Chemokines and Chemokine Receptors: Positioning Cells for Host Defense and Immunity

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Abstract

Chemokines are chemotactic cytokines that control the migratory patterns and positioning of all immune cells. Although chemokines were initially appreciated as important mediators of acute inflammation, we now know that this complex system of approximately 50 endogenous chemokine ligands and 20 G protein–coupled seven-transmembrane signaling receptors is also critical for the generation of primary and secondary adaptive cellular and humoral immune responses. Recent studies demonstrate important roles for the chemokine system in the priming of naive T cells, in cell fate decisions such as effector and memory cell differentiation, and in regulatory T cell function. In this review, we focus on recent advances in understanding how the chemokine system orchestrates immune cell migration and positioning at the organismic level in homeostasis, in acute inflammation, and during the generation and regulation of adoptive primary and secondary immune responses in the lymphoid system and peripheral nonlymphoid tissue.

INTRODUCTION

PTX: pertussis toxin

CCL: CC chemokine ligand

CCR: CC chemokine receptor

CXCR: CXC chemokine receptor

CXCL: CXC chemokine ligand

S1P1: sphingosine-1-phosphate receptor1

HSC: hematopoietic stem cell

CAR: CXCL12 abundant reticular

Chemokines are chemotactic cytokines that control the migratory patterns and positioning of immune cells. Chemokine function is critical for all immune cell movement ranging from the migration required for immune cell development and homeostasis, to that required for the generation of primary and amnestic cellular and humoral immune responses, to the pathologic recruitment of immune cells in disease. Chemokines constitute the largest family of cytokines, consisting of approximately 50 endogenous chemokine ligands in humans and mice (**Table 1**). Chemokine receptors constitute the largest branch of the γ subfamily of rhodopsin-like seven-transmembrane receptors. Chemokine receptors are differentially expressed on all leukocytes and can be divided into two groups: G protein–coupled chemokine receptors, which signal by activating pertussis toxin (PTX)-sensitive G_i-type G proteins, and atypical chemokine receptors, which appear to shape chemokine gradients and dampen inflammation by scavenging chemokines in a G protein–independent manner. There are approximately 20 signaling chemokine receptors and 5 nonsignaling chemokine receptors (**Table 2**). In this review, we focus on recent advances in understanding how the chemokine system orchestrates immune cell migration and positioning in homeostasis, in acute inflammation, and during the generation and regulation of adoptive primary and secondary immune responses in the lymphoid system and peripheral nonlymphoid tissue. Much has been learned about the chemokine system since this topic was last reviewed in these pages (1), and we refer the reader to other reviews that cover topics not covered in this review, such as the role of chemokines in inflammatory disease, in cancer, and as drug targets (2–4).

CHEMOKINE CONTROL OF IMMUNE CELL RESIDENCE IN PRIMARY LYMPHOID ORGANS

The development and differentiation of immune cell precursors occurs in the primary lymphoid organs—the bone marrow and thymus—and these processes are under the fine control of chemokines. T cell development in the thymus depends on the interaction of epithelial-derived CCL21, CCL25, and CXCL12 with CCR7, CCR9, and CXCR4, respectively, expressed on T cell progenitors (5). T cell progenitors first enter the thymus in response to CCL21, CCL25, and CXCL12, which are produced by the thymic epithelium and neighboring structures. Double-negative thymocytes are guided past the corticomedullary junction by CCR7- and CXCR4-mediated signals. CCR9 expression then guides these cells into the subcapsular zone. Subsequent developmental progression from double-positive through single-positive thymocytes occurs in concert with their migration into the medulla, which is dependent on CCR7 and perhaps another yet to be defined chemokine. Mature thymocytes then upregulate the sphingosine-1-phosphate receptor 1 (S1P1), which allows them to ultimately migrate out of the thymus and into the blood in response to sphingosine-1-phosphate (S1P) produced by thymic pericytes at the corticomedullary junction (5).

In contrast to the situation in the thymus, in the bone marrow the homeostatic retention and development of immune cells appears to be largely dependent on CXCL12/CXCR4 interactions (**Figure 1**). Hematopoietic stem cells (HSCs) are located in close apposition to CXCL12 abundant reticular (CAR) cells, which produce large amounts of CXCL12 that binds to CXCR4 on HSCs. This interaction retains HSCs within the bone marrow and indirectly maintains HSC populations by promoting their retention within HSC niches (6). The induced deletion of either CAR cells or CXCR4 reduces the number of HSCs in the bone marrow by 50% (7). This observation also illustrates a possible role for CXCL12/CXCR4 interactions in the release of HSCs from the bone marrow, which constitutes an important mechanism for the rapid peripheral moblization of HSCs

Table 1 Chemokines[a]

Chemokine	Other names	Receptor	Key/main immune function[b]
CXCL1	GROα, MGSA, mouse KC	CXCR2	Neutrophil trafficking
CXCL2	GROβ, MIP-2α, mouse MIP2	CXCR2	
CXCL3	GROγ, MIP-2β	CXCR2	
CXCL4	PF4	?	Procoagulant
CXCL5	ENA-78, mouse LIX	CXCR2	Neutrophil trafficking
CXCL6	GCP-2 (no mouse)	CXCR1, CXCR2	
CXCL7	NAP-2	CXCR2	
CXCL8	IL-8 (no mouse)	CXCR1, CXCR2	
CXCL9	Mig	CXCR3	Th1 response; Th1, CD8, NK trafficking
CXCL10	IP-10	CXCR3	
CXCL11	I-TAC	CXCR3	
CXCL12	SDF-1	CXCR4	Bone marrow homing
CXCL13	BLC, BCA-1	CXCR5	B cell and Tfh positioning LN
CXCL14	BRAK	?	Macrophage skin homing (human)
Cxcl15	Lungkine (mouse only)	?	?
CXCL16		CXCR6	NKT and ILC migration and survival
CCL1	I-309, mouse TCA3	CCR8	Th2 cell and Treg trafficking
CCL2	MCP-1, mouse JE	CCR2	Inflammatory monocyte trafficking
CCL3	MIP-1α	CCR1, CCR5	Macrophage and NK cell migration; T cell–DC interactions
CCL4	MIP-1β	CCR5	
CCL5	RANTES	CCR1, CCR3, CCR5	
Ccl6	C-10, MRP-1 (mouse only)	Unknown	?
CCL7	MCP-3, mouse Fic or MARC	CCR2, CCR3	Monocyte mobilization
CCL8	MCP-2	CCR1, CCR2, CCR3, CCR5 (human); CCR8 (mouse)	Th2 response; skin homing (mouse)
Ccl9/10	MIP-1γ, MRP-2 (mouse only)	Unknown	?
CCL11	Eotaxin-1	CCR3	Eosinophil and basophil migration
Ccl12	MCP-5 (mouse only)	CCR2	Inflammatory monocyte trafficking
CCL13	MCP-4 (no mouse)	CCR2, CCR3, CCR5	Th2 responses
CCL14	HCC-1 (no mouse)	CCR1	?
CCL15	Leukotactin-1, HCC-2, MIP-5 (no mouse)	CCR1, CCR3	?
CCL16	HCC-4, NCC-4, LEC (no mouse)	CCR1, CCR2, CCR5	?
CCL17	TARC	CCR4	Th2 responses, Th2 cell migration, Treg, lung and skin homing
CCL18	PARC, DC-CK1 (no mouse)	CCR8	Th2 response; marker AAM, skin homing
CCL19	ELC, MIP-3β	CCR7	T cell and DC homing to LN
CCL20	MIP-3α, LARC	CCR6	Th17 responses; B cell and DC homing to gut-associated lymphoid tissue
CCL21	SLC, 6CKine	CCR6, CCR7	T cell and DC homing to LN
CCL22	MDC	CCR4	Th2 response, Th2 cell migration, Treg migration

(*Continued*)

Table 1 (*Continued*)

Chemokine	Other names	Receptor	Key/main immune function[b]
CCL23	MPIF-1, MIP-3 (no mouse)	Unknown	?
CCL24	Eotaxin-2, MPIF-2	CCR3	Eosinophil and basophil migration
CCL25	TECK	CCR9	T cell homing to gut; thymocyte migration
CCL26	Eotaxin-3	CCR3, CX3CR1	Eosinophil and basophil migration
CCL27	CTAK	CCR10	T cell homing to skin
CCL28	MEC	CCR3, CCR10	T cell and IgA plasma cell homing to mucosa
XCL1	Lymphotactin α, SCM-1α	XCR1	Cross-presentation by $CD8^+$ DCs
XCL2	Lymphotactin β, SCM-1β (no mouse)	XCR1	
CX3CL1	Fractalkine	CX3CR1	NK, monocyte, and T cell migration

[a]Table is modified from Reference 256.
[b]Abbreviations: AAM, alternatively activated macrophages; DC, dendritic cell; ILC, innate lymphoid cell; LN, lymph node; NK, natural killer; Tfh, T follicular helper cell; Th, T helper cell; Treg, regulatory T cell.

after injury or stress. Blockade of CXCR4 on HSCs is sufficient to prevent their bone marrow engraftment and to promote HSC mobilization in mice and humans (8, 9). A common method of stem cell mobilization in humans, G-CSF treatment, promotes the destruction of CXCL12 in the bone marrow and the appearance of peripheral blood HSCs with low CXCR4 expression (10). It remains unclear which mechanism—CXCL12 availability or CXCR4 expression—constitutes the main factor dictating the homeostatic release of HSCs. However, given the small number of circulating HSCs, any downregulation of CXCR4 must be either transient or very uncommon to allow for the rapid return of HSCs to the bone marrow and for the few HSCs to be in the circulation.

CXCL12/CXCR4 interactions are necessary for normal bone marrow development of multiple immune cell lineages, including B cells, monocytes, macrophages, neutrophils, natural killer (NK) cells, and plasmacytoid dendritic cells (pDCs) (11). CXCL12/CXCR4 interactions promote the retention of both developing and mature immune cells within the bone marrow. Thus, blockade of CXCR4 signals using antagonists leads to abnormal and increased mobilization of neutrophils into the peripheral blood (8). As neutrophils mature in the bone marrow, their expression of CXCR4 progressively decreases, permitting neutrophil release from the bone marrow and their positioning in blood and peripheral tissues (12). Normal downregulation of CXCR4 appears to play an important role in controlling bone marrow residence of neutrophils under homeostatic conditions, but other chemokines may be involved. When compared with wild-type neutrophils in gnotobiotic mice, CXCR2-deficient neutrophils exhibit enhanced bone marrow retention and reduced numbers in the peripheral blood (13). However, this likely plays a minor role in neutrophil homeostasis because neutrophils doubly deficient in both CXCR2 and CXCR4 exhibit the CXCR4-deficient phenotype of constitutive mobilization (13). Indeed, the primary importance of CXCR4 in neutrophil homeostasis has been illustrated in patients with warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome. This syndrome is most commonly caused by mutations in CXCR4 that enhance responsiveness to CXCL12 either by impairing CXCR4 internalization and chemokine-induced desensitization or by leading to enhanced or sustained chemokine signaling (14, 15). Neutrophils are unable to normally decrease responsiveness to CXCL12 and are therefore trapped within the bone marrow, resulting in peripheral neutropenia.

NK: natural killer

pDC: plasmacytoid DC

WHIM: warts, hypogammaglobulinemia, infections, and myelokathexis

In contrast to granulocytes, homeostatic release of monocytes from the bone marrow is dependent on both CXCR4 and CCR2 signaling. Although CCR2 expression varies among mature

Table 2 Chemokine receptors[a,b]

Receptor	Immune cell expression	Key immune function
G protein–coupled receptors		
CXCR1	Neutrophil > monocyte, NK, mast cell, basophil, $CD8^+$ T_{EFF}	Neutrophil trafficking
CXCR2	Neutrophil > monocyte, NK, mast cell, basophil, $CD8^+$ T	B cell lymphopoiesis, neutrophil egress from bone marrow, neutrophil trafficking
CXCR3	Th1, $CD8^+$ T_{CM} and T_{EM}, NK, NKT, pDC, B cell, Treg, Tfh	Th1-type adaptive immunity
CXCR4	Most (if not all) leukocytes	Hematopoiesis, organogenesis, bone marrow homing
CXCR5	B cell, Tfh, Tfr, $CD8^+$ T_{EM}	B and T cell trafficking in lymphoid tissue to B cell zone/follicles
CXCR6	Th1, Th17, γδ T, ILC, NKT, NK, plasma cell	ILC function, adaptive immunity
CCR1	Monocyte, macrophage, neutrophil, Th1, basophil, DC	Innate immunity, adaptive immunity
CCR2	Monocyte, macrophage, Th1, iDC, basophil, NK	Monocyte trafficking, Th1-type adaptive immunity
CCR3	Eosinophil > basophil, mast cell	Th2-type adaptive immunity, eosinophil distribution and trafficking
CCR4	Th2, skin- and lung-homing T, Treg > Th17, $CD8^+$ T, monocyte, B cell, iDC	Homing of T cells to skin and lung, Th2-type immune response
CCR5	Monocyte, macrophage, Th1, NK, Treg, $CD8^+$ T, DC, neutrophil	Type 1 adaptive immunity
CCR6	Th17 > iDC, γδ T, NKT, NK, Treg, Tfh	iDC trafficking; GALT development, Th17 adaptive immune responses
CCR7	naive T, T_{CM}, T_{RCM}, mDC, B cell	mDC, B cell, and T cell trafficking in lymphoid tissue to T cell zone, egress of DC and T cells from tissue
CCR8	Th2, Treg, skin T_{RM}, γδ T, monocyte, macrophage	Immune surveillance in skin, type 2 adaptive immunity, thymopoiesis
CCR9	Gut-homing T, thymocytes, B, DC, pDC	Homing of T cells to gut, GALT development and function, thymopoiesis
CCR10	Skin-homing T cell, IgA-plasmablasts	Humoral immunity at mucosal sites, immune surveillance in skin
XCR1	Cross-presenting $CD8^+$ DC, thymic DC	Antigen cross-presentation by $CD8^+$ DCs
CX3CR1	Resident monocyte, macrophage, microglia, Th1, $CD8^+$ T_{EM}, NK, γδ T cell, DC	Patrolling monocytes in innate immunity, microglial cell and NK cell migration, type 1 adaptive immunity
Atypical (nonsignaling) receptors		
ACKR1 (DARC; Duffy)	RBC, LEC	Chemokine transcytosis, chemokine scavenging
ACKR2 (D6)	LEC, DC, B cell	Chemokine scavenging
ACKR3 (CXCR7)	Stromal cells, B cell	Shaping chemokine gradients for CXCR4
ACKR4 (CCRL1; CCX-CKR)	Thymic epithelium	Chemokine scavenging

[a]Table is modified from Reference 256.

[b]Abbreviations: DC, dendritic cell; GALT, gut-associated lymphoid tissue; iDC, immature dendritic cell; ILC, innate lymphoid cell; LEC, lymphatic endothelium; NK, natural killer; NKT, natural killer T; RBC, red blood cell; T_{CM}, central memory T cell; T_{EFF}, effector T cell; T_{EM}, effector-memory T cell; Tfh, T follicular helper cell; Tfr, follicular regulatory T cell; Th, T helper; T_{RCM}, recirculating memory T cell; Treg, regulatory T cell; T_{RM}, resident-memory T cell; > indicates higher relative level of receptor expression.

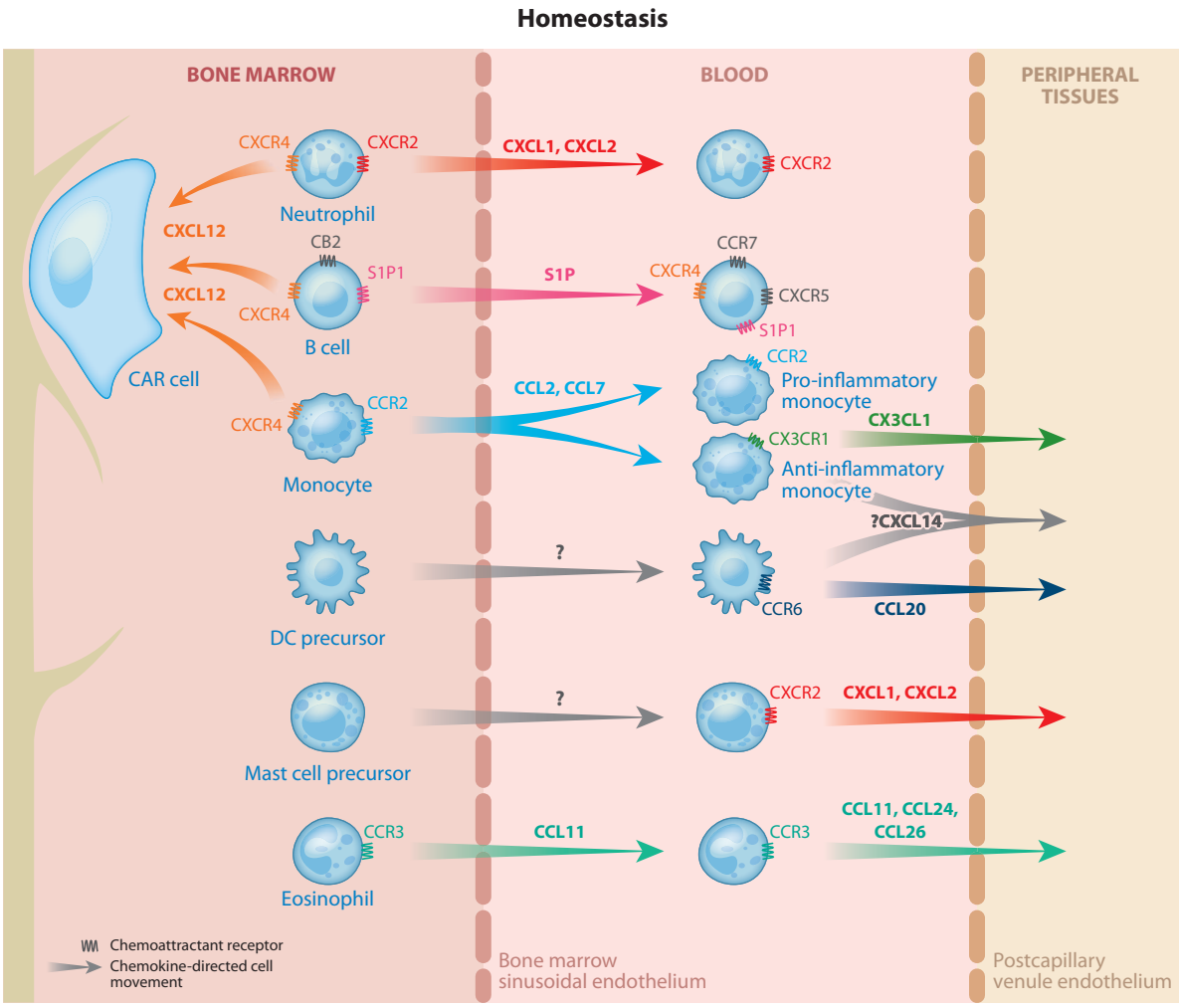

Figure 1

Chemokines control homeostatic immune cell trafficking between the bone marrow, blood, and peripheral tissues. Developing neutrophils, B cells, and monocytes are retained in the bone marrow by CAR cell–derived CXCL12. DC precursors, mast cell precursors, and developing eosinophils are retained in the bone marrow by unknown mechanisms. In the absence of CXCR4 signaling or in the presence of CXCR2 signaling, neutrophils exit the bone marrow and enter the blood. B cells enter the bone marrow sinusoids via CB2 signaling and exit into the blood via S1P1 signaling. B cells can then enter lymphoid structures utilizing CCR7, CXCR4, and CXCR5 signals. Monocytes enter the blood in response to CCR2 signaling as well as decreased CXCR4 signaling. Monocytes differentiate into pro-inflammatory ($CCR2^+$) and anti-inflammatory ($CX3CR1^+$) monocytes. Anti-inflammatory monocytes can enter the periphery by following CX3CL1 gradients. DC precursors enter the blood by unknown mechanisms and can exit into the periphery by following CCL20 gradients. In humans, CXCL14 gradients may also play a role in the migration of anti-inflammatory monocytes and DC precursors into the peripheral tissues. Mast cell precursors exit the bone marrow via unknown mechanisms and migrate into the gut following CXCR2-mediated signals. Eosinophils enter the blood and exit into the peripheral tissues following CCR3 signaling via CCL11 and CCL24 (humans and mice) as well as CCL26 (humans). (Abbreviations: CAR, CXCL12 abundant reticular; CB2, cannabinoid receptor 2; DC, dendritic cell; S1P1: sphingosine-1-phosphate receptor 1.)

monocyte subsets, early monocytes are a homogeneous subset with expression of both CXCR4 and CCR2 (16). CXCL12 interacts with CXCR4 to retain monocytes in the bone marrow. Blockade of CXCR4 induces a small increase in the number of peripheral blood monocytes (17). This is in contrast with the robust release of granulocytes induced by CXCR4 blockade and indicates that another signal is necessary for monocyte mobilization. That other signal appears to be mediated by CCR2. Loss of CCR2-mediated signals prevents the exit of monocytes from the bone marrow; in mice deficient in CCR2 or its ligands CCL2 and CCL7, or in the case of pharmacological blockade of CCR2, decreases occur in circulating monocytes, with concomitant increases in bone marrow monocytes (17, 18). Therefore, monocyte mobilization from the bone marrow appears to be governed by opposing forces of CXCR4- and CCR2-mediated signaling. The main source of CCR2 ligands under homeostatic conditions remains unclear.

Homeostatic control of B cell residence and release from the bone marrow also depends on CXCL12/CXCR4 interactions. B cell progenitor development is dependent on CXCR4, and conditional B cell CXCR4 deficiency leads to the premature exit of immature B cells from the bone marrow (19). This early exit, prior to functional maturation, is associated with reduced numbers of naive B cells as well as with defective B cell function (19). As developing B cells progress into immature B cells, they reduce CXCR4 levels and migrate, using the cannabinoid receptor 2, into the bone marrow sinusoids (20). Finally, exit of immature B cells into the peripheral blood is partially dependent on S1P1-mediated migration, given that the number of immature and naive B cells is reduced in the peripheral blood and spleen, but not in the lymph nodes (LNs), of B cell–specific S1P1-deficient mice (21).

SLO: secondary lymphoid organ

LTi: lymphoid tissue inducer

FDC: follicular dendritic cell

FRC: fibroblastic reticular cell

APC: antigen-presenting cell

CHEMOKINE CONTROL OF IMMUNE CELL LOCALIZATION IN SECONDARY LYMPHOID ORGANS

Secondary lymphoid organs (SLOs) include the LNs, spleen, and Peyer's patches. SLOs develop in specific locations along the lymphatics, where hematopoietically derived lymphoid tissue inducer (LTi) cells cluster in response to CXCL13 production by mesenchymal cells. Activated via IL-7 and/or TRANCE/TRANCER interactions, LTi cells express lymphotoxin-$\alpha 1\beta 2$ ($LT\alpha 1\beta 2$), which binds to the lymphotoxin-β receptor ($LT\beta R$) on stromal organizer cells. This interaction induces stromal organizer cells to upregulate expression of adhesion molecules and chemokines (specifically CCL19, CCL21, and CXCL13), and this upregulation promotes the attraction and entry of immune cells into the nascent LNs. Together, the combination of LTi cells and the chemokines induced by them provide the necessary signals for development and maintenance of SLOs. Mice deficient in LTi cells do not develop SLOs, whereas mice with deficiencies in downstream chemokines have defective SLO development, albeit to lesser degrees (22). These observations have solidified a role for the LTi cell as the master inducer of SLO development, with chemokines playing a downstream role in mediating cellular recruitment and the architectural separation of cell types.

In mature SLOs, the homeostatic production of chemokines plays an essential role in maintaining the architecture of SLOs. Networks of follicular dendritic cells (FDCs) in the B cell follicles produce CXCL13, which promotes the homeostatic localization of B cells via CXCR5. Within the spleen, marginal zone B cells depend on CXCR7 expression for their homeostatic positioning. Treatment of mice with CXCR7 antagonists leads to decreased numbers of splenic marginal zone B cells (23). At the same time, fibroblastic reticular cells (FRCs) within the T cell area produce CCL19, CCL21, and CXCL12, which promote the entry and localization of T cells and dendritic cells (DCs) in this region via CCR7 and CXCR4. Thus, even in the absence of an immune response, naive lymphocytes and antigen-presenting cells (APCs) actively migrate and are

retained in the SLOs. As we discuss below, this localization can be enhanced during an immune response. Activated DCs can directly produce soluble CCL19 and surface-bound CCL21, and they can promote further CCL21 production by FRCs. Together, this promotes further influx of CCR7-positive cells, thereby promoting cellular interactions (24).

TLO: tertiary lymphoid organ

iBALT: inducible bronchus-associated lymphoid tissue

CHEMOKINE CONTROL OF IMMUNE CELL LOCALIZATION IN TERTIARY LYMPHOID ORGANS

Tertiary lymphoid organs (TLOs) are characterized by the organization of distinct T and B cell zones and by their location in the periphery at sites of chronic inflammation. Unlike LTi cells' central role in inducing SLO development, their role in TLO development is unclear. Although LTi cells can be seen in association with TLOs, TLOs can still develop in the absence of LTi cells (25). In a model of chronic intranasal lipopolysaccharide (LPS) administration, mice developed inducible bronchus-associated lymphoid tissue (iBALT) independently of LTi cells (though they were induced to migrate to the site) as well as independently of CCR2 and CCR6. Instead, LTα-LTβR interactions as well as CXCL13, CCL19, and CCL21 were necessary for organization of iBALT. LPS induced IL-17 production, which promoted CXCL13 and CCL19 expression (25). This study underscores an important role for inflammatory cytokines in directly inducing chemokine expression, sidestepping the LTi cell. Similar results have been seen in other model systems of cytokine overexpression (26). Inflammatory cytokines induce expression of the lymphocyte-attracting chemokines CXCL13 and CCL19, which act together to promote lymphocyte entry into TLOs. LTα1β2 expression on recruited lymphocytes (or DCs) can then bind to LTβR on stromal cells, stabilizing and promoting further chemokine production and TLO induction and providing a positive feedback loop independent of further inflammatory cytokine production. The existence of a cytokine-free positive feedback loop is underscored in studies in which TLO development has been engineered by ectopic expression of lymphotoxin, CXCL13, CCL19, or CCL21 (27). Thus, the primary role for chemokines in initiating, promoting, and ultimately enforcing TLO formation is becoming clearer. Given the role of TLOs in local immune activation in infection and pathological autoimmune responses, this chemokine feedback loop may present a fruitful area for targeting and attenuating autoimmune responses.

CHEMOKINE CONTROL OF HOMEOSTATIC IMMUNE CELL LOCALIZATION IN THE PERIPHERY

Effective immune surveillance depends on the localization of immune cells throughout the body, not just in lymphoid organs. Chemokines appear to play a role in this homeostatic localization, although the precise mechanisms remain unclear given that many of the experimental methods used to study immune cell homeostasis depend on perturbing the host's homeostasis. However, there appear to be general pathways utilized by cells that remain in the peripheral tissues, blood, or the lymphoid organs (**Figure 1**). These stereotypical pathways are discussed below, as are recent insights into chemokine control of immune cell homeostasis.

Neutrophils

After release from the bone marrow, neutrophils remain in the blood, where they await inflammatory stimuli that would promote their migration into peripheral tissue. Neutrophils are also seen in the spleen, liver, and lung at steady state, although it is unclear whether they actively home to these tissues or are simply seen there because of the highly vascularized state of those organs.

Neutrophils have been reported to be specifically sequestered in the capillaries of these tissues, and recent data using multiphoton intravital microscopy of the lung showed evidence for homeostatic entry of neutrophils into the lung interstitium (28). The concept of sentinel neutrophils being recruited into peripheral tissue in homeostasis is intriguing, and in homeostasis neutrophils may be actively crawling along and patrolling the lung vasculature, but not the spleen or liver (29). These marginated neutrophils may be retained in the lung vasculature via CXCR4. A recent study showed that plerixafor, a CXCR4 antagonist, promotes the release of marginated neutrophils from the lung (30). CXCR4 may also control neutrophil elimination. Neutrophils persist for only hours in the peripheral blood, so many neutrophils must be eliminated from the peripheral blood on a routine basis. Neutrophils increase their expression of CXCR4 with senescence, which leads to their entry back into the bone marrow, where they ultimately undergo apoptotic cell death (31). Whether this occurs in specific bone marrow niches or in association with other cells remains unknown.

LTB_4: leukotriene B_4

Eosinophils and Basophils

Under homeostatic conditions, eosinophils are sparsely scattered throughout the peripheral tissues and blood, with the gastrointestinal tract making up the main reservoir of eosinophils. Eosinophils can first be found within the gastrointestinal tract at embryonic day 19, and they continue to populate peripheral tissues throughout adult life. This baseline migration into the periphery is largely dependent on the production of CCL11 (eotaxin-1) by stromal cells and immune cells (32). CCL11 promotes not only eosinophil residence in peripheral tissues but also their release from the bone marrow (33). CCL11 binds to CCR3, which is highly expressed on human and murine eosinophils. CCR3 is also expressed on human, but not murine, basophils. Human and murine basophils also express CCR2 (34). However, the role of chemokines in the homeostatic localization of basophils remains unknown.

Mast Cells

Mast cells are found throughout all vascularized tissues, where they play roles in acute inflammation and as effector cells in type 2 immune responses. Although mast cells are generally assumed to be long-lived cells in the periphery, they are continually replenished from mast cell precursors that exit the bone marrow and home to peripheral sites, where they differentiate into connective tissue and mucosal mast cells. Mast cell precursors express CXCR2, CXCR4, CCR3, and CCR5 (35). These precursors likely follow epithelial- and fibroblast-derived stem cell factor (SCF) for entry into peripheral tissues. SCF binds to the c-kit receptor on mast cells and mast cell precursors, activating them and inducing their chemotaxis independent of PTX blockade of G protein–coupled signaling (36). There may be additional tissue-specific signals. Intestinal homing of mast cell precursors depends on their expression of the $\alpha4\beta7$ integrin and CXCR2 (37). However, CXCR2 deficiency leads to defects only in intestinal mast cells, which may indicate that different chemokines may be required for their entrance into other peripheral tissues. The precise chemokines are unclear, given that chemokine receptor expression varies greatly among the in vivo subsets (38). What is clear is that local production of CXCR2 and c-kit ligands, as well as of inflammatory mediators such as leukotriene D_4 (LTD_4) and B_4 (LTB_4), will induce entry of mast cell precursors across the endothelium into the peripheral tissues (39). Whether additional chemokine signals exist remains unclear.

Monocytes

Once monocytes exit the bone marrow, they can be divided into proinflammatory and anti-inflammatory subsets based on their expression of CCR2 and CX3CR1, respectively. Using

an adoptive transfer model, the proinflammatory ($CCR2^+$) monocyte population was shown to be largely restricted to the peripheral blood and spleen. Conversely, the anti-inflammatory ($CX3CR1^+$) population was distributed in the peripheral blood and nonlymphoid organs of recipient mice (40). This is likely because of differential homeostatic expression of CCR2 and CX3CR1 ligands; in the absence of inflammation, there is little expression of CCR2 ligands in the periphery, whereas CX3CL1 is homeostatically expressed by endothelium in various tissues (41). $CX3CR1^+$ monocytes may follow additional chemokine signals as they enter the periphery and develop into tissue macrophages under homeostatic conditions. CXCL14 is homeostatically produced by human fibroblasts in the skin and lamina propria, and tissue macrophages can be found in close association with CXCL14-producing cells (42). However, the full role of CXCL14 and its receptor, which remains to be identified, is not yet fully appreciated.

Dendritic Cells

Under homeostatic conditions, DCs are thought to develop in situ from specific DC precursor cells that first populate the periphery. The chemokines that regulate transit of DC precursors into peripheral tissues such as the skin and gut epithelium are largely unknown, although CCL2, CXCL14, and CCL20 have been implicated. Mice deficient in CCR2 on hematopoietic cells have decreased numbers of dermal langerin-positive DCs (43). However, langerin-negative dermal DCs are still present and functional, indicating that additional chemokine pathways guide DC precursors. In humans, CXCL14 is highly expressed in healthy skin around dermal blood vessels and epidermal keratinocytes and promotes chemotaxis of monocytes and DC precursors (44). CXCL14 attracted $CD14^+$ DC precursors into in vitro–derived human epidermal equivalents, where they differentiated into langerin-positive DCs (44). Further characterization of this pathway has been difficult, though, because mouse CXCL14 does not appear to play the same role. CXCL14-deficient mice have normal homeostatic development and placement of macrophages, monocytes, and DCs (45). Finally, CCL20 has been shown to promote migration of $CD14^+$ cells and in vitro–derived Langerhans cells, and CCR6-deficient mice lack DCs in the subepithelial dome of Peyer's patches (46). However, homeostatic positioning within the skin is unaffected, indicating that this may be important in the setting of inflammation or microbial stimulation (47).

Once DCs are in the periphery, it is unclear whether their retention is an active or passive process. Immature, resident DCs express various chemokine receptors (e.g., CCR1, CCR2, CCR5, CCR6, CXCR1, CXCR2, and CXCR4) that may play roles in the peripheral retention of DCs, but their precise roles remain unclear (48). Chemokine-mediated guidance into the draining LN is better understood. Under homeostatic conditions, DCs use a CCR7-dependent process to migrate via the afferent lymphatics into the draining LN (48). In skin-draining LNs, CCR7 is necessary for migration of the dermal langerin-positive DC subset (49). Similarly, gastrointestinal and lung-resident DCs spontaneously migrate into mesenteric and mediastinal LNs, respectively, via a CCR7-dependent process, underscoring the role of CCR7 in homeostatic migration of peripheral DCs (50). These populations have been described as semimature owing to their increased surface expression of MHC class II and CCR7. CCR7 deficiency does not completely block other DC subsets from entering the draining LN, however, which may reflect different sources for these DCs (i.e., blood) (49). Once in the draining LN, the ultimate role of these semimature DCs is unknown, although evidence suggests that they play important roles in tolerance to inhaled and ingested antigens (50).

Lymphatic vessels in peripheral tissues (i.e., nonlymphoid organs) produce CCL21-Leu, which promotes migration of $CCR7^+$ cells first into the lymphatic capillaries and then through increasingly large lymphatic vessels as they approach the draining LN. Unlike migration from the

comparatively fast-flowing (i.e., high shear flow) bloodstream, this migration may not be dependent on integrins, given that subcutaneously injected pan-integrin-deficient DCs are able to migrate normally into the draining LN (51). Once in the lymphatics, CCL21-Ser and CCL19 appear to play an additive role in DC migration into the subcapsular sinus of the draining LN; decreased migratory DCs are found in the LNs of *plt/plt* mice, which lack CCL21-Ser and CCL19 (52). Once in the subcapsular sinus, DCs then follow CCL19 and CCL21-Ser gradients, which are produced homeostatically by FRCs in the LN and promote localization of DCs into the T cell area. It remains unclear whether these ligands provide overlapping or distinct signals. Experiments using 3-D microfluidic systems indicate that DCs will preferentially migrate toward the bound CCL21 if presented with equal amounts of CCL19 and CCL21 (53). Whether this is relevant in vivo remains to be determined.

HEV: high endothelial venule

DCs can be divided into multiple subsets based on their differential expression of cell-surface markers, their function, and their location within the host. Just as CCR7 guides DCs into the T cell zones where they can interact with and induce T cell responses, the EBI2 receptor also plays a role in positioning of DC subsets. EBI2 is the receptor for 7α,25-dihydroxycholesterol, and under steady-state conditions it is highly expressed on splenic $CD4^+$ DCs and is expressed on other subsets after LPS stimulation. Expression of EBI2 is necessary for normal positioning of $CD4^+$ DCs in the bridging channels of the spleen and the subsequent immune response to particulate antigens (54). This underscores the importance of homeostatic positioning of DCs, given that defective positioning of these splenic $CD4^+$ DCs leads to defective adaptive immune priming. Further studies are necessary to illustrate the interactions and relative roles of CCR7- and EBI2-mediated signaling in DC function and placement.

As previously discussed, S1P interacts with the S1P1 receptor to promote egress of lymphocytes from the primary lymphoid organs under homeostatic conditions. Its role in DC homeostasis is less clear. Mature DCs express S1P1 and migrate toward S1P. In vivo, blockade of S1P1-mediated signaling does not impact the number of splenic DCs but does lead to modest decreases in migration of mature skin-resident DCs to the draining LN after fluorescein isothiocyanate (FITC) skin painting (55). In the murine spleen, S1P1 blockade leads to the redistribution of immature $CD4^+$ DCs from the bridging channels to the marginal zone, but it does not affect the localization of mature DCs into the T cell area (55). Thus, S1P1 interactions may promote distinct DC localizations in different lymphoid organs.

Lymphocytes

Naive lymphocytes constantly circulate from the blood to the SLOs and back into the blood. As has been extensively reviewed, their recirculation, location in SLO, and function are exquisitely dependent on chemokine and integrin interactions (56). Most naive lymphocytes enter the LNs through the high endothelial venules (HEVs). Naive T cells express CCR7 and CXCR4. CCR7 binds to CCL21, which is produced by the HEV and presented on the luminal endothelium, and to CCL19, which is produced by the FRC network and transcytosed across the HEV for presentation on the luminal endothelium. Mice deficient in either CCR7 or CCL19 and CCL21 expression exhibit two- to fivefold decreases in naive T cells in the LN (57, 58). CXCR4 on naive T cells also promotes LN entry by binding to CXCL12, which is produced by the FRCs and transcytosed and presented on the HEV lumen. This is a minor signal compared with CCR7, as mice deficient in CXCR4 exhibit defects in naive T cell homing only in the absence of CCR7 signaling (59). Once they have entered the LN, T cells follow gradients of CCL19 and CCL21 into the T cell area, which allows them to scan DCs (56). Prolonged signaling of CCL19 via CCR7 ultimately leads to downregulation of that receptor. In the absence of CCR7-mediated retention signals,

T cells migrate toward S1P gradients, which lead them out of the LN into the efferent lymph. Once in the efferent lymph and/or the blood, S1P1 is desensitized by sustained S1P signaling and CCR7 is upregulated again, allowing the cycle to begin anew (56).

Naive B cells express CCR7, CXCR4, and CXCR5. Unlike naive T cells, B cells show only a modest defect in LN trafficking in the absence of CCR7-mediated signaling (58). CXCR4-deficient B cells can normally enter the LN; however, B cells deficient in both CXCR4 and CCR7 signaling display defects in LN homing owing to defective attachment to the HEV (59, 60). This defect is not complete, indicating that another chemokine may promote LN entry. Naive B cells also express CXCR5, which binds to the CXCL13 that is produced by FDCs and can be presented after transcytosis by the HEV (61). CXCR5-deficient B cells are largely restricted from the Peyer's patches, but this appears to play little to no role in B cell homing to other SLOs (59, 60). Once they have entered the LN, B cells migrate to follicles in response to CXCL13 gradients (60). Just like T cells, continued signaling via CXCR5 promotes its downregulation, while at the same time S1P1 expression increases, thus promoting B cell exit. In addition to S1P, CXCR4 also plays a role in B cell exit from the Peyer's patches (62). Whether other chemokines may play a similar role for B cell exit from other lymphoid organs remains to be determined.

Treg: regulatory T cell

T_{CM}: central memory T cell

T_{EM}: effector memory T cell

ILC: innate lymphoid cell

NKT: natural killer T

Regulatory T Cells

The Foxp3$^+$ regulatory T cells (Tregs) are important mediators of immune responses. In the absence of infection or inflammation, Tregs are widely distributed throughout most lymphoid and nonlymphoid tissues (63). In mice, disruption of chemokine receptor expression can change this widespread distribution and result in pathogenic inflammatory responses. For instance, deletion of CCR4 in Tregs prevents their homeostatic accumulation in the skin and lung and results in inflammation in these specific organs (63). In humans, the baseline expression of chemokine receptors on circulating Tregs changes with development. In neonatal cord blood, most Tregs are naive and express the LN-homing receptors CCR7, CXCR4, and L-selectin as well as the gut-homing receptors CCR9 and $\alpha4\beta7$ (64, 65). By three years of age, most Tregs in human blood appear to have a memory phenotype and, similar to memory T cells, can be found as either CCR7- and L-selectin-positive central memory T (T_{CM})-like cells or CCR7-negative effector memory T (T_{EM})-like cells. These memory Tregs have mostly downregulated the gut-homing CCR9 and $\alpha4\beta7$ and instead have acquired skin-, lung-, and inflammation-homing chemokine receptors, such as CXCR3, CCR2, CCR4, CCR5, CCR6, and CCR8 (64, 65). This change from naive and LN- and gut-homing to memory and skin-, lung-, and inflammatory-homing phenotypes suggests that in the first few years of life many circulating Tregs encounter self or microbial antigens in the gut and then are either sequestered in the gut or are activated in such a way that circulating Tregs acquire a skin-, lung-, and inflammation-homing phenotype over time (65).

Innate Lymphocytes

Innate lymphocytes include the newly expanding group of innate lymphoid cells (ILCs) as well as natural killer T (NKT) cells and $\gamma\delta$ T cells. ILCs are a group of innate lymphocyte–like cells that are notable for not undergoing recombination of antigen receptors and clonal selection. These cells play important roles in bridging the innate and adaptive immune responses and are generally found throughout the periphery, taking part in immune surveillance. Much remains to be determined about the role of chemokines in directing the homeostatic and inflammatory migration patterns of ILCs, but certain things are known. Group 1 ILCs include ILC1 and the classical NK cells. As has been recently reviewed by others, human NK cells express CXCR1, CXCR2, CXCR3,

CXCR4, and CX3CR1, which promote their homeostatic localization in peripheral tissues as well as their migration under inflammatory conditions (66, 67). CXCR4, and possibly CX3CR1, provide important signals for NK cell retention within the bone marrow. Their egress is promoted by S1P5 signaling and loss of CXCR4-mediated retention (68, 69). Whether the mature NK cell remains within the bloodstream or populates the periphery under homeostatic conditions may depend on the expression of specific chemokine receptors. CXCR6 is expressed on hepatic NK cells and may be important for their homing and maintenance within the liver (67). Alternatively, $CD56^{high}$ NK cells also express CCR7, allowing for homeostatic migration to the LNs (70).

PRR: pattern-recognition receptor

Less is known about the chemokine guidance of other innate lymphocytes. Group 2 ILCs include ILC2, also referred to as natural helper cells or nuocytes. ILC2 express CXCR4 as well as CXCR6 and CCR9, which promote their homeostatic distribution in the skin and gut, respectively (71, 72). Group 3 ILCs include ILC3 and LTi cells. LTi cells express CXCR5 and CCR6, and their CCR6 expression allows LTi cells to migrate into the intestinal epithelium in response to epithelial-derived CCL20 and β-defensins, which are produced in response to commensal bacteria (73). CD1d-restricted NKT cells express CXCR3, CXCR6, CCR5, and CCR6 (74). CXCR6 promotes NKT cell residence in peripheral tissues including the liver and the lung, whereas the other chemokine receptors may more generally target NKT cells to sites of inflammation (75). Likewise, CCR8 expression on dermal-resident γδ T cells promotes their residence in the skin, while circulating γδ T cells express the inflammatory chemokine receptors CCR1, CCR2, CCR3, CCR5, CXCR1, CXCR2, and CXCR3 (76). Both their peripheral localization and homeostatic expression of inflammatory chemokine receptors make innate lymphocytes primed to respond to acute inflammation.

CHEMOKINE CONTROL OF INNATE RESPONSES DURING ACUTE INFLAMMATION

Acute inflammation is a complex process characterized by the coordinated movement of effector cells to the site of inflammation as well as the exit of immune cells from peripheral sites to the draining lymphoid organs to initiate the immune response. Such a coordinated movement of cells requires the induced expression of inflammatory chemokines and their respective receptors on target cells. This process is tied to immune cell activation and first begins at the site of pathogen encounter. For the purposes of this review, we explore the role of chemokines during an acute inflammatory response in a stereotypical peripheral tissue (**Figure 2**).

Resident Immune Cells

Mast cells play an important role in acute inflammation because they express a wide variety of pattern-recognition receptors (PRRs) and contain large granules of preformed inflammatory mediators. This allows them to immediately respond to inflammatory signals as opposed to requiring transcriptional activation and production of mediators. Mast cell granules are heterogeneous and can undergo "piecemeal degranulation," meaning that specific granule components can be released depending on the stimulus (i.e., FcεRI versus PRR activation). In mouse models of airway hyperreactivity, mast cells have been shown to release CCL1, which induces migration of CCR8-expressing Th2 effector cells to the airway epithelium (77). Alternatively, LPS stimulation of murine peritoneal mast cells leads to immediate release of CXCL1- and CXCL2-containing granules, but not histamine-containing granules, as well as to transcriptional activation of CXCL1 and CXCL2. This promotes early neutrophil recruitment that is abolished in mast cell–depleted mice, but not macrophage-depleted mice (78). However, neutrophil recruitment is normal at 4 h

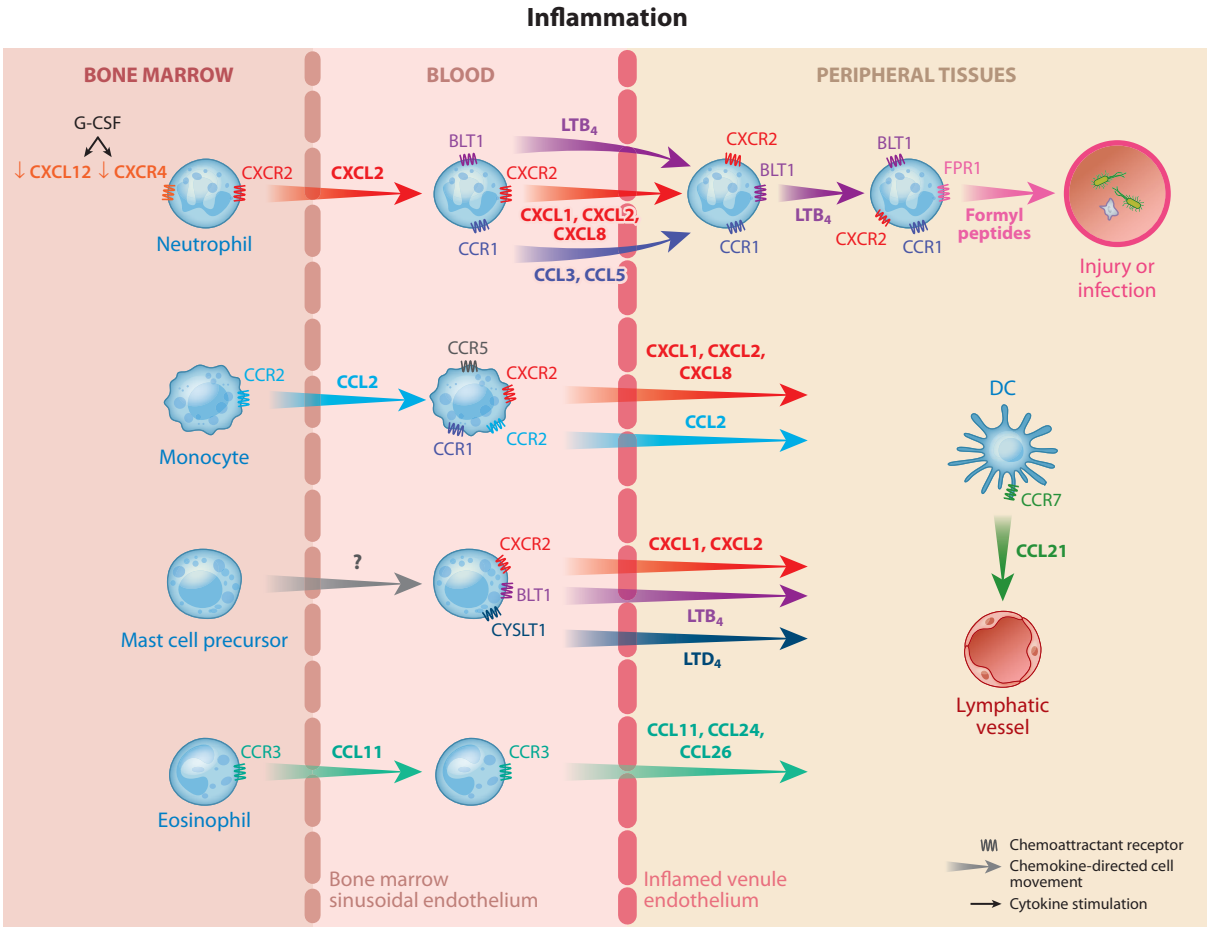

Figure 2

Chemokines control innate immune cell trafficking between the bone marrow, blood, and peripheral tissues in inflammation. During inflammation, systemic levels of G-CSF lead to decreased CXCL12 production by the bone marrow and to decreased CXCR4 expression by developing neutrophils. Loss of CXCR4-mediated retention and the presence of CXCR2-mediated exit signals promote the release of neutrophils into the blood. The BLT1, CCR1 (or CXCR1 in humans), and CXCR2 receptors on neutrophils bind to their ligands, which are soluble or bound to glycosaminoglycans on the luminal surface of the endothelium. This signaling promotes the exit of neutrophils into the peripheral tissues. Once in the periphery, activated neutrophils promote additional neutrophil entry by releasing CXCR2 ligands themselves as well as cytokines (*not shown*), which activates the endothelium to express additional CCR1 and CXCR2 ligands. In the tissue, neutrophils may follow CCR1 (mouse), CXCR1 (humans), CXCR2, and BLT1 signaling to the source of infection or injury. Additionally, exposure to bacterial or mitochondrial formyl peptides may promote neutrophil entry to sites of infection or cell death. Monocytes migrate out of the bone marrow by following CCL2 gradients that may be produced in response to systemic pathogen-associated molecular patterns such as TLR ligands. Monocytes adhere to inflamed endothelium via CXCR2 signals and migrate to the periphery by following CCR2 signaling. Mast cell precursors are able to migrate to the periphery via CXCR2, BLT1, or CYSLT1 signaling. Eosinophils leave the bone marrow and migrate to the periphery via CCR3 signaling. Finally, mature tissue-resident DCs upregulate CCR7 and migrate into afferent lymphatic vessels by following CCL21 gradients to exit peripheral tissue. (Abbreviations: BLT1, leukotriene B_4 receptor 1; CYSLT1: cysteinyl leukotriene receptor 1.)

after LPS injection, indicating that macrophage-mediated production is sufficient to promote neutrophil influx by that time (78).

Mast cell degranulation provides an important immediate source of chemokines to initiate neutrophil recruitment. However, the amount of preformed mediators is limited; sustained neutrophil influx, therefore, depends on the transcriptional production and release of chemokines. Although epithelium and stromal cells express PRRs, tissue-resident macrophages, DCs, and mast cells appear to be the major sources of acute inflammatory cytokines and chemokines. PRR stimulation of murine macrophages leads to production of a wide range of chemokines including, but not limited to, CXCL1, CXCL2, CXCL8, CCL2, CCL3, CCL4, and CCL5. In response to various stimuli, mast cells have been shown to produce CCL2, CCL3, CCL4, CCL5, CCL11, CCL20, CXCL1, CXCL2, CXCL8, CXCL9, CXCL10, and CXCL11 (79). Finally, viral exposure of DCs leads to production of many of the same chemokines as well as CXCL16 (80). Depletion of any of these cell types results in impaired inflammatory cell migration, although the degree of impairment is dependent on the model used. Whether this inflammatory chemokine production is universal or specific to certain pathogens (i.e., bacterial versus helminth infection) remains unclear.

EAE: experimental autoimmune encephalitis

Another source of chemokine production in the tissue occurs in response to inflammatory cytokines that are released by local immune cells. In addition to chemokines, acute inflammatory cytokines such as TNF and IL-1 are released by activated mast cells, DCs, and macrophages. These cytokines can stimulate other local immune cells or activate local epithelial cells. Cytokine-activated epithelium can produce a host of chemokines, including CCL2, CCL3, CCL4, CCL5, CXCL1, CXCL2, CXCL3, CXCL5, and CXCL8 (81). At the same time, cytokines can alter chemokine production and expression by local endothelial cells. In experimental autoimmune encephalitis (EAE), IL-17 stimulation of brain endothelial cells leads to abluminal expression of CXCR7, which acts as a "sink" for CXCL12. In the absence of endothelial CXCL12, leukocytes can enter the brain and induce disease pathogenesis (82). Together, the release of preformed chemokines from mast cells and the induction of chemokines by PRRs and cytokines promote immune cell entry until the resolution of the inflammatory stimulus.

Neutrophils

One of the earliest cells targeted by the chemokines of acute inflammation is the neutrophil. Neutrophils express many chemokine receptors, including CXCR2 and CCR1 (and CXCR1 in humans). Neutrophils also express chemotactic receptors for complement, lipid mediators, and bacterial products such as *N*-formyl-methionyl-leucyl-phenylalanine. Together, these receptors promote motility, provide directional information, and may promote neutrophil effector function. However, chemokines also appear to play an essential role in mediating neutrophil release in response to peripheral inflammation. Neutrophils persist in the periphery for only about 6 h. Therefore, increased release of neutrophils from the bone marrow as well as increased myelopoiesis are necessary to sustain normal neutrophil numbers during an infection. In the setting of inflammation, systemic levels of G-CSF increase, which promotes increased neutrophil production as well as bone marrow exit (83). G-CSF treatment decreases CXCL12 production in the bone marrow and CXCR4 expression on neutrophils, thus interrupting CXCL12/CXCR4-based neutrophil retention (84, 85). G-CSF also promotes increased expression of CXCL2 in bone marrow endothelium, actively promoting neutrophil exit (13).

Once in the peripheral blood, neutrophils migrate to areas of acute inflammation by first interacting with and then transmigrating across the activated endothelium. Endothelial cells are activated by local production of inflammatory cytokines, such as TNF, IL-1, and IL-17, and this activation leads to activation-induced expression of P-selectin, E-selectin, and integrins.

ACKR: atypical chemokine receptor

BLT1: leukotriene B_4 receptor 1

Neutrophils bind to these selectins and integrins, slowing their movement and causing them to roll along the endothelium. Neutrophil adhesion then occurs when the rolling neutrophils bind to CXCL1, CXCL2, or CXCL8 on the luminal surface of the endothelium. There are two established sources for these luminal chemokines. First, cytokine-activated endothelial cells can directly produce the chemokines. Alternatively, distantly produced chemokines that reach the basal surface of the endothelium can be taken up and transcytosed to the luminal surface. Thus, the endothelium is able to "pass on" the directional and cell-specific information produced by immune cells at the site of inflammation as well as amplify cellular entry by making its own chemokines. Such chemokine transcytosis by endothelial cells appears to be mediated at least in part by the Duffy antigen receptor for chemokines (DARC, also known as atypical chemokine receptor, or ACKR1) (86). Along with heparan sulfate, DARC presents bound chemokine on the luminal surface of the endothelium. This immobilization is necessary for chemokines to bind to and activate immune cells and then to promote immune cell transmigration (87). This process is also under tight control; luminal immobilized chemokines are rapidly endocytosed and destroyed so that the endothelium can ultimately limit acute inflammatory cell entry (88). Although the entire endothelium may be permissive to inflammatory cell entry, recent data have shown that there are hotspots for neutrophil entry that correspond to the localization of pericytes (89). These pericytes may not only provide structural entry points, but may also upregulate adhesion molecules and produce chemokines after activation via their PRRs (90). This directly promotes immune cell migration and emphasizes an important role for pericytes as innate immune sensors and promoters of the acute inflammatory response.

Once they have transcytosed, neutrophils follow multiple chemoattractant gradients as they migrate through the interstitium to the acute inflammatory source. Chemokines make up one class of chemoattractants, but neutrophils can also respond to a wide variety of chemoattractant molecules that include lipid mediators, bacterial products, and complement fragments. All these chemoattractants play important roles in mediating directional migration, and recent work has shown that these agents may play important and nonredundant roles. Using a mouse model of autoimmune arthritis, we and others have shown that LTB_4 acting through its G protein–coupled signaling receptor BLT1 on neutrophils is necessary for neutrophil recruitment into the joint (91, 92). However, LTB_4 is essential only for the initial entry of neutrophils into the joint. Once inside the joint, neutrophils are activated by immune complexes to produce IL-1β. This inflammatory cytokine then activates local synoviocytes as well as macrophages and endothelial cells to produce neutrophil-active chemokines, such as CCL3, CCL4, CCL5, CXCL1, and CXCL2. These chemokines bind to the CCR1 and CXCR2 receptors on neutrophils, promoting additional neutrophil entry and amplifying the acute inflammatory response (93).

The different classes of chemoattractants can amplify immune cell recruitment to the site of tissue injury by acting sequentially in time as well as in space. Chemokine gradients are formed when viable cells produce and release protein or lipid chemoattractants. But how do innate immune cells migrate to sites of necrotic injury where there are no viable cells? In a model of sterile thermal tissue injury, CXCL2 gradients form around the area of tissue injury and promote neutrophil chemotaxis toward the injury site (94). However, neutrophil chemotaxis from the surrounding area into the actual site of tissue necrosis occurred in response to endogenous formyl peptides. Endogenous formyl peptides are produced by mitochondria and can be released upon cellular damage (95). These mitochondrial formyl peptides bind to the formyl peptide receptor 1 (FPR1) and promote neutrophil chemotaxis to the site of cellular injury (94, 95). Spatial separation between chemoattractants may be sufficient for them to play sequential roles, but there may also be a hierarchy of chemotactic molecules. Formyl peptides and complement fragments may be able

to supersede signaling from chemokines by using alternative signaling pathways, such as p38 mitogen-activated protein kinase as opposed to phosphatidylinositol 3-kinase pathways (96).

In addition to creating sequential waves of neutrophil recruitment, different chemoattractant molecules may be able to amplify the directional signaling of other chemoattractants. FPR1 stimulation on neutrophils leads to neutrophil LTB_4 production, which then feeds back in an autocrine and paracrine fashion and promotes further neutrophil migration toward formyl peptides (97). Indeed, neutrophil-derived LTB_4 production was recently shown to be essential for the coordinated chemotaxis and clustering (i.e., swarming) of neutrophils at sites of tissue damage in vivo (98). Finally, although chemoattractant molecules can promote and amplify cellular migration, different forms of chemokines can also play distinct roles in migration. For example, CXCL8 can exist in both monomeric and dimeric forms. CXCL8 dimers reportedly promote initial neutrophil recruitment, whereas the monomers are responsible for sustained recruitment (99). This may be due to differential binding to CXCR2, although the precise mechanism remains to be determined.

After migrating to the inflammatory focus, neutrophils act as terminal effector cells. They remove pathogens via phagocytosis and restrict pathogens by functionally trapping them in extruded DNA that forms neutrophil extracellular traps. At the same time, they also produce cytokines and chemokines that promote additional inflammatory cell influx. Activated neutrophils produce CCL3, CCL4, CCL5, CCL20, CXCL1, CXCL8, CXCL9, and CXCL10. Thus, they can promote and amplify the initial acute inflammatory response by promoting the influx of additional immune cells (100).

Monocytes

Along with neutrophils, inflammatory monocytes are targeted early in the setting of acute inflammation by activated endothelium. Monocytes utilize different chemoattractant molecules for their migration, however. CCL2 and CCL7 are rapidly produced by stromal and immune cells after PRR activation or after cytokine stimulation. CCL2 dimerizes and binds to extracellular matrix glycosaminoglycans, thus establishing a stable gradient for $CCR2^+$ inflammatory monocytes (87). CCL2 and CCR2 interactions are essential for normal inflammatory monocyte migration into peripheral tissues (101). However, CCL2 may also direct monocyte recruitment far from the site of acute inflammation. Some CCL2 remains soluble and enters the draining LN via the afferent lymph, where it can bind to HEVs and induce inflammatory monocyte entry into the draining LN (102). CCL2 has also been detected in the serum during infection, where it may play a role in mobilizing inflammatory monocytes from the bone marrow (16). Given the mechanisms in place to remove excess systemic chemokines, it remains unclear whether enough systemic CCL2 reaches the bone marrow niche. An alternative hypothesis is that small amounts of pathogen-associated molecular patterns, such as Toll like receptor (TLR) ligands, enter the systemic circulation during infection and activate PRRs on bone marrow niche cells (103). This leads to local CCL2 production by bone marrow cells, which then promotes monocyte egress (103). Given the heterogeneity of acute inflammatory stimuli that induce monocyte egress, it is likely that multiple mechanisms contribute to the chemokine-dependent mobilization of inflammatory monocytes.

Inflammatory monocytes express many additional chemokine receptors that may play specific and nonredundant roles in adhesion and migration. For example, monocytes express CXCR2, but they do not migrate in response to CXCL8. Instead, CXCL8/CXCR2 interactions were shown to be necessary for the firm adhesion of monocytes to the vascular endothelium in an atheroma model (104). Subsequent chemoattractants, such as CCL2, then promote directional migration into the lesion. However, CXCR2 may promote chemotaxis when bound to its atypical ligand macrophage migration inhibitory factor (MIF). MIF binds to CD74 and CXCR2 on monocytes

and macrophages, leading to CXCR2 signaling and integrin-dependent chemotaxis of monocytes, which is necessary for the maintenance of atherosclerotic plaques (105). In addition to CXCR2, CCR1 and CCR5 can promote monocyte adhesion and transmigration across endothelial cells in vitro (106). Finally, transfer experiments have shown that CCR6 is necessary for the migration of inflammatory monocytes into cutaneous tissues after immunization (107). Nonetheless, the CCR6 ligand CCL20 does not induce monocyte migration in vitro, so the role of CCL20/CCR6 interactions in monocyte migration remains unclear (16).

The fractalkine receptor CX3CR1 is a well-established marker of anti-inflammatory or patrolling monocytes. However, it is also present to a lesser degree on proinflammatory monocytes. CX3CR1 plays an essential role in promoting integrin-mediated adhesion within the vessel, which allows for the patrolling phenotype of anti-inflammatory monocytes (108). CX3CR1 may also provide a prosurvival signal to anti-inflammatory monocytes (109). This role as a survival signal may underlie the proatherogenic role of CX3CL1 and the observation that polymorphisms associated with decreased CX3CR1 expression decrease the risk of atherogenesis (110). Finally, CX3CR1 may play a role in the normal function of inflammatory monocytes. CX3CR1 is necessary for early migration of inflammatory monocytes into the spleen after *Listeria* infection in a competitive adoptive transfer model (108).

Eosinophils

Although neutrophils and monocytes are the main constituents of the acute inflammatory response, many other cells are signaled to enter sites of acute inflammation; their influx may depend on exposure to precise stimuli. Eosinophils express the chemokine receptors CCR1 and CCR3, allowing them to respond to a wide variety of chemokines, including the eotaxins (CCL11, CCL24, and CCL26). In asthma models, CCL11, CCL24, and CCL26 are robustly induced in the lung upon exposure to IL-4 and IL-13 (111). These chemokines appear to play overlapping roles. CCR3 and CCL11 both promote eosinophil migration to peripheral tissues, as mice deficient in either show defects in eosinophil migration to the lung in allergic asthma models (112, 113). CCL24 and CCL26 also induce eosinophil migration, although some data suggest that CCL24 specifically promotes lung accumulation of eosinophils and that CCL26 may support late and prolonged eosinophil recruitment (114, 115). Eosinophil recruitment may be further promoted by thymic stromal lymphopoietin (TSLP). Eosinophils stimulated with TSLP increase their expression of ICAM-1 and CD18, thereby promoting eosinophil adhesion (116). Interestingly, TSLP-stimulated eosinophils also produce CCL2, CXCL1, and CXCL8, promoting further influx of inflammatory cells (116). To prevent excessive eosinophilic inflammation, CCL11 activity in vivo is regulated by CD26, a surface-associated protease. CD26 cleaves CCL11, leading to a protein that can bind and desensitize CCR3 but cannot activate it (117). This may play an important role in preventing excess inflammation, given that CD26-deficient mice exhibit enhanced inflammation in an OVA-induced asthma model (118).

Dendritic Cells

Once activated by inflammatory cytokines or PRR ligation, DCs undergo a maturation process, downregulate expression of immature chemokine receptors, and upregulate CCR7 expression (119). They follow stable gradients of CCL21, which is produced by the lymphatic endothelium and released into the periphery where it binds to glycosaminoglycans (120). CCR7-mediated signals increase the mobility and directional migration of mature DCs toward the lymphatic endothelium (121). There, DCs enter the lumen of the lymphatic vessel via gaps in the basement

membrane beneath the lymphatic endothelial cells (122). Once in the lumen, they crawl along following the direction of lymphatic flow until they reach the collecting lymphatics, at which point they freely flow with the lymph to the draining LN (121).

There are two main sources for mature DCs in the draining LN: peripheral DCs entering via the afferent lymph and monocyte-derived DCs entering via the HEV. Interstitial fluid from the inflamed periphery is rich in chemokines, and this chemokine-rich fluid flows into the afferent lymph and the draining LN. There, the chemokines are transported and displayed on the luminal side of the HEV, promoting inflammatory cell entry from the blood (102). This includes the entry of inflammatory monocytes via CCR2 that can differentiate into mature monocyte-derived DCs and appear to play an important role in Th1 differentiation (123). Thus, even in the absence of local chemokine production, the HEV can promote entry of inflammatory cells. This mechanism is likely to play an important role only early in the immune response, given that mature DCs produce large amounts of cytokines and chemokines to promote additional cell entry.

Innate Lymphocytes

As discussed above, innate lymphocytes express inflammatory chemokine receptors under homeostatic conditions, which allow them to rapidly migrate and respond to inflammatory stimuli. In murine models of *Toxoplasma gondii* and cytomegalovirus infection, NK cells migrated to sites of acute inflammation using the CCR5 receptor (124, 125). Likewise, CXCR3 promoted NK cell migration into the heart in an orthotopic transplant model and into the liver in a hepatitis model (126, 127). The infiltration of NK cells into peripheral tissue then serves as a feed-forward mechanism and promotes further cell migration via production of the IFN-γ-inducible CXCR3 ligands CXCL9 and CXCL10. Similarly, in a murine model of acute hepatitis, NKT cells migrated into the acutely inflamed liver via a CXCR6-dependent process, where they promoted local accumulation of monocytes and macrophages (128). Chemokines also link innate and adaptive immune responses. CX3CR1 expression on NK cells was necessary for their migration into the brain in a model of EAE (129). Blockade of this migration led to an enhanced disease phenotype, given that NK cells provide an early source of IFN-γ that inhibited the formation of pathogenic Th17 cells (130). Thus, chemokine-mediated cell migration may promote important cellular interactions between innate and adaptive immune cells.

CHEMOKINE CONTROL OF ADAPTIVE IMMUNE RESPONSES

The adaptive immune response is initiated in the SLOs with the arrival of antigen or mature DCs. In the LN and spleen, chemokines have an essential role in modulating this adaptive response by promoting the initial priming of lymphocytes and guiding their differentiation and phenotype. In the sections below, we review recent advances in the role of chemokines in $CD4^+$ and $CD8^+$ T cell priming and memory formation and in the activation of B cells (**Figure 3**).

Lymphocyte Patrolling in the Lymph Node

During the initiation of adaptive immunity, antigen-loaded DCs and naive T cells must localize to the same region. This colocalization is promoted by CCR7-mediated migration of mature DCs and T cells into the T cell area of the draining LN. However, the rarity of antigen-specific T cells requires naive T cells to be able to quickly scan and interact with many DCs scattered throughout the T cell region of the LN. Theoretically, the random walk migration of lymphocytes upon the FRC network could provide a "guided randomness" that would increase this scanning ability of

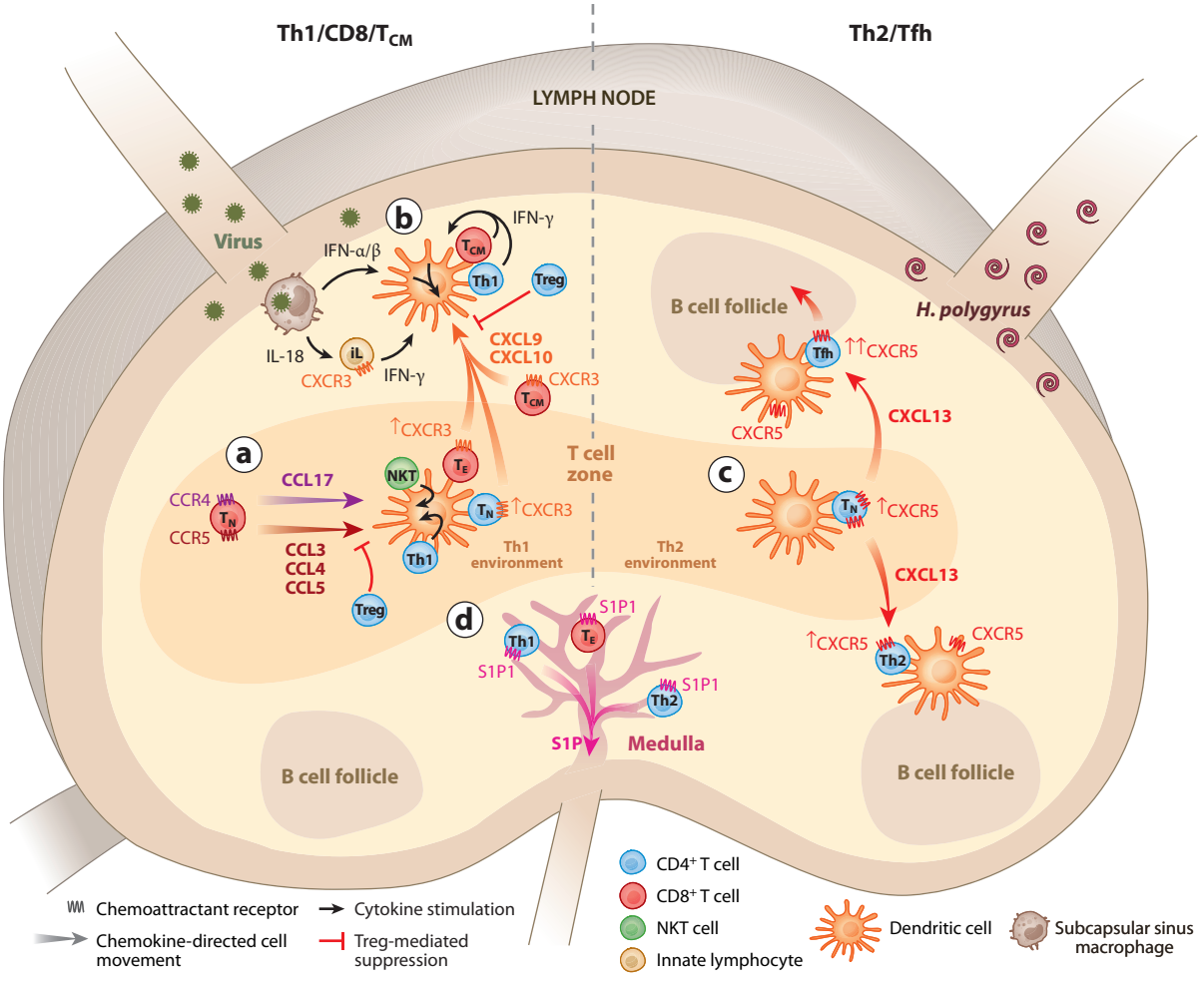

Figure 3

Chemokines guide the positioning of immune cells in the lymph node for the generation of primary and secondary immune responses. (*a*) During a primary immune response, CD8$^+$ T cells are guided to DCs that have been licensed by CD4$^+$ T cells, as well as possibly other CD8$^+$ T cells and activated natural killer T (NKT) cells. The chemokine signals that DCs produce include CCL3, CCL4, CCL5, and CCL17, which attract naive CD8$^+$ T cells (T$_N$) via CCR5 and CCR4. Tregs can influence this process by modulating production of the CCR5 ligands. Activated CD8$^+$ T cells upregulate CXCR3 and likely migrate to the periphery of the lymph node, where high concentrations of CXCL9 and CXCL10 are produced. (*b*) Model of CD4$^+$ Th1 and CD8$^+$ central memory T cell (T$_{CM}$) responses to viral infection. Subcapsular sinus (SCS) macrophages produce type I interferons (IFN-α/β) upon infection, resulting in the production of CXCL10, which attracts IFN-γ-producing CD4$^+$ Th1 and CD8 T$_{CM}$ cells. The CD4$^+$ T cells are recruited from the T cell zone as they are activated in a Th1 environment and upregulate CXCR3, whereas the CD8 T$_{CM}$ cells may be recruited from the T cell zone or are already prepositioned in the periphery of the lymph node by unknown signals. In addition, cytokines such as IL-18 activate innate lymphocytes (iL) positioned near the SCS to produce additional IFN-γ. The high levels of IFN-γ in this peripheral region result in the production of CXCL9 by stromal cells and DCs, thus providing a positive feedback loop for recruitment of CXCR3$^+$ cells to this region. CD4$^+$ Th1 and CD8$^+$ T$_{CM}$ cells acquire activation signals in this region, and may also act to control virally infected cells and prevent viral spread. The production of CXCL9 and CXCL10 may be modulated by Tregs during infection via unknown mechanisms. (*c*) In the course of a Th2 response, naive CD4$^+$ T cells acquire CXCR5, allowing them to interact with CXCR5$^+$ DCs that are present in perifollicular regions. Signals at this site appear to direct the formation of both Th2 and T follicular helper (Tfh) cells. Tfh cells express higher levels of CXCR5 and migrate into the B cell follicle, whereas Th2 cells express lower levels of CXCR5 and exit the lymph node. (*d*) Activated effector cells upregulate the S1P1 receptor and migrate out of the lymph node via an S1P gradient.

lymphocytes (131). Computer modeling suggests, however, that the modulation of lymphocyte velocity is what most affects that ability of a T cell to find a cognate DC (132). Chemokines promote such increases in cellular velocity. Blockade of T cell chemokine receptor signaling using PTX results in a 50% decrease in velocity and an 80–90% decrease in motility coefficient within the LN (133, 134). Much of the increase in velocity may be driven in vivo by FRC-produced and -bound CCL19 and CCL21 and can be modulated by DCs that have the ability to cleave the C terminus of CCL21. This cleavage releases CCL21 from the scaffold and into a soluble form, altering the balance of bound versus soluble chemokine and thereby affecting the ability of CCL21 to mediate haptotaxis (135). In the absence of CCL19 and CCL21 or CCR7, T cell migration still occurs, but the velocity of these cells decreases by 20–30%.

CXCL13/CXCR5 interactions appear to play an analogous role in B cells; the deletion of CXCR5 partially decreases B cells' velocity, with a reduction of the motility coefficient by 26% in the T cell zone and 55% in the follicle (60). Interestingly, the discrepancy demonstrated between the almost total lack of migration seen in the PTX-treated cells and the moderate reductions of velocities seen with single chemokine receptor deletion suggests that other chemokines or chemoattractant signaling via G protein–coupled receptors may be important. Candidate chemoattractants have included CXCL12/CXCR4 interactions and the lipid mediator thromboxane A2; however, the data have not shown clear roles for these chemoattractants in baseline T cell motility in the LN in vivo (136, 137). One alternative candidate promoting lymphocyte motility may be 7α,25-dihydroxycholesterol and its receptor EBI2 because this system has been recently implicated in lymphocyte migration within the LN, although further studies are needed to confirm its impact on lymphocyte velocity (138).

CD8$^+$ T Cell Priming

During the early priming steps of an effector CD8$^+$ cytotoxic T lymphocyte (CTL) response, chemokine signaling via CCR5 and CCR4 promotes optimal activation of naive CD8$^+$ T cells. Although CCR5 is not expressed on naive T cells, it is quickly upregulated by unknown stimuli in a subset of CD8$^+$ lymphocytes upon LN entry (139). When CD4$^+$ T cells interact with DCs presenting their cognate antigen, both the DC and the CD4$^+$ T cell produce the CCR5 ligands CCL3 and CCL4. These chemokines bind to CCR5 on naive CD8$^+$ T cells, promoting their migration to CD4$^+$ T cell/DC clusters (139). This results in enhanced contacts between CD4$^+$ T helper cell–licensed DCs and naive CD8$^+$ T cells and promotes increased quantity and quality of the CD8 memory response. Chemokine-guided clustering may also occur in the absence of licensing by CD4$^+$ T cells. The interaction of antigen-specific CD8$^+$ T cells with mature DCs induces CCR5-mediated recruitment of additional naive CD8$^+$ T cells, which may help to prime unrelated CD8$^+$ T cells in settings of low antigen frequency (140). Alternatively, CCR4 may play a role similar to that of CCR5 in the setting of NKT cell activation, given that the injection of α-galactosylceramide enhances the priming of CD8$^+$ T cells via a mechanism that depends on CCL17 expression by DCs and CCR4 expression on CD8$^+$ T cells. These CCL17/CCR4 interactions result in increased contact duration and directionality of CD8$^+$ T cells toward DCs and are synergistic with the CCR5-directed recruitment by CD4$^+$ T cells (141). Thus, depending on the environment and accessory cells encountered during priming, there may be at least two chemokine pathways recruiting CD8$^+$ T cells to DCs. Whether CCR4 and CCR5 ligands are in a soluble form or presented on the membrane of the DC remains unclear. Indeed, at least in vitro, various chemokines such as CCL21, CXCL10, and CCL5 can be presented on the surface of DCs and instigate antigen-independent tethering between T cells and DCs (142). Further studies are required to determine the contribution of soluble versus bound chemokine signaling

and whether chemokine-mediated signaling promotes the T cell–T cell interactions that were recently described to be required for optimal $CD8^+$ T cell differentiation (143).

VV: vaccinia virus

LCMV: lymphocytic choriomeningitis virus

The above studies examined the $CD8^+$ T cell response to nonreplicating antigen, but chemokine signaling may also play a role during viral infection. During vaccinia virus (VV) infection in mice, VV infects both macrophages and DCs in the LN parenchyma and results in their rapid release of the CCR5 ligands CCL3, CCL4, and CCL5 (144). Using antibody-mediated neutralization, the authors showed that these chemokines mediated the preferential recruitment of $CD8^+$ T cells away from the macrophage-rich region to the DC-rich peripheral interfollicular region of the LN within 6–8 h post-infection. Presumably, this would result in optimal $CD8^+$ T cell priming, as was seen in experiments using nonreplicating antigens. Recently, however, another group did not find a role for CCR5 in the initial antigen encounter and clustering with DCs by naive $CD8^+$ T cells during modified VV Ankara infection (145). The reason for these discrepancies may be the different viral strains used or cell populations transferred; therefore, the precise role of CCR5-mediated signaling in $CD8^+$ T cell priming interactions during infection is not completely clear. Taken together, however, the above results suggest that chemokine receptors and their ligands are important in the early $CD8^+$ T cell–DC priming interaction during the response to vaccination and likely infection.

$CD8^+$ T Cell Effector Versus Memory Formation

As the initial $CD8^+$ T cell priming events occur, inflammatory chemokine receptors such as CXCR3 and CCR5 become stably expressed on activated $CD8^+$ T cells (146). Interestingly, the expression of CXCR3 and to a lesser extent CCR5 on $CD8^+$ T cells has recently been found to have a profound effect on the generation of memory cell subsets (147–149). Using various infectious models, the absence of CXCR3 expression on $CD8^+$ T cells was shown to significantly increase the generation of memory cells while reducing the output of short-lived effectors. This effect was surprisingly large, with antigen-specific secondary memory cells doubly deficient in CXCR3 and CCR5, accounting for nearly 20% of the total polyclonal $CD8^+$ T cell pool even 270 days after secondary challenge with influenza virus (148). Because the strength and duration of antigen presentation and the inflammatory environment has a significant effect on formation of CD8 memory, the role of CXCR3 in the positioning of $CD8^+$ T cells within inflammatory environments was analyzed. Indeed, although wild-type $CD8^+$ T cells clustered with $CD11c^+$ DCs in the splenic marginal zone and the B cell–T cell boundary after VV or lymphocytic choriomeningitis virus (LCMV) infection, CXCR3-deficient $CD8^+$ T cells predominantly localized to the T cell area (147, 149). This CXCR3-mediated positioning in the marginal zone likely exposes $CD8^+$ T cells to a high concentration of antigen and cytokines that are expressed by infected macrophages and DCs, which promotes the effector cell formation that is lost in the absence of CXCR3. However, this effect may be infection specific; 10 days after influenza infection, CXCR3 appears to play a role in the activation of antigen-specific cells only in the lung and not in the SLOs (148). During influenza infection, wild-type $CD8^+$ T cells localized to the large airways, which are thought to be the sites of greatest viral infection and replication, whereas CXCR3-deficient $CD8^+$ T cells localized to the interstitial spaces of the lung. Thus, CXCR3 may promote the trafficking of $CD8^+$ T cells to highly inflammatory microenvironments with increased antigen presentation, thereby promoting effector cell differentiation. Differences in $CD8^+$ T cell positioning during viral infections may be dependent on the site of viral replication—SLO-resident macrophages and DCs in the case of LCMV and VV, versus the lung-resident cells in the case of influenza. Therefore, CXCR3 signaling on $CD8^+$ T cells likely acts after the initial priming stages to promote effector cell development by bringing antigen-specific cells to sites of infection, where they detect

further antigen and inflammatory signals. This environment then optimizes the balance of newly activated $CD8^+$ T cells for effector function at the expense of memory generation.

The data presented above therefore suggest that chemokines sequentially influence the priming and fate decisions of naive $CD8^+$ T cells (**Figure 3**). In such a model, CCR7 and its ligands CCL19 and CCL21 recruit naive T cells and mature APCs to the LN and then promote their mobility to optimize rare antigen-specific naive $CD8^+$ T cell and cognate antigen-loaded DC encounters. During these early transient encounters, unknown signals upregulate the expression of various chemokine receptors, including CCR5, CCR4, and CXCR3, and downregulate CCR7 on the $CD8^+$ T cells. CCR5 and CCR4 ligands are then produced by DCs that have come into contact with antigen-specific or activated $CD4^+$ T cells, $CD8^+$ T cells, and/or NKT cells, resulting in additional recruitment of naive $CD8^+$ T cells. The activation of CXCR3 signaling then brings newly activated $CD8^+$ T cells to sites of ongoing infection, where the high levels of antigen and inflammatory cytokines can be expected to promote effector differentiation at the expense of memory formation.

$CD4^+$ Th1 Priming

Recent studies have begun to shed light on how chemokines also modulate $CD4^+$ T cell priming, differentiation, and activation. $CD4^+$ T cell helper subsets express specific chemokine receptors; Th1 cells, for example, preferentially upregulate the chemokine receptors CCR5, CXCR3, and CXCR6 (150). For CXCR3, this upregulation is especially rapid and occurs early during $CD4^+$ T cell activation in SLOs (151). The ligands for CXCR3, CXCL9 and CXCL10, are also expressed in the LN early during Th1 differentiation and play an important role in $CD4^+$ Th1 priming (152). For example, in an experiment in which mice were either injected with antigen-pulsed mature DCs or immunized with antigen and adjuvant, CXCR3 was required for optimal Th1 differentiation. The Th1 response to antigen-pulsed DCs required the production of CXCL10 by the DCs and expression of CXCR3 on the $CD4^+$ T cells, which resulted in a significant increase in the formation of stable contacts between these cells types in the LN. During immunization, optimal Th1 differentiation required CXCL9 expression by radio-resistant stromal cells, predominantly in the interfollicular areas, and CXCL10 expression by radio-sensitive cells, predominantly in the medullary region, which promoted the migration of $CD4^+$ T cells from the T cell zone into the interfollicular and medullary regions of the LN. These findings indicate that CXCR3 interactions with both CXCL9 and CXCL10 promote Th1 differentiation by promoting stable contacts with DCs as well as by placing these $CD4^+$ T cells into potential niches of high IFN-γ production. Indeed, a recent report documented that innate lymphocytes such as NK, NKT, $\gamma\delta$ T, and innate-like $CD8^+$ T cells are prepositioned in these peripheral regions of the LN where local cytokine production can mediate rapid IFN-γ production by these innate cell types (153). CXCR3 is highly expressed on NK and NKT cells as well as on innate-like $CD8^+$ T cells and thus may serve to further cluster these IFN-γ-producing cells with DCs that are priming $CD4^+$ T cells (154–156). Additionally, CXCR3 recruits pDCs, and these cells have been shown to augment Th1 responses by cytokine production (157, 158). The requirement for CXCR3-mediated peripheralization for optimal Th1 activation may explain why *plt/plt* mice, which have a deficiency of the CCR7 ligands CCL19 and CCL21 in the LN, have delayed but enhanced Th1 cell responses (159). Although these mice may have alterations in the recruitment of tolerogenic DCs or Tregs to the LN, another explanation could be that the absence of these CCR7 ligands tips the balance from retention of $CD4^+$ T cells in the T cell zone toward peripheralization to the interfollicular and medullary regions leading to enhanced Th1 priming. Beyond CXCR3, CCR2 may augment the Th1 response by recruiting IL-12-producing inflammatory monocytes into the LN (123). It is interesting to

speculate that this feed-forward loop may also be terminated by the CXCR3-mediated recruitment of effector and memory $CD8^+$ T cells, which can kill antigen-bearing DCs (160). Alternatively, as we discuss below, Tregs may alter the expression of CXCR3 and its ligands in the reactive LN.

Tfh: T follicular helper cell

$CD4^+$ Th2 and Tfh Priming

Recent studies also suggest that chemokine guidance optimizes Th2 and T follicular helper (Tfh) cell differentiation in the LN (161). Th2 and Tfh cell responses during *Heligmosomoides polygyrus* infection were reduced with the antibody-mediated neutralization of CXCL13 or deletion of CXCR5 on both DCs and $CD4^+$ T cells. This chemokine signaling directed $CD4^+$ T cell migration out of the T cell zone and into the perifollicular regions of the LN, where activation of the Th2 and Tfh cell subsets was optimized. In a different Th2 model mediated by FITC skin sensitization, CCL22 was found to be upregulated on draining Langerhans cells in the T cell zone, and newly activated $CD4^+$ T cells migrated in response to this CCR4 ligand (162). In addition, the other CCR4 ligand, CCL17, was strongly upregulated in mature Langerhans cells and DCs upon entry into the LN (163). Thus, both CXCR5 and CCR4 may promote Th2 responses. However, a more recent study did not find a role for CCR4 in the early priming of Th1 or Th2 cells, but instead found defects in the formation of $CD4^+$ memory T cells in the lung and skin (164). In any case, chemokines appear to play an important role in optimizing the differentiation of Th1, Th2, and Tfh $CD4^+$ T cells by recruiting priming $CD4^+$ T cells out of the T cell zone and into the more peripheral intrafollicular and perifollicular regions of the LN (**Figure 3**). Therefore, one unanswered question is how the microenvironment of peripheral regions of the LN differs from the T cell zone during these priming events. If these differences are due to local concentrations of skewing cytokines, it remains to be determined what these cytokines are and what accessory cells are producing them in these microdomains. Furthermore, it will be of great interest to determine if there are other such pathways for other T cell subsets, such as Th17, Th22, and Th9 cells.

B Cell Activation

As with T cells, the distribution of B cells is mediated by chemokines and other chemoattractant molecules in a highly orchestrated response to antigen. Within the follicles, CXCL13/CXCR5 interactions in naive B cells promote their movement upon the FDC and marginal reticular cell scaffold (60). This brisk migration, coupled with the positioning of the follicle near the subcapsular sinus, allows the naive B cell to sample antigen presented on various cell types and in specialized regions of the follicle. Once the B cell encounters antigen, it transiently arrests, but within a few hours it upregulates the receptors CCR7 and EBI2 while maintaining expression of CXCR5 (165, 166). This alteration in chemoattractant receptors then guides the B cell to the edge of the follicle at the T/B cell border (165, 167). Meanwhile, activated $CD4^+$ Tfh cells downregulate CCR7 and upregulate CXCR5, which brings the Tfh cells to this same interfollicular region and T/B cell border (161). Upon interaction with Tfh cells at the T/B cell border, B cells downregulate CCR7 but retain CXCR5 and further upregulate EBI2, which causes them to relocate into the outer follicle. The B cells destined for the germinal center then downregulate EBI2 and as a result of S1P2 signaling show reduced responsiveness to other chemoattractants. Together, these promote clustering of B cells in the germinal center (166, 168). The germinal center itself is divided into a dark zone, where B cell proliferation and hypermutation occurs, and a light zone, where B cell survival and selection signals are provided. As B cells proliferate within the dark zone, they downregulate CXCR4 and upregulate CXCR5, causing migration into the light zone, where CXCL13 is predominately expressed. Based on unknown signals received in the light zone, a subset of B cells reexpress CXCR4 and traffic back to the dark zone via CXCL12 to undergo further somatic hypermutation

and proliferation. This process results in a bidirectional B cell migration between the two zones that allows for multiple cycles of proliferation and selection (169, 170). Primed Tfh cells stably express CXCR5, allowing them to gain entry into the germinal center, where they can promote the survival and selection of germinal center B cells. Thus, as for T cells, chemokine signaling is essential for the activation of B cells in the LN.

CHEMOKINE CONTROL OF EFFECTOR CELL FUNCTION

Migration to Peripheral Tissues

Most effector lymphocytes must exit the LN to perform their immune functions. The regulation of effector lymphocyte egress from the LN is similar to what we have described for naive T cells above and has recently been thoroughly reviewed (171). During an immune response, CCR7 and S1P1 are both downregulated on the activated T cells, and the production of the CCR7 ligand CCL21 is also reduced (172). T cells then preferentially regain S1P1 signaling compared with CCR7-mediated signaling three days after response to antigen and after about four cell divisions (172). This change in receptor expression changes the balance from CCR7-mediated retention signals to S1P1 exit signals and thus allows for the most activated terminal effectors to leave the LN and effect an immune response in the periphery. As effector T cells exit the LN, they are guided by chemokines to sites of infection and inflammation in order to perform their immune functions (**Figure 4**). After activation, effector $CD8^+$ T cells continue to downregulate LN-homing CD62L and CCR7 and induce the expression of chemokine receptors, such as CXCR3, CCR5, CXCR6, CCR6, CXCR1, and CX3CR1, to varying extents depending on their state of differentiation. These induced chemokine receptors then mediate effector $CD8^+$ T cell migration to sites of inflammation (146, 173, 174). Likewise, effector $CD4^+$ T cells also express an inflammatory chemokine receptor repertoire that is dependent on their effector differentiation (150). For example, Th1 cells express CCR5, CXCR3, and CXCR6, whereas Th2 cells express CCR4, CCR8, CX3CR1, and CRTH2. Th17 cells express high levels of CCR6 and CCR4 but are CCR10 negative, whereas Th22 cells express CCR4, CCR6, and CCR10 (175, 176). The newly described Th9 subset appears to express a disparate range of chemokine receptors, including CCR3, CCR6, and CXCR3 (177). Although the preferential expression of these chemokine receptors is apparent in vitro, there is some overlapping expression in vivo, which is not surprising given the relative plasticity between the various $CD4^+$ T cell subsets (178). For example, both Th1 and Th2 cells can express the chemokine receptor CCR4 in vivo and require this receptor for either Th1 or Th2 granulomatous responses in the lung (179). Effector $CD4^+$ T cells may also augment the local production of chemokines to enhance local $CD8^+$ effector T cell recruitment. This concept was recently elegantly demonstrated during vaginal infection with herpes simplex virus (HSV)-2 (180). In this model, $CD4^+$ T cells recruited to the vagina produced IFN-γ that promoted the local production of CXCL9 and CXCL10 and the enhanced recruitment of activated $CD8^+$ T cells to the tissue via the chemokine receptor CXCR3. Thus, the production of inflammatory chemokines in peripheral tissues may result in $CD4^+$ T cell recruitment that can then further modulate the local milieu of chemokines to optimize $CD8^+$ T cell recruitment.

In addition to general inflammatory chemokine receptor expression, the site of priming appears to imprint effector T cells with the ability to traffic to particular organs (see review in Reference 181). Much of the available data focus on homing to the gut, skin, and lung. In the gut, $CD103^+$ DCs and stromal cells from Peyer's patches and mesenteric LNs produce retinoic acid that triggers expression of $\alpha4\beta7$ and CCR9 on T cells (182). The interaction of $\alpha4\beta7$ and CCR9 with their cognate ligands—endothelial-derived MAdCAM and epithelial-derived

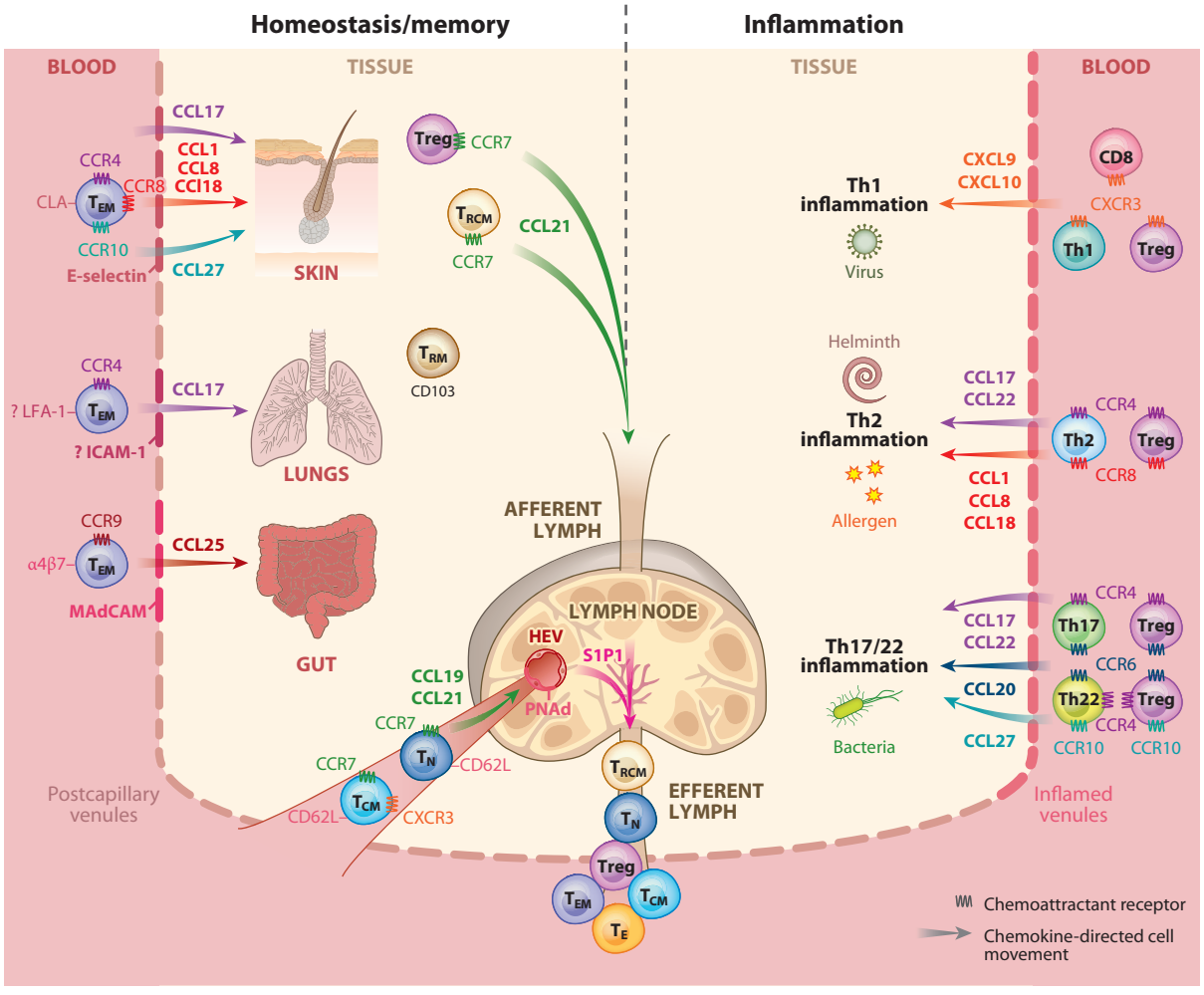

Figure 4

Chemokines orchestrate the trafficking patterns of effector and memory T cell subsets. (*Left*) The trafficking of T cell subsets at homeostasis. Naive T cells (T_N) express CCR7 and CD62L and migrate between the blood and secondary lymphoid organs (SLOs). Central memory T cells (T_{CM}) have a trafficking pattern similar to that of naive T cells and also express CCR7 and CD62L, allowing for lymph node access via interactions with CCL19, CCL21, and PNAd presented on high endothelial venules (HEVs). Effector memory T cells (T_{EM}) are found in the blood and are characterized by a lack of CCR7 expression and instead are imprinted with tissue-homing chemokine receptors and adhesion molecules. These trafficking molecules allow for the specific migration of T_{EM} cells into various tissues based on each tissue's unique chemokine and adhesion molecule expression. Resident-memory T cells (T_{RM}) express CD103 and persist for long periods within the tissues, whereas recirculating memory T cells (T_{RCM}) enter the tissues but then return to the SLO in a CCR7-dependent manner. Regulatory T cells (Tregs) appear to have a similar distribution to effector and memory T cells and likely form populations that are tissue resident and recirculating. (*Right*) The recruitment of effector cells to inflamed tissues. As naive $CD4^+$ T cells differentiate into Th1, Th2, and Th17/22 helper subsets they express distinct repertoires of chemokine receptors that allow for the specific T cell subsets to be recruited to tissues undergoing specific types of inflammation. Once in the tissue, the recruited T cells produce T helper cell type–specific cytokines that further increase specific chemokine production by the tissue in a feed-forward mechanism that results in further effector T helper cell subset trafficking into the tissue. Like effector $CD4^+$ T cells, Tregs also differentiate into heterogeneous subsets that express distinct chemokine receptor repertoires. This chemokine expression on Tregs allows for their specific recruitment to the areas of inflammation where they may serve to dampen inflammatory responses by modulating the feed-forward mechanism described above.

CCL25—directs effector T cells to the lamina propria and epithelium of the small intestine (183). Conversely, T cells primed within the skin-draining inguinal LN preferentially migrate to the skin (184). This cutaneous migration is mediated by cutaneous lymphocyte antigen (CLA), the mouse E/P-selectins, and the chemokine receptors CCR4, CCR8, and CCR10. CCR10 expression, and thus skin homing, may be controlled by vitamin D3. In humans, vitamin D3 is metabolized by DCs from the skin to its active form, which induces the expression of CCR10 on T cells while downregulating the gut-homing integrin $\alpha4\beta7$ (185). However, this pathway may not be relevant in mice, which lack cutaneous vitamin D3 production and do not upregulate CCR10 in response to vitamin D3 (185). Recently, investigators demonstrated that CCR8 and CLA are upregulated on T cells that are primed in the presence of keratinocytes, suggesting that T cells may be recruited to inflamed skin by CCR4 and CCR10 but then, once within the skin, receive retention signals via CCR8 and CLA (186).

Preferential homing of T cells into the lung has also been described (187, 188). Lymphocyte function-associated antigen-1 promotes effector $CD8^+$ T cell migration to the noninflamed lung, whereas CCL5 ligand expression promotes the transmigration of these cells into the pulmonary interstitium (187). More recently, lung imprinting has been described; T cells primed in vitro by lung DCs preferentially traffic back to the lung via a process partially dependent on CCR4, both at homeostasis and with inflammatory stimuli (189). Interestingly, during intranasal immunization, lung DCs were also able to imprint $CD4^+$ T cells with the gut-homing integrin $\alpha4\beta7$ and CCR9, suggesting that there may be some mucosal cross talk mediated by lung DCs (190). Accordingly, this imprinting is likely a plastic process, given the evidence that cells can be reprogrammed and that local infection or immunization results in the distribution of effector cells to a wide range of nonlymphoid tissue (184, 191, 192). This widespread distribution may be reconciled with the idea of tissue tropism by the observation that activated $CD8^+$ T cells often traffic to distant LNs in the days following VV infection (193). These activated cells may then acquire additional homing molecules associated with the microenvironment of the LN or tissue that they have entered following dissemination (193). In addition, during inflammation, the upregulation of inflammatory chemokines in the tissue (e.g., CXCR3 ligands) and of their corresponding receptors on activated effector T cells (e.g., CXCR3) also likely accounts for the systemic distribution of effector T cells.

Modulation of Effector Cell Function Within Tissues

Once within the nonlymphoid tissue, chemokines continue to influence effector cells. For example, during *Toxoplasma gondii* infection, treatment with anti-CXCL10 antibodies resulted in a decrease in the number of $CD8^+$ T cells and an increase in the parasite burden in the brains of chronically infected mice (194, 195). Direct observation of the $CD8^+$ T cells within the explanted brain by multiphoton microscopy revealed a significant decrease in the average cell velocity within the tissue upon treatment with either PTX or CXCL10 antibody. CXCL10 was thought to increase velocity of $CD8^+$ T cells by promoting a generalized Lévy walk pattern with a mix of long trajectories and short random movements that could potentially be more efficient in finding sites of ongoing infection (194). In other settings, investigators have found that chemokines can provide important survival signals in peripheral tissue. For example, CX3CR1 was found to be important for the development of allergic airway inflammation in mice. Blockade of this pathway did not affect T cell migration to the lung but was required for maintenance and survival of a population of $CD4^+$ Th2 cells in the inflamed airways (196). Finally, in addition to regulating the velocity of migration and survival within tissue, chemokines also control tissue retention by controlling lymphocyte egress via the afferent lymph. CCR7 expression on $CD4^+$ and $CD8^+$

T cells is essential for these cells' egress from the lung in both asthma and influenza infection and from resting and acutely inflamed skin (197–199). In addition, with chronic inflammation, T cells can leave the skin in a PTX-sensitive, but CCR7-independent, pathway, suggesting that other chemokines can also mediate tissue exit (200). In addition to exit signals provided by CCR7, there may be retention signals provided by inflamed tissues. One such retention signal may be provided by the S1P1 pathway (201), but it likely includes other chemokines constitutively expressed in the tissue; the full delineation of these signals awaits further study. Finally, atypical nonsignaling scavenging chemokine receptors also play a role in titrating retention versus exit signals. For example, D6 (ACKR2) expressed on lymphatic endothelium scavenges inflammatory chemokines but not homeostatic chemokines (e.g., CCL19 and CCL21), allowing preferential binding of CCL19 and CCL21 to the afferent lymphatics and thus the preferential exit of CCR7-bearing cells (202).

CHEMOKINE CONTROL OF MEMORY CELL FUNCTION

In most infections, the pathogen is effectively cleared by the immune response, and the expanded effector T cells undergo a phase of contraction by apoptosis, leaving approximately 5–10% of the responding population as a heterogeneous pool of long-lived memory cells. Chemokines play an ongoing and critical role in these memory cell populations by determining their migration and surveillance properties (**Figure 4**).

Central Memory and Lymph Node Surveillance

Over a decade ago, investigators recognized that the heterogeneous pool of memory cells circulating in blood could be subdivided based on the differential expression of chemokine receptors and homing molecules (203). T_{CM} cells were defined by the coexpression of LN-homing CD62L and CCR7 and their recirculation through the blood and SLOs, similar to naive cells. Unlike naive cells, however, these cells express inflammatory chemokine receptors such as CXCR3 and have high levels of CXCR4 that may serve as a CCR7-independent means of LN entry (204). Recently, the expression of CXCR3 on T_{CM} has been found to have a critical role for their function (205). The $CD8^+$ T_{CM} response was directly compared with naive $CD8^+$ T cells after injection of antigen-pulsed DCs or infection with LCMV. Surprisingly, the response to antigen-pulsed DCs was similar in naive T and T_{CM} cells except for an enhanced IFN-γ response by the T_{CM}. However, when the same comparisons were made during LCMV infection, T_{CM} had higher CD69 expression, proliferation, and IFN-γ production compared with the naive cells. This brisk activation was mediated by the specific recruitment of $CXCR3^+$ T_{CM} from the T cell zone to the medulla, interfollicular area, and subcapsular sinus of the LN—regions of the LN with a predominance of LCMV-infected macrophages. Such trafficking to the LN periphery depended on a cascade of cytokine and chemokine signaling that resulted in the local production of the CXCR3 ligands. These chemokines would then recruit additional $CXCR3^+$ T_{CM} to the periphery of the LN and sites of active LCMV infection in a feed-forward mechanism that resulted in their quick activation and expansion in response to infection. Similar results were seen using modified VV Ankara, except in this study the transferred T_{CM} were already positioned in the cortical and medullary ridge prior to infection (145). Upon viral rechallenge, T_{CM} were then further recruited to the subcapsular sinus in a similar feed-forward mechanism involving CXCR3/CXCL9 interactions. The reason for the observed differences in the prepositioning of the T_{CM} cells is unclear. However, these studies have demonstrated that $CD8^+$ T_{CM} are either prepositioned or recruited to microenvironments of the LN in a CXCR3-dependent manner that allows for enhanced recall responses to viruses that can establish an infection within the LN. Thus, CCR7-mediated LN homing coupled with CXCR3

expression on T_{CM} positions these cells to quickly control the spread of virus within the lymphatic system upon rechallenge (**Figure 3**).

T_{RM}: resident-memory T cell

T_{RCM}: recirculating memory T cell

T Effector and Resident Memory Cells in Peripheral Surveillance

Although T_{CM} preferentially migrate through the blood and SLOs, where they are positioned to respond quickly to pathogens that access and replicate within the LN, other memory subsets are classified on the basis of their migration properties within nonlymphoid tissue (**Figure 4**). Effector memory T cells (T_{EM}) are characterized by the lack of LN-homing CD62L and CCR7 expression and the lack of tissue retention molecules, such as CD103 and CD69. These cells therefore preferentially migrate between the blood and spleen compartments and enter peripheral organs, such as the lung, liver, intestinal tract, and kidney (191, 192). However, they may also have access to peripheral LNs via CD62L-independent mechanisms, including CD62P and CXCR3 during inflammation and $\alpha4\beta1$ and E-selectin in the resting state (160, 206, 207). Compared with T_{CM}, T_{EM} proliferate less and are skewed toward greater production of effector cytokines such as IFN-γ (203). Thus, these cells have been hypothesized as functioning to survey the peripheral tissue and enact a quick effector response to tissue infection.

More recently, another subset of long-lived memory cells, termed resident-memory T cells (T_{RM}), has been defined by their preferential tissue residence. As recently reviewed, these cells have been definitively identified in the skin, vaginal mucosa, central nervous system, salivary gland, lung, and intestine and are characterized by the increased expression of CD103 and CD69 and a lack of CD62L and CCR7 (208). Interestingly, recent studies have demonstrated the importance of chemokines in the development and maintenance of $CD8^+$ T_{RM}. For example, the development of $CD8^+$ T_{RM} in the skin during HSV infection is dependent upon localization of $CD8^+$ T_{RM} precursors to the epidermis by CXCR3-CXCL10 interactions, which counteract a CCR7-dependent tissue exit signal (209). Further, $CD8^+$ T_{RM} are maintained in the tissue by downregulation of S1P1 (210). It should be noted that within most of these tissues, $CD4^+$ and $CD8^+$ memory T cells localize to distinct compartments. For example, in the skin, a slow-moving population of $CD8^+$ T cells patrols the epidermis and hair follicles, whereas $CD4^+$ memory T cells traffic more rapidly through the dermis and then back into the circulation (211, 212). These recirculating memory T cells (T_{RCM}) appear to have a phenotype distinct from T_{CM}, T_{EM}, or T_{RM}, with $CCR7^{int\text{-}pos}$, $CD62L^{int}$, $CD103^{+/-}$, $CD69^-$, $CCR4^{+/-}$, and E-selectin$^+$ expression, which may suggest that they represent a distinct memory cell subset (213). In any case, T_{RM} appear uniquely positioned in the peripheral tissue to quickly enact effector functions and provide protection from reinfection or reactivation of latent virus (214, 215). Exactly how T_{RM} mediate their protective response has not been fully elucidated, but recent data have suggested that a key function of these cells is their rapid and robust expression of chemokines. In the lung, for instance, influenza-specific memory $CD4^+$ T cells can greatly enhance the early production of inflammatory chemokines, such as CXCL9, CXCL10, CCL2, and CXCL1, upon heterologous viral challenge, resulting in reduced titers and improved viral control (216). In the female reproductive tract, $CD8^+$ T_{RM} quickly produce IFN-γ in response to cognate antigen. This results in robust local production of chemokines, such as CXCL9, which recruits circulating memory $CD8^+$ T cells independent of their antigen specificity (217). Thus, a major function of both $CD4^+$ and $CD8^+$ T_{RM} may be the rapid and robust local production of chemokines in mucosal tissues upon the recognition of previously encountered antigen. Such chemokine production would then recruit circulating memory T cells, newly activated effector T cells, or other leukocyte subsets, such as monocytes, neutrophils, and NK cells, for enhanced protection to rechallenge with the same or heterologous viruses.

CHEMOKINES IN REGULATORY T CELL FUNCTION

GVHD: graft-versus-host disease

Tfr: follicular regulatory T cell

Chemokine-directed trafficking also critically influences the Treg response (**Figure 4**). Under homeostatic conditions, a subset of Tregs utilize CCR7 to migrate and localize to the T cell areas of the LN and spleen (218, 219). CCR7-mediated localization within the T cell zone appears to be critical for the maintenance of a SLO Treg population by providing access to sites of paracrine-expressed IL-2 (219). During the induction or priming phase of an immune response, CCR7-mediated positioning of Tregs is also essential for their suppressive function. Treg expression of CCR7 is necessary for the control of $CD4^+$ T cell proliferation in models of immunization, cutaneous contact hypersensitivity, inflammatory bowel disease, and the priming phase of allergic airway inflammation (220–222). $CD62L^+$ $CCR7^+$ Tregs that home to the LN have enhanced suppressive activity compared with peripherally homing Tregs in models of autoimmune disease and graft-versus-host disease (GVHD) (223, 224). During cardiac transplant, Treg expansion required the migration of alloantigen-presenting pDCs into the draining LNs, which then produced CCL17, resulting in a CCR4-mediated colocalization of $Foxp3^+$ Tregs with these APCs and their activation (225). CCR4 also plays a role in mediating the LN accumulation of Tregs in a transfer model of inflammatory bowel disease; CCR4-deficient Tregs demonstrate decreased numbers in the mesenteric LN and a lack of colitis control (226).

Once within the LN, Tregs alter the chemokine-mediated positioning of other cells in order to influence the immune response. Tregs may influence optimal $CD8^+$ T cell priming by modulating the expression of the CCR5 ligands CCL3, CCL4, or CCL5 (227). Deletion of Tregs during $CD8^+$ T cell priming resulted in increased expression of CCL3 and CCL4 by DCs, which increased stable $CD8^+$ T cell–DC interactions in the setting of a low-avidity antigen. This resulted in the activation and expansion of a low-avidity $CD8^+$ T cell response at the expense of high-avidity memory cells (227). Therefore, deletion of Tregs increased CCL3 expression and impaired memory responses to *Listeria monocytogenes* infection (227). Tregs may also impact $CD4^+$ T cell priming. As previously discussed, optimal Th1 differentiation depends on CXCR3-mediated signals. Tregs inhibit stable contacts between $CD4^+$ T cells and DCs and can influence the expression of CXCR3 ligands in the draining LN during genital HSV-2 infection (228–230). In addition, in a model of diabetes, Tregs have been found to prevent the expression of CXCR3 on effector T cells (231). Whether Tregs directly affect T cell priming via CXCR3 remains to be determined. Finally, a subset of $CXCR5^+$ Tregs called follicular regulatory T cells (Tfr) migrates to the germinal center, where they control affinity maturation and differentiation of B cells (232, 233). In addition to this mechanism of B cell control, CCL4/CCR5 interactions may control the antibody response by promoting the interactions of Tregs with B cells and APCs within the LN (234). In sum, these results suggest that chemokine-guided positioning of Tregs within the LN plays an essential role in Treg-mediated modulation of initial priming and germinal center responses.

Strong evidence also suggests an important role for the chemokine-guided migration of Tregs to peripheral tissues to control immune responses. Like their $CD4^+$ T cell counterparts, naive-like Tregs upregulate the expression of inflammatory chemokines, such as CCR2, CCR4, CCR5, CCR6, CCR8, and CXCR3, and downregulate lymphoid-homing CCR7 after priming with cognate antigen in the presence of adjuvant (235). Interestingly, this switch to expression of inflammatory receptors appears to be accelerated when compared with naive $Foxp3^-$ $CD4^+$ T cells, thus allowing for the rapid accumulation of Tregs in the affected tissue (235). In addition, multiple studies have demonstrated the importance of inflammatory chemokine receptor expression on Tregs in controlling pathology. For example, expression of the inflammatory receptor CCR5 on Tregs is critical for their migration to the affected tissue and their function in infections with *Leishmania major* and fungal pathogens (236, 237). During GVHD, CCR5 mediates accumulation

of Tregs in GVHD target organs, whereas CCR8 promotes Treg maintenance by allowing for their tolerogenic interactions with donor $CD11c^+$ APCs (238, 239). In an islet allograft transplant model, adoptively transferred Tregs required CCR2, CCR4, CCR5, and the P- and E-selectins to migrate from the blood to the inflamed transplanted tissue (240). These Tregs then migrated from the tissue in a CCR2-, CCR5-, and CCR7-dependent fashion into the draining LN, where they could suppress antigen-specific T effector cell activation and proliferation. Such sequential migration from the allograft to the LN was required for full Treg suppressive function, but it is unclear if this sequence of migration is applicable to other situations and endogenous Tregs.

Recently, multiple studies have examined the heterogeneous upregulation of inflammatory chemokine receptors on human and murine Tregs. Tregs that express CXCR3 resemble Th1 subsets in that they express the transcription factor Tbet in addition to Foxp3 and can produce IFN-γ as well as IL-10. CXCR3 expression was dependent on Tbet and IFN-γR (241), and a recent study also suggested that IL-27 can directly promote CXCR3 expression on Tregs in tissues (242). This acquisition of CXCR3 on Tregs is important in certain situations. In EAE, CXCR3-deficient mice have increased chronic disease scores that are likely secondary to altered positioning of Tregs within the central nervous system (243). Similarly, during inflammation of the liver from autoimmune hepatitis, expression of CXCR3 was required for recruitment of Tregs to this organ (244). Similar chemokine receptor skewing occurs in Th2-, Th17-, Tfh-, and Th22-like Treg subsets (232, 245). IRF4-deficient Tregs have reduced CCR8 expression and defects in controlling Th2 inflammation (246). Expression of the Th2-associated chemokine receptor CCR4 on Tregs is important for their control of the effector phase of murine models of Th2-associated allergic asthma (222, 247). Stat3 is a transcription factor associated with Th17 responses, and Stat3-deficient Tregs have reduced CCR6 expression (248). These Stat3-deficient Tregs are immunosuppressive in vitro but are unable to control intestinal Th17 responses in vivo, suggesting that CCR6-mediated trafficking of Tregs is essential for their function. Supporting this idea, CCR6 expression on Tregs was found to be important for trafficking to the central nervous system during EAE and to tissues infected with *Leishmania major* (249, 250).

Before this understanding of the heterogeneity of the Treg population, investigators had found that the basal repertoire of chemokines in a nonsegregated human memory Treg population had an increased expression of CCR4 and CCR8 when compared with both naive and memory $CD25^-CD4^+$ T cells (251). Although this could represent a fundamental difference in the Treg chemokine receptor profile compared with non-Foxp3-expressing $CD4^+$ T cells, this difference could also represent skewing of the Treg population toward a Th2-like subset (246). Furthermore, localized priming can imprint Tregs with specific tissue–homing adhesion molecules, as Tregs primed in the peripheral LNs go on to express skin-homing E- and P-selectins, whereas activation within the mesenteric LNs results in the expression of gut-homing $\alpha4\beta7$ (252). Disruption of gut-selective homing of Tregs via blockade of $\alpha4\beta7$ and MAdCAM1 or genetic deletion of CCR9 prevented their ability to establish oral tolerance (253), although the dependence on CCR9 appears to be dose related (254). Finally, there is also evidence that Tregs may segregate into a resident-memory population, but the chemokine repertoire of this population is unknown (255). Thus, there appears to be a heterogeneous population of Tregs with chemokine and adhesion molecule repertoires that mimic many of the effector and memory populations thus far identified for $Foxp3^-CD4^+$ T cells (**Figure 4**). These data suggest that Tregs co-opt the differentiation and chemokine guidance of effector $CD4^+$ T cells to migrate both to the lymphoid organs to suppress effector cell priming and to the peripheral tissues to suppress ongoing inflammation and recall responses. However, more work is needed to parse out the sequence and timing of these events and how the Tregs function within particular microenvironments.

CONCLUSION

Chemokines were first appreciated as important chemoattractants for innate immune cells, such as neutrophils and macrophages, and for the generation of acute inflammatory responses. However, over time, as we have detailed in this review, it has become apparent that the chemokine system is also critical for the trafficking patterns, migratory behavior, cellular interactions, and positioning of T cells, B cells, and DCs necessary to generate primary and secondary adaptive immune responses. As we have discussed, chemokines play important roles in the priming of naive T cells by influencing their cellular interactions and positioning in lymphoid tissue. Chemokines also play important roles in the central cellular fate decisions of the adaptive immune responses, such as effector and memory differentiation. In addition, Tregs not only are influenced by chemokine gradients but also influence chemokine expression, which is one way in which they exert their suppressive functions. As our understanding of the biological scope and complexity of this intricate system of chemotactic cytokines and receptors has grown, so too has its importance to the field of immunology.

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