is activated, it upregulates S1P1 and CXCR5 to migrate to the T:B interphase (upper left panel). Follicular DC (FDC) and FRC that produce CXCL12, CXCL13, and BAFF attract B cells to interact with FDC by employing its BCR. Once the B cell is activated, it upregulates S1P2, CXCR5, and CCR7 (lower right panel). Activated T and B cells migrate to the T:B cell interphase, where the B cell presents antigen to the Th, and both migrate to the germinal center following a CXCL13 gradient to induce antibody production (upper right panel).

induces migration of Tfh to the T and B cell interfollicular region. Tfh interacts with B cells expressing both CXCR5 and CCR7 at this interphase [64,67].

CCL21 is an essential chemokine for naïve $CD8^+$ T cells to home at the LN [64]. Once T cells and DCs entry to the LN and interact, they produce CCL3 and CCL4, both known to participate in CCR5-dependent naïve $CD8^+$ T cell recruitment toward APC [68]. The initial contacts between a $CD8^+$ T cell and APC are weak; however, once the T cell recognizes antigen on MHC-I molecules by its TCR, LFA-1 activates and binds firmly to ICAM-1 on APC [64]. Pro-inflammatory cytokines can also induce C-type lectin expression on $CD8^+$ T cells; this molecule forms a complex with S1P1 to inhibit S1P recognition, which causes cytotoxic T cell permanence in the LN. This inhibition prevents the $CD8^+$

T cell from leaving the LN while it becomes fully activated. After activation, cytotoxic T cells proliferate and regain S1P1 expression that leads them outside the LN [64,69].

B cells employ CXCR5 and CXCR4 to follow CXCL13 and CXCL12 gradients produced by follicular DC (FDC) and fibroblastic reticular cells (FRCs) to attract them to the germinal centers (GCs) [70]. Also, FDC produces B-cell-activating factor belonging to the TNF family (BAFF) to promote B cell survival [70]. CXCL13 and BAFF guide B cells to encounter unknown peptides presented on MHC by APCs to become activated. Upon activation by APC, B cells upregulate CCR7 and migrate toward the interphase of the B cell follicle and the T cell zone. The interactions between B and T cells induce the proliferation of B cells, which can either migrate to the GC or differentiate into short-living plasma cells (PCs) to produce an initial wave of antibodies for the initial control of an infectious disease [67]. To generate a GC, Tfh and activated B cells decrease CCR7 expression and upregulate S1P receptor 2 (S1P2) to migrate toward the follicle center. At the GC, particularly in the dark zone, B cells experience clonal expansion and somatic hypermutation. At the light zone, affinity maturation and class-switch recombination occur. Centroblasts (rapidly dividing B cells) at the dark zone express CXCR4 and recognize CXCL12 produced by stromal cells. This axis retains centroblasts at the dark zone during somatic hypermutation and isotype change. Once achieved, the B cells (known as centrocytes) downregulate CXCR4 to migrate toward the light zone following a CXCL13 gradient through CXCR5. At this point, B cells will experience positive selection signals. Selected B cells that survive the maturation of affinity proliferate, differentiate, and migrate from the GC to other SLO or the BM. These cells can become long-lived PCs that depend on the CXCR4–CXCL12 axis and can produce and secrete antibodies for prolonged periods. Once the infection resolves, B cells at the GC differentiate to memory B cells. In case of a new challenge by the same pathogen, they rapidly react to produce antibodies to mount a more effective immune response.

T cell diapedesis

T cells as myeloid cells extravasate to damaged or infected tissues; however, there is a great degree of heterogeneity regarding phenotype and functions among T cell repertoires, including memory and migration patterns that depend on activation [62]. Some parallelisms apply concerning selectin ligands and the integrins T cells employ during diapedesis, even with this degree of heterogeneity. After leaving the LN, T cells travel through peripheral circulation until they reach an inflamed tissue and employ PSGL-1 and L-selectin to tether and roll over the activated endothelium expressing E- and P-selectin. It is worth noting PSGL-1 forms heterodimers with CD44, sialophorin, or the T cell immunoglobulin and mucin domain 1. The cytokines that induce differentiation toward a T helper (Th) 1 or 2 (Th1 or Th2) phenotype modifies PSGL-1 glycosylation patterns that ultimately influence its affinity for the selectins on EC [62]. Like myeloid cells, and according to integrin mechanics and signaling, chemokine recognition on EC induces the high-affinity conformation of LFA-1 and VLA-4, resulting in firm arrest. Effector $CD8^+$ T cells upregulate CXCR3 and are recruited by T $CD4^+$ cells by producing IFNγ, which induces CXCL9 and CXCL10 production at the infection site [71]. During firm adhesion, the GTPase Ras-related protein 1 (as in myeloid cells) is essential to induce LFA-1 and VLA-4 activation. Another molecule that influences integrin-mediated firm adhesion is the integrin-associated protein associated in *cis* to these molecules and is indispensable to activate the integrins mentioned above [72]. T cells at the central nervous system crawl in an LFA-1- and VLA-4-dependent manner, following CXCL12, CCL19, and CCL21 gradients, and perform extravasation paracellularly or transcellularly. T cell transmigration involves VLA-4, homotypic and heterotypic interactions by PECAM-1, JAM-A/B, and CD99. T cells display tropism for a tissue depending on the patterns of chemokine receptor expression. For example, $CD8^+$ T cells expressing the α4β7 integrin and CCR9 have a predilection to home the gut, while T cells expressing E-selectin and CCR4 will prefer homing the skin [64]. These patterns of expression and homing capacities dictate T cell responses to infection and govern infiltration in other types of disease such as T cell acute lymphoblastic leukemia or multiple sclerosis [62,64,73].

Plasma cell homing

After completing class-switch recombination and affinity maturation, the B cells differentiate into plasmablasts and, in turn, PCs to produce immunoglobulins of different isotypes, each with specific functions at different tissues. To effectively produce antibodies, the PC can either stay at the LN where it aroused or home a specific niche such as the skin, mucosa, or the BM. These imply that plasmablasts abandon the LN by modulating chemokine receptor expression to ensure tissue-specific homing. At least for multiple myeloma PC rolling, PSGL-1 interacts with P-selectin on EC from the BM. To home the BM, VLA-4 expressed by the PC binds VCAM-1 on the BM ECs; also, to achieve this, the integrin α4β7 on these cells must interact with mucosal addressin cell adhesion molecule-1

(MADCAM-1), but also with fibronectin and CD44. The CXCL12−CXCR4 and the LPAM−CD44 axes contribute to PC maintenance at the BM [74]. IgA-producing PCs possess distinctive migratory patterns to home specific tissues. For example, PCs generated at intestinal LN express high levels of VLA-4 and can recognize MADCAM-1 to home the intestine. Other mucosas different from the intestine do not express MADCAM-1 but VCAM-1 instead. Apart from these integrins, chemokines also play an indispensable role in achieving homing to the tissues mentioned above. For example, a PC that expresses CCR9 recognizes CCL25 and, therefore, traffic to the intestinal epithelium and endothelium; conversely, CCR10 recognition of CCL28 induces PC trafficking to salivary glands, mammary glands, and large intestine [75]. Thus, PCs also have a predilection for specific tissues, which depend on chemokine receptor, adhesion molecule expression, and the antibody isotype they produce.

Innate lymphoid cell migration

Natural killer (NK) cells, ILC1, ILC2, and ILC3 constitute the ILCs. NK cells exert cytotoxicity to virus-infected or tumoral cells by balancing the inhibitory or activating signals. When NK cells receive more activating signals than the inhibitory ones, they degranulate enzymes such as granzymes and perforins that create holes in the target cell to kill it. In PB, for simplicity, there are mainly two kinds of NK cells: $CD56^{dim}$ (mainly cytotoxic) and $CD56^{bright}$ (IFNγ producers); however, among these two populations, it was shown that more than 30,000 phenotypes exist just for PB NK cells [76]. $CD56^{bright}$ cells express CCR7, CXCR3, CXCR4, and L-selectin, concordant with their presence at the LN. $CD56^{dim}$ express CXCR4 and CXCR1, CXCR2, and CX3C chemokine receptor-1 and enable them to arrive at inflamed tissues. S1P also governs NK cell trafficking, but unlike other leukocytes, they use S1P5 to exert this function; a balance in the signaling of this receptor and CXCR4 also dictates their egress from the BM [76,77]. Cytotoxic NK cells are more represented in the BM, lung, and spleen, among other tissues. In contrast, $CD56^{bright}$ cells are more abundant at mucosas, the liver, and kidneys [76]. By their expression of CCR7 and L-selectin, NK cells home the SLO through the lymphatic circulation and recognize CCL19 and CCL21, just like T- and B-cells or DCs.

Skin inflammation is known to recruit NK cells that express CCR8 but lack CCR7. Besides, in psoriatic patients, NK cells are recruited to the skin by employing CXCR3 and CCR5, which recognize CXCL10 and CCL5, respectively. NK cells are also recruited to the lungs during aspergillosis in a CCR2-dependent fashion; during vaccinia virus infections, macrophages produce CCL2 attracting NK cells through the same receptor [78,79]. On the other hand, NK cells are fundamental for tumor immunosurveillance. The high frequency of these cells within a tumor has been correlated to better overall survival in patients diagnosed with solid tumors [80]. NK cells need to degrade extracellular matrix components to reach cancer cells. The extracellular matrix degradation is thought to be enhanced by CXCL12 within the tumor microenvironment as this chemokine induces MMP secretion and adhesion to VCAM-1 and ICAM-1. Tumors can evade NK cytotoxicity, for example, in endometrial cancer, which produces low amounts of CXCL12 and CCL27, reducing NK cell recruitment and function [81]. According to chemokine receptor expression patterns, these are just a few examples of how NK cells denote preference to arrive infected or transformed tissues.

ILCs are very important for the differentiation of Th1 cells. For example, ILC1 produces IFNγ and TNFα to combat intracellular pathogens; ILC2 produces IL-4, IL-5, IL-9, and IL-13 to combat helminths and help to acquire a Th2 phenotype; ILC3 can produce IL-17A, GM-CSF, and also TNFα to induce a Th17 phenotype and combat extracellular pathogens. While NK cells locate at most organs, ILC1 home at the intestine, ILC2 are mainly represented at the skin, while ILC3 can also be found at the intestine and at peripheral LN. NK cells and ILC, like other lymphocytes, use CCR7, CCR9, and the integrin α4β7 to home SLO and the intestines. Like T cells, these subpopulations can modify their chemokine receptor expression patterns and home to other organs [82]. Infection and inflammatory events can modify the presence of ILC within a given tissue. For example, during a *Citrobacter rodentium* infection, ILC3 augments while ILC2 decreases its intestine proportions [82]. It is also likely that other infectious agents can modulate the presence of these cells at a given organ. If the BM is being instructed to produce and release ILC or its progenitors, and if other organs contribute to these differences is currently unknown. However, some hints suggest ILC progenitors generate ILCs through differentiation in SLO but can also arrive at this organ from other tissues. As T- and B-cells acquire a repertoire of chemokine receptors and adhesion molecules to home different organs, this is also the case for ILC1 and ILC3, which acquire CCR7 to home the mesenteric LN. There, DC produces retinoic acid, and upon its recognition, ILC1 and ILC3 switch chemokine receptor expression to CCR9 and the integrin α4β7 to promote ILC1 and ILC3 homing at the intestine. In contrast, ILC2 acquires these receptors during differentiation at the BM [83].

As ILCs are recently described [84], much remains to be studied regarding their migration under inflammatory conditions and infection. One potential problem

compared to other lymphocytes is that ILCs are poorly represented throughout the body [82]. It seems some parallelisms to other lymphoid cells can be assumed concerning adhesion, extravasation, and migration as the mechanisms of integrin activation, in general, do not vary substantially among leukocytes; however, this speculation needs to be proved.

The resolution of inflammation

Once the immune system has controlled infection and/or inflammation, a tissue must recover its stability and function as it heals. The resolution of inflammation is a process that involves lipid mediators, proteins, and autacoids, endogenous molecules produced on demand to exert their function at the same tissue. These molecules can sequester pro-inflammatory cytokines, inhibit leukocyte migration at inflammatory sites, and promote neutrophil apoptosis. Prostaglandin E2 and lipoxin A_4 can induce neutrophil reverse migration away from an injury site in vivo while its speed is not affected [85,86]. Lipoxins can perturb integrin-mediated signaling, directly affecting migration, adhesion, and transendothelial migration [87]. These events tempt to inhibit neutrophil function and induce apoptosis so that macrophages can clear these cells from the already recovering tissue. While the role of resolvins and lipoxins is subject of intense investigation, much remains to be described regarding other leukocytes. For example, resolvins control pro-inflammatory cytokine production by T cells [88]; however, it is unknown if T cells or other lymphocytes can modify their migratory behaviors during the resolution of inflammation.

Conclusions

The cytoskeleton comprises actin filaments, microtubules, and intermediate filaments; which are coordinated by the proteins that interact with it. It is the necessity of responding to external factors that induce cytoskeletal remodeling for proper migration. Optimal cellular movement is governed by chemokine receptors activating signaling pathways that depend on GTPases upon recognizing their cognate ligands. These molecular switches activate and deactivate integrins in practically all the steps of leukocyte recruitment. The tissue production of chemoattractants, the adhesion molecules expressed under normal and inflammatory conditions, and the chemokine receptor and adhesion molecule expression patterns a leukocyte presents govern leukocyte tropism and recruitment patterns. In this respect, it appears that certain chemokine receptor−chemokine axes dictate specific events in different tissues. For example, the CXCR4−CXCL12 axis governs BM entrance and exit, a fundamental process under normal and emergency conditions; L-selectin, CCR7, and CCR9 on leukocytes recognize CD34, Gly-CAM-1, and the chemokines CCL19 and CCL21 to home to the LN. The S1P−S1P1 axis is responsible for trafficking from distinct SLO and the thymus to PB. Under inflammatory conditions, cells can modify their patterns of chemokine receptors and adhesion molecule expression to arrive at a given tissue and exert effector functions. However, exaggerated leukocyte recruitment can be detrimental as activated leukocytes can severely damage the inflamed tissue. In this regard, we now understand that an organism counts with different recently discovered mechanisms to turn off the immune response once the noxious agent has been cleared. In this respect, we are beginning to better understand how antiinflammatory mediators can affect leukocyte migration and function. It is essential to know all mechanisms of leukocyte migration and how to control them since several pathological conditions are aggravated by excessive leukocyte recruitment. Moreover, some cells in oncological diseases also exploit these mechanisms to prevail.

Glossary

Adherent junctions A region at the plasma membrane of two cells interacting with specific proteins linked to the actin cytoskeleton.
Autacoids Molecules produced locally in a tissue that exert a rapid effect.
Chemokine Low-molecular-weight proteins with the capacity to attract leukocytes in a concentration-dependent manner.
Cytoskeleton Biopolymer composed of actin fibers, microtubules, and intermediate filaments that confer rigidity and movement to a cell.
Diapedesis Cascade series of events that culminates with the extravasation of a leukocyte through endothelial layers at different tissues.
Extracellular matrix Group of proteins and polysaccharides that are excreted and surround cells to conform a tissue.
Extravasation The migration of a leukocyte or any other cell through an intact or inflamed vessel.
G-protein-coupled receptor Receptor conformed by seven transmembrane regions that, upon ligand recognition, change its conformation to activate G proteins.
Glycoprotein Protein bound to carbohydrate molecules.
Gradient The progressive augmentation or decrease in the concentration of a substance within a given area.
GTPase Low-molecular-weight enzyme that binds GTP and catalyzes it to GDP and inorganic phosphate.
Homing Ability of a cell to return to the organ of origin.
Intravasation Entry of a leukocyte or other cells to peripheral or lymphatic circulation.
Leading edge The frontal section of a leukocyte in movement.
Myelopoiesis The process by which a hematopoietic stem cell produces myeloid cells.
Niche An area of a tissue that provides a cell with all the requirements to maintain its functionality.
Pericyte Cells surrounding postcapillary venules that participate in producing soluble factors to guide leukocytes during the diapedesis cascade.

Polarization A change in shape before cell movement; it implies the formation of the leading edge and a uropod.

Resolvin Lipid-derived mediators with antiinflammatory properties.

Stromal cell A type of cell that conforms to different types of connective tissue.

Tight junctions Continuous protein network surrounding a tissue that seals the space between cells and forms a barrier to separate two tissues.

Uropod The rear portion of a moving cell.

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