

Molecular Determinants Controlling Homeostatic Recirculation and Tissue-Specific Trafficking of Lymphocytes

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Key Words

Lymphoid tissues, secondary · High endothelial
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Abstract

The homeostasis of the immune system is maintained by the recirculation of naïve lymphocytes through the secondary lymphoid tissues, such as the lymph nodes, Peyer's patches, and spleen. Upon insult by pathogens or antigens, lymphocytes become activated, and the regulated trafficking of these cells results in the integration of systemic and regional immune responses. The exquisite specificity of such lymphocyte trafficking is determined by tissue-specific guidance signals expressed by the endothelial cells of postcapillary venules, combined with counterreceptors expressed by the circulating lymphocytes. The high endothelial venules can selectively guide naïve lymphocytes into the lymph nodes and Peyer's patches by expressing a unique combination of vascular addressins, lymphocyte-specific chemokines, and chemokine-binding molecules. The inflamed postcapillary venules in extralymphoid tissues, such as the skin and intestinal lamina propria, also use a distinct array of endothelial adhesion molecules and tissue-

selective chemokines, and support the recruitment of effector and memory lymphocytes that express the appropriate receptors for tissue-specific trafficking. In this review, we summarize the present understanding of the homeostatic recirculation of naïve lymphocytes through the secondary lymphoid tissues and the specific targeting of antigen-experienced lymphocytes into the effector sites. We also revisit some previous studies that reported apparently conflicting observations.

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Introduction

The immune system protects the host from invasion by a myriad of species of harmful microbes in the external environment. Whereas pathogens can enter the body via a variety of routes to initiate infection, pathogen-derived antigens are invariably delivered to the secondary lymphoid tissues, such as the lymph nodes (LNs), Peyer's patches (PPs), and spleen. Naïve lymphocytes continuously recirculate through these secondary lymphoid tissues [1–5], and upon antigen encounter, become activated and undergo a reprogramming of their trafficking properties. Most antigen-experienced lymphocytes traffic through the secondary lymphoid organs, but they can also

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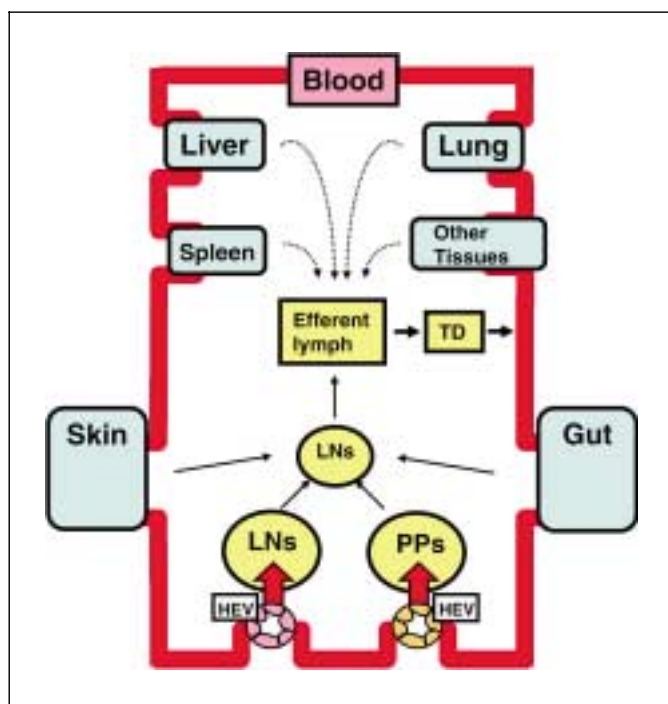


Fig. 1. Lymphocyte recirculation between the blood and lymphoid/nonlymphoid tissues. Lymphocytes in the blood circulation enter secondary lymphoid tissues, such as LNs and PPs, through HEVs and subsequently return to the blood via the efferent lymph. Only a small number of lymphocytes enter nonlymphoid tissues under physiological conditions. Upon encountering antigen in the secondary lymphoid tissues, lymphocytes acquire the ability to migrate into inflamed effector sites, such as the skin and intestinal lamina propria. TD = Thoracic duct.

migrate to extralymphoid effector sites, such as the intestinal lamina propria and inflamed skin [6]. Thus, the integration of systemic and regional immune responses depends on the regulated trafficking of lymphocytes¹ [3–6].

In this review, we will summarize recent advances in our understanding of the homeostatic recirculation of naïve lymphocytes and tissue-specific targeting of antigen-experienced lymphocytes into the effector sites. In addition, we will revisit some previous studies that reported apparently conflicting but often neglected observations in the field of lymphocyte trafficking.

¹ To describe the emigration of lymphocytes from the blood into an organ, sometimes the term lymphocyte ‘homing’ is used. However, most mature lymphocytes never return to the primary lymphoid tissues where they were born (home) in their lifetime, but they preferentially migrate through secondary lymphoid tissues. Thus, in most cases, it is more accurate to use the term lymphocyte ‘migration’ or ‘trafficking’ [134].

Homeostatic Recirculation of Naïve Lymphocytes

Circulating naïve lymphocytes continuously enter the secondary lymphoid tissues, and subsequently return to the circulation via efferent lymph [7] (fig. 1). This process, called ‘lymphocyte recirculation’, which serves as a major homeostatic mechanism for the immune system, was first demonstrated by Gowans [8]. This trafficking route is highly specific to lymphocytes, as granulocytes and monocytes do not recirculate between the blood and the lymph. When entering LNs and PPs, naïve lymphocytes selectively adhere to and transmigrate through the high endothelial venules (HEVs) [1, 5, 9]. In the LNs, it is estimated that one in every four lymphocytes entering the HEVs leaves the blood and migrates into the tissue [10].

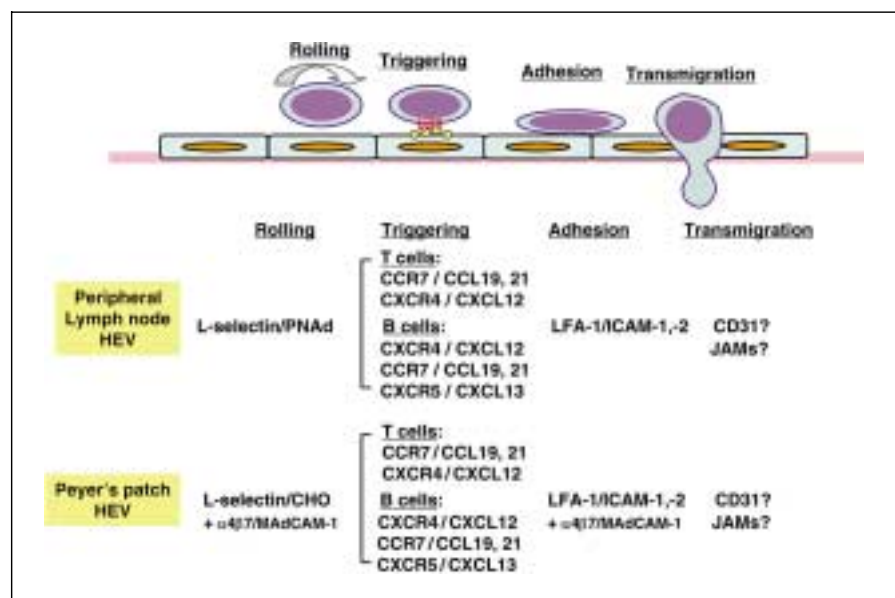
HEVs are also present in the tonsils, appendix, and bronchus [11] and nasal-associated lymphoid tissues [12], but not the spleen, and the HEVs in these tissues also support the extensive trafficking of naïve lymphocytes. HEVs are readily distinguishable from conventional venules by their cuboidal and tall endothelial cells, thick basement membrane, and prominent perivascular sheath [1, 5, 9].

While HEVs share some common features with ordinary venules, they specifically express tissue-specific adhesion molecules for naïve lymphocytes (called ‘vascular addressins’ in this context). HEVs in peripheral LNs express peripheral node addressins (PNAd) [13], whereas those in PPs express mucosal addressin cell adhesion molecule-1 (MAdCAM-1) [14]. The expression of these adhesion molecules is highly dependent on the lymphoid microenvironment and disappears rapidly upon in vitro culture [1, 15]. In addition, HEVs specifically express a variety of trafficking molecules with distinct functions, which coordinately constitute molecular determinants for the recirculation of naïve lymphocytes [16–18].

Determinants in Homeostatic Lymphocyte Recirculation: Lessons from HEVs

Trafficking of lymphocytes is controlled by a series of at least four molecularly distinct adhesion and signaling events [19]. These cascade reactions begin with lymphocyte rolling (step 1) on the vascular endothelium through villous-associated adhesion receptors, such as L-selectin [20] and integrin $\alpha 4 \beta 7$ [21]. This is followed by the triggering of pertussis toxin-sensitive G-protein-coupled receptors by chemoattractants (step 2). Chemoattractant binding, in turn, induces intracellular signals for the acti-

Fig. 2. Multiple adhesion cascades in lymphocyte-HEV interaction. In peripheral LN HEVs, lymphocytes initiate rolling via L-selectin/PNAd interactions. Subsequently, T cell adhesion is triggered by the CCR7/CCL19, 21 and CXCR4/CXCL12 pathways. In contrast, B cell adhesion is triggered by CXCR5/CXCL13 in combination with the CCR7/CCL19, 21 and CXCR4/CXCL12 pathways. These lymphocytes firmly adhere to the luminal surface of HEVs mainly via LFA-1/ICAM-1, 2 interactions. In PP HEVs, L-selectin/carbohydrate (probably not PNAd) and the integrin $\alpha 4\beta 7$ /MAdCAM-1 interactions mediate lymphocyte rolling. Chemokines trigger lymphocyte adhesion, which is dependent on integrin $\alpha 4\beta 7$ /MAdCAM-1 and LFA-1/ICAM-1, 2 interactions. The precise mechanisms underlying the transmigration step remain unclear.



vation-dependent firm adhesion of lymphocytes [22, 23] (step 3), in which the leukocyte integrin LFA-1 (CD11a/CD18) plays a vital role [24]. Finally, lymphocytes transmigrate across endothelial cells (step 4) and penetrate the underlying basement membrane to gain access to the parenchyma of the tissue. The cascade of reactions between the circulating lymphocytes and HEVs is summarized in figure 2. In each step of the sequential interactions, HEV-specific trafficking molecules, including multiple classes of L-selectin ligands, lymphocyte-specific chemokines, chemokine-binding proteins, and junctional adhesion molecules, play important roles in naïve lymphocyte recruitment.

Multiple Classes of L-Selectin Ligands Expressed in HEVs Control Lymphocyte Rolling

L-selectin expressed in lymphocytes mediates the initial rolling by recognizing sulfated and sialylated *O*-linked oligosaccharides presented by certain carrier proteins [25–27]. The L-selectin ligands in LN HEVs, collectively termed PNAd, consist of a group of sialomucins, including GlyCAM-1 [28], CD34 [29], podocalyxin [30], and sgp200 [31] (fig. 3a). In PP HEVs, MAdCAM-1 modified with L-selectin-reactive sugar chains is also important for lymphocyte rolling [32]. The absence of overt defects in lymphocyte trafficking across HEVs in CD34-deficient and CD34/GlyCAM-1 double-deficient mouse strains suggests that the multiple sialomucins cooperatively mediate L-selectin-dependent lymphocyte rolling in vivo

[33]. Podocalyxin-deficient mice show perinatal lethality, and no information on the effect of this defect on lymphocyte trafficking is currently available [34]. Recently, two novel endothelial sialomucins, endomucin [35] and endoglycan [36, 37], have been added to the list of L-selectin ligands in HEVs. Both endomucin and endoglycan can support L-selectin-dependent cell rolling in vitro upon the appropriate posttranslational modifications [37, Kanda et al., unpubl. data].

L-selectin-reactive oligosaccharides are synthesized by the coordinated actions of multiple enzymes including $\alpha(1,3)$ -fucosyltransferase (FucT)-IV [38], FucT-VII [39], and the sulfotransferase HEC-GlcNAc6ST (also known as LSST) in vivo [40]. Because the monoclonal antibody (mAb) MECA-79 abrogates L-selectin-mediated lymphocyte rolling by specifically recognizing L-selectin-reactive oligosaccharides in LN HEVs [13, 41], glycoproteins carrying MECA-79-reactive oligosaccharides have been considered to be the relevant L-selectin ligands in HEVs. In addition, studies by Rosen and colleagues [40] suggest the presence of another class of ligands for L-selectin. They found in HEC-GlcNAc6ST-deficient mice that luminal staining of HEVs by the MECA-79 mAb was almost completely eliminated, but significant abluminal staining remained. Under these conditions, lymphocyte migration to the peripheral LNs was only moderately (~50%) reduced, and the remaining lymphocyte migration was indeed L-selectin dependent. Based on these observations, they proposed that, in addition to the classical

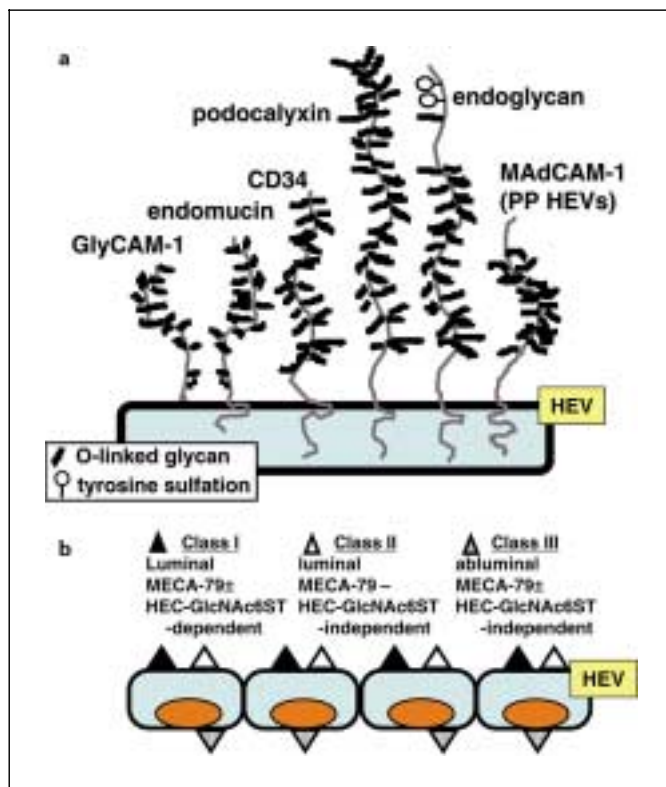


Fig. 3. L-selectin ligands expressed by HEVs. **a** LN HEVs express multiple sialomucins, including GlyCAM-1, CD34, podocalyxin, endoglycan, and endomucin. They are heavily modified with O-linked glycans that are reactive with L-selectin. Endoglycan can bind L-selectin via the tyrosine sulfation and sialyl Lewis X modification at the N-terminal region. In PP HEVs, MAdCAM-1 modified with L-selectin-reactive oligosaccharides also serves as a functional L-selectin ligand. **b** The proposed three classes of L-selectin ligands expressed by LN HEVs. In this model, the class 1 ligands (MECA-79⁺/HEC-GlcNAc6ST-dependent) and the class 2 ligands (MECA-79⁻/HEC-GlcNAc6ST-independent) are expressed in the luminal aspect of HEVs, whereas the class 3 ligands (MECA-79⁻/HEC-GlcNAc6ST-independent) are selectively expressed in the abluminal aspect of HEVs.

MECA-79 mAb-reactive L-selectin ligands (class 1 ligands), there must be a class of MECA-79 mAb-unreactive L-selectin ligands in the luminal surface of HEVs (referred to as class 2 ligands) as well as another class of L-selectin ligands (class 3 ligands) that is specifically expressed in the abluminal aspect of HEVs [40, 42] (fig. 3b).

M'Rini et al. [43] recently showed that MECA-79-negative L-selectin ligands are indeed expressed in the venules specifically located in the medulla of LNs. T cells rolled along and migrated through these venules in an L-

selectin-dependent manner [43], indicating the physiological relevance of the new class of L-selectin ligands expressed in these venules.

Although whether or not the MECA-79-negative L-selectin ligands identified by M'Rini et al. correspond to the class 2 ligands proposed by Rosen and colleagues [40] remains to be determined, these observations clearly indicate that at least two structurally distinct L-selectin ligands are expressed in the luminal surface of HEV-like blood vessels in a segmentally confined manner and regulate lymphocyte rolling.

Lymphocyte-Specific Chemokines Induce Lymphocyte Adhesion to HEVs

Chemokines Directing the Trafficking of Naïve T Cells into the LNs and PPs

Because the LFA-1 expressed in circulating lymphocytes is inactive in ligand binding, it must be activated to induce the firm adhesion of lymphocytes [22]. CC chemokine ligand 21 (CCL21, also known as SLC), a ligand for CC chemokine receptor 7 (CCR7), is expressed by the majority of HEVs in LNs and PPs, and triggers the firm adhesion of T cells to HEVs in vivo by activating LFA-1 [44–46]. Another CCR7 ligand, CCL19 (ELC), is also expressed in HEVs and induces T cell adhesion [47]. While CCL21 is abundantly produced by HEVs [44], CCL19 appears to be produced by stromal cells in the area surrounding HEVs and is transported to the luminal surface of the HEVs [47]. Importantly, a spontaneous mutant mouse strain, *plt/plt* (*plt*; paucity of LN T cells) [48], which lacks CCL21 and CCL19 in its HEVs [49, 50], shows grossly defective T cell adhesion to HEVs [45, 46], and subcutaneous injections of CCL21 [45] or CCL19 [47] can restore the luminal chemokine presentation and T cell adhesion in HEVs in these mice. In vitro studies showed that CCL21 induces integrin activation and T cell arrest on immobilized ICAM-1 [51, 52] and MAdCAM-1 [53] under flow conditions. These observations demonstrate that the CCR7 ligand chemokines are critical for triggering T cell arrest in HEVs, which is obligatory for T cell entry into LNs. The relative contribution of CCL21 and CCL19, however, remains to be determined.

CXCL12 (SDF-1), a ligand for CXCR4, also has been shown to play a role in T cell trafficking via HEVs [54]. The migration of CXCR4-deficient T cells to LNs and PPs was significantly impaired when they were transferred into *plt/plt* mice but not wild-type mice, indicating that the CXCL12-CXCR4 interaction plays a role in T cell migration across HEVs in collaboration with CCR7 pathways. Interestingly, the migration of CXCR4-defi-

cient T cells was more severely affected in mice carrying a *plt* mutation on the BALB/c background than on the C57BL/6 background, suggesting that some chemokine pathways can be regulated by strain-specific genetic modifications in mice [54]. Although CXCL12 is expressed as a protein on the luminal aspect of HEVs, its mRNA is not detectable there. Thus, like CCL19, CXCL12 is probably produced elsewhere and transported to HEVs [54].

Chemokines Directing Trafficking of Naïve B Cells into the LNs and PPs

CXCL12 also functions as a potent chemoattractant for mature B cells [55]. Okada et al. [54] reported that the CXCL12-CXCR4 pathway is required for B cell trafficking via HEVs, in cooperation with CCR7 ligands. They showed that the migration of CXCR4-deficient B cells into LNs and PPs was significantly suppressed in *plt/plt* mice but not in wild-type mice. In addition, a B cell chemokine, CXCL13 (BLC), which binds to CXCR5, also contributes to B cell trafficking across HEVs. Okada et al. showed that the entry of CXCR5-deficient B cells into PPs was selectively suppressed, whereas the migration into LNs was hardly affected. The combined desensitization of CCR7 and CXCR4 in CXCR5-deficient B cells further diminished the migration of the B cells into PPs. Under their experimental conditions, CXCL13 was specifically detected in the PP HEVs near follicles but undetectable in the other HEVs of PPs and LNs. Therefore, Okada et al. concluded that B cell entry into LNs requires CCL21, CCL19, and CXCL12, whereas the entry into PPs needs CXCL13 together with CCL21, CCL19, and CXCL12.

However, a study by Ebisuno et al. [56] is apparently in conflict with the study of Okada et al. in several respects. They observed that CXCL13 was detectable in the majority (~90%) of the HEVs in both LNs and PPs. Luminal expression of CXCL13 was detected in ~50% of the HEVs in LNs and PPs. In vivo migration studies showed that B cell migration to the LNs was significantly impaired in CXCL13-deficient mice. These results indicate that the CXCL13 expressed on the luminal surface of HEVs plays a critical role in the B cell trafficking across HEVs into both LNs and PPs. There are several possible explanations for the apparent discrepancy between the studies by Okada et al. and Ebisuno et al., including differences in the sensitivity of chemokine detection and in the time points used to determine lymphocyte migration. Like CCL19 and CXCL12, CXCL13 seems to be produced mainly by non-HEV cells and transported to the HEVs [54, 56].

Chemokine-Binding Molecules Expressed in and around HEVs

Although chemokines expressed by HEVs play pivotal roles in lymphocyte trafficking, the mechanisms regulating their spatial distribution and functions at the HEVs are largely unknown. We found that a variety of chemokine-binding molecules are expressed in and around HEVs [17, 18] (fig. 4a). In the luminal surface of vascular endothelial cells, proteoglycans are thought to play a role in chemokine presentation under blood flow [57]. Heparan sulfate proteoglycan(s) appears to be expressed in HEVs, given that an mAb against heparan sulfates reacts with HEVs and its reactivity is abrogated by heparitinase treatment [Tanaka, unpubl. data]. However, its ability to present chemokines to lymphocytes at the HEVs is unknown.

We also found that a promiscuous chemokine receptor, DARC (Duffy antigen receptor for chemokines) is abundantly expressed in the lumen of HEVs [58]. DARC is a member of the heptahelical chemokine receptor family. Because DARC lacks in its cytoplasmic portion the DRY motif, which is required for G protein coupling [59], it is unlikely to transmit chemokine signals and hence may function as a scavenger receptor [60]. We showed that DARC downregulates the activities of CXCL1 (KC) and CCL2 (MCP-1) by binding and sequestering them [58]. Interestingly, HEVs express an inflammatory chemokine, CXCL1, at the mRNA and protein levels, although the HEVs do not permit the trafficking of monocytes and neutrophils under physiological conditions [Ebisuno and Tanaka, unpubl. data]. These observations suggest that HEVs possess regulatory machineries that can functionally downregulate CXCL1 in situ and that DARC may contribute to this process. However, because DARC-deficient mice show no obvious abnormality in lymphocyte trafficking into the LNs under physiological conditions [58], DARC appears to be dispensable for the homeostatic lymphocyte trafficking into LNs. Molecules other than DARC, such as proteoglycans and/or sialomucins, may also be involved in the sequestration of inflammatory chemokines at HEVs.

In the basal lamina of HEVs, mac25/angiomodulin (AGM), which was previously identified as insulin-like growth factor-binding protein-related protein-1 [61], is expressed [62]. In contrast to the observation by Girard et al. [63] that mac25/AGM is localized to the luminal and basolateral aspects of HEVs, our studies indicated that it is selectively expressed in the basal lamina [62]. The mac25/AGM is unique in that it can selectively bind certain chemokines, such as CXCL10 (IP-10), CCL5

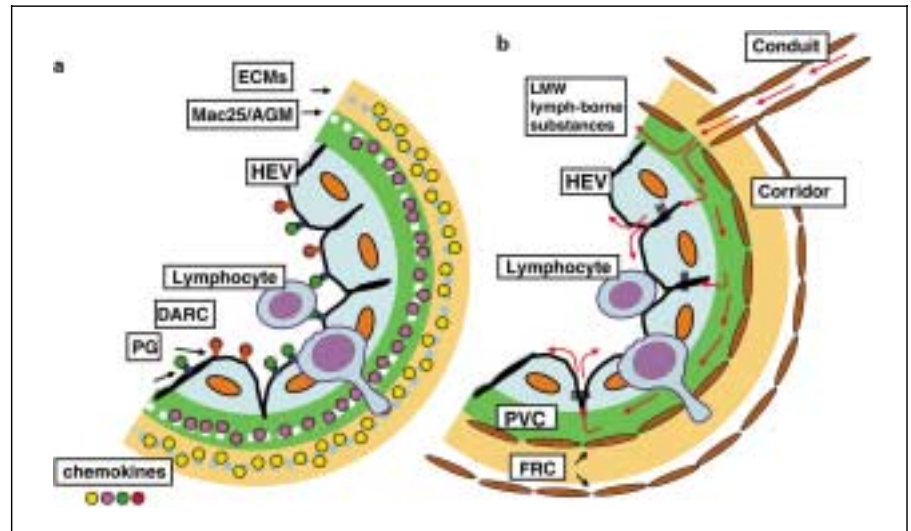


Fig. 4. Chemokines, chemokine-binding proteins, and the FRC networks at HEVs. **a** A schematic diagram showing the expression of multiple chemokines and chemokine-binding proteins at HEVs. HEVs express multiple chemokines (green, pink, yellow, red). At the luminal surface of HEVs, proteoglycans (PG, blue) may capture and present lymphoid chemokines, such as CCL21/SLC (green), whereas DARC (brown) may capture and scavenge inflammatory chemokines, such as CXCL1/KC (red). In the basal lamina of HEVs, mac25/AGM (white-dotted circle) can capture lymphoid chemokines, such as CXCL10/IP-10 (pink), and ECM components (light blue-dotted circle) also bind chemokines (yellow). Lymphocytes may respond to these matrix-presented chemokines sequentially during

the extravasation process. **b** Fibroblastic reticular cell (FRC) networks around HEVs. Pericytic cells that are probably of FRC origin surround HEVs in a concentric manner, forming 'corridors' around the HEVs, which appears to provide a three-dimensional scaffold for the attachment and migration of lymphocytes and antigen-presenting cells. An ECM-filled channel 'conduit' is also ensheathed by FRCs and allows the specific delivery of low molecular weight (LMW) lymph-borne substances from the subcapsular sinus to the perivascular channel (PVC) of HEVs. Lymph-borne substances reaching the PVC are subsequently transported to the luminal surface of HEVs. The mechanisms controlling this unidirectional transport are still incompletely characterized.

(RANTES), and CCL21 [64], and colocalizes with CXCL10 and CCL21 in the basal lamina of HEVs. Furthermore, it could present these chemokines to receptor-expressing cells in vitro [64]. Therefore, mac25/AGM is likely to present certain chemokines in the basal lamina of HEVs. In addition, in the basal lamina of HEVs, some extracellular matrix components can also capture certain chemokines. For instance, fibronectin binds CXCL12 [65], whereas some laminins specifically bind CCL21 [Yang and Tanaka, unpubl. data].

Collectively, a number of chemokine-binding molecules, including proteoglycans, DARC, mac25/AGM, and various extracellular matrix components, are expressed with distinct topology in the region of HEVs (fig. 4a). If chemokines are differentially captured and displayed by these chemokine-binding proteins, extravasating lymphocytes bearing the appropriate chemokine receptors would recognize these chemokines sequentially, which would expedite the directional movement of lymphocytes from the inside to the outside of the HEVs.

Transendothelial Migration of Lymphocyte and HEV Junctions

Although most lymphocytes transmigrate through the lateral junctions of HEVs [66, 67], the mechanism of this process is currently unknown. An in vitro study with cytokine-stimulated human endothelial cells demonstrated that the exposure to hydrodynamic shear flow promoted a robust transendothelial migration of CD3⁺ T cells in cooperation with CXCL12 immobilized on the apical endothelial surface [68]. This suggests that fluid shear-induced mechanical signals coupled with chemokine-induced G protein-dependent signals play an important role in the transendothelial migration of lymphocytes. In addition, CD31 [69] and CD99 [70] have been reported to act at distinct steps of monocyte transmigration across cytokine-activated endothelial cells. However, the physiological relevance of these in vitro observations to the lymphocyte transmigration across HEVs remains to be determined.

Table 1. Proposed nomenclature of JAM proteins and related molecules [135]

Name	Species	Original name	GenBank accession
JAM-A	mouse	JAM	U89915
	human	JAM	AF111713
	human	JAM	AF154005
	human	F11R	AF207907
JAM-B	mouse	VE-JAM	AF255911
	mouse	JAM-3	AJ291757
	human	VE-JAM	AF255910
	human	JAM-2	AY016009
JAM-C	mouse	JAM-2	AJ300304
	human	JAM-3	AF356518
ESAM	mouse	ESAM	AF361882
	human	ESAM	AF361746
CAR	mouse	CAR	NM_009988
	human	CAR	NM_001338
A33	mouse	A33	AF247659
	human	A33	U79725

JAM = Junctional adhesion molecule; F11R = F11 receptor; ESAM = endothelial cell-selective adhesion molecule; CAR = Cox-sackie- and adenovirus receptor.

Unlike the typical, fully sealed endothelium, attachments between adjacent HEVs are mediated by discontinuous 'spot-welded' junctions, which have been implicated in the highly efficient lymphocyte extravasation at HEVs [66]. Recent studies suggest that a new class of junctional adhesion molecules contributes to the distinct functions of HEV junctions. They are the recently identified Ig superfamily adhesion molecules, the JAM (junctional adhesion molecule) family members (table 1)². We found in mice that the JAM family members, including JAM-A, JAM-B, JAM-C, and a JAM-related molecule ESAM are expressed in HEVs [Nishitani and Tanaka, unpubl. data]. Aurrand-Lions et al. [71] reported that JAM-C is preferentially expressed in mouse HEVs and lymphatic vessels, but not in the brain vasculature. Interestingly, they found that upon transfection, JAM-C increased the paracellular permeability of MDCK epithelial cells as compared with

untransfected or JAM-A-transfected MDCK cells [72]. In addition, the overexpression of JAM-C in tEnd.1 endothelial cells promoted lymphocyte transmigration in response to CXCL12 [73]. These results suggest that JAM-C may function as a structural component of cellular junctions in HEVs and regulate the permeability of HEVs. In addition, HEVs express other molecules associated with tight junctional complexes, such as claudin-4 and claudin-5 [Nishitani and Tanaka, unpubl. data]. HEVs also express homophilic adhesion molecules associated with adherence junctional complexes, such as cadherin-2, cadherin-5 (VE-cadherin), cadherin-13, nectin-1, nectin-2, and nectin-3 [Nishitani and Tanaka, unpubl. data]. When lymphocytes transmigrate through HEVs, these molecules are likely to disengage in a coordinated manner, but how this is accomplished with the right timing and topology remains to be determined.

Although the lymphocyte behavior inside of the lymphoid tissues has been only partially characterized, recent studies with two-photon laser microscopy provide new insights into this issue [74, 75]. Miller et al. [74] reported that within inguinal LNs, T cell receptor-transgenic CD4⁺ T cells moved in apparently random directions immediately after extravasation. Mempel et al. [75] reported that T cell receptor-transgenic CD8⁺ T cells showed highly variable and time-dependent motility after entry into the popliteal LNs. It remains to be determined, however, what adhesive and chemotactic signals control the lymphocyte trafficking pathways within lymphoid organs.

Impact of Ongoing Immune Responses on HEV

Functions: Role of the Conduit System

HEVs are equipped with a reticular network known as the 'conduit', which runs from the subcapsular sinus to the abluminal side of HEVs and serves as a specialized delivery route for lymph-borne molecules [76–78] (fig. 4b). The conduit, which is ensheathed by fibroblastic reticular cells, appears to permit only low-molecular-mass substances, such as cytokines and chemokines, to gain access to HEVs [78]. It has been hypothesized that bulk fluid flow carries lymph-borne substances in the conduit, allowing their directional transport to HEVs and that soluble factors reaching the abluminal side of HEVs are then transported to the luminal surface [76–78] (fig. 4b). Thus, under physiological conditions, the conduit system is likely to contribute to the HEV-directed transport of certain lymphoid chemokines, such as CCL19, CXCL12, and CXCL13, which are mainly produced by non-HEV cell components within lymphoid tissues. During immune responses, this specialized transport system appears

² Because JAM molecules have been cloned by several investigators, their nomenclature is confusing. Thus, we follow in this review a recent recommendation for the nomenclature of the JAM family proteins and JAM-related molecules, as summarized in table 1 [135].

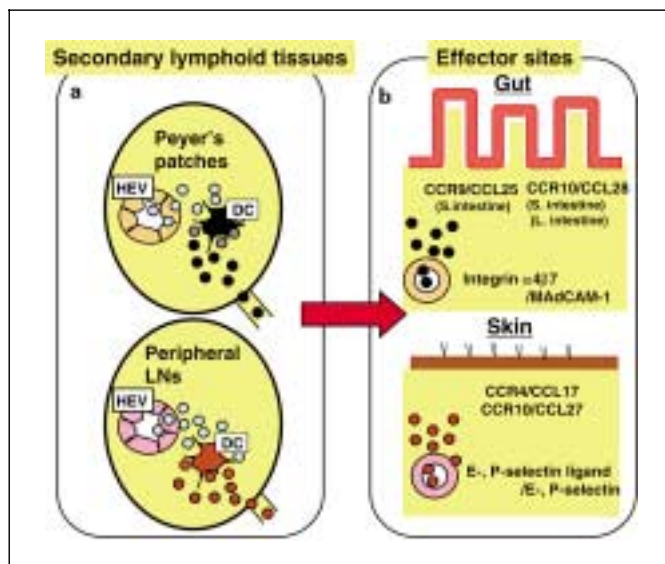


Fig. 5. Tissue-specific targeting of antigen-experienced lymphocytes to the effector sites. Upon antigen encounter in secondary lymphoid tissues, lymphocytes are reprogrammed to migrate to the extralymphoid tissues, such as the skin and gut. The gut-tropic lymphocytes express high levels of integrin $\alpha 4\beta 7$, which mediates their rolling and firm adhesion to intestinal venules by interacting with MAdCAM-1. These lymphocytes are guided into the small and large intestine by CCR9/CCL25 and CCR10/CCL28, respectively. The skin-tropic lymphocytes express E- and P-selectin ligands, allowing lymphocyte rolling on the postcapillary venules in the inflamed skin. They then migrate into the skin in response to CCR4/CCL17 and CCR10/CCL27 signaling. In the induction of the tissue tropism of lymphocytes, tissue-specific DCs may play a critical role.

to support the selective delivery of certain proinflammatory chemokines to HEVs, influencing leukocyte recruitment into the secondary lymphoid tissues.

Palframan et al. [79] reported that the inflammatory chemokine CCL2 (MCP-1), produced in inflamed skin, is transported to HEVs and promotes monocyte recruitment into the LNs. In a similar local inflammation model, Janatpour et al. [80] reported that a subset of HEVs predominantly located in the parafollicular region in inflamed LNs acquired CXCL9 (MIG) expression and preferentially supported CXCR3-expressing monocyte recruitment. This was apparently dependent on TNF produced in the LN parenchyma, because neither the upregulation of CXCL9 expression and nor monocyte recruitment was observed in the TNF α -deficient mice [80]. The LN microenvironment thus appears to influence the nature of the molecules expressed in HEVs by producing humoral factors such as TNF α . Collectively, the chemo-

kine repertoire displayed in HEVs appears to be affected by ongoing immune responses via at least two mechanisms: remote control by peripheral inflammatory sites and immediate control by the adjacent LN parenchyma.

Lymphocyte Trafficking into the Spleen

The spleen is a major secondary lymphoid organ that collects antigens from the blood. The lymphoid compartment of the spleen, which is known as the white pulp, is surrounded by the marginal zone, where lymphocytes entering the spleen are released from splenic arterioles [81].

Lymphocyte migration into the splenic white pulp requires adhesive interaction through lymphocyte integrins LFA-1 and $\alpha 4\beta 1$ and their ligands expressed by the sinus lining cells in the marginal zone [82]. This process also requires pertussis toxin-sensitive G α i signaling. These observations suggest that chemokine receptor signaling and the integrins LFA-1 and $\alpha 4\beta 1$ are functionally linked to promote lymphocyte entry into the splenic white pulp, indicating close parallels between lymphocyte entry into the splenic white pulp and that into the LNs and PPs. It should be noted, however, that selectins are not required for lymphocyte entry into the splenic white pulp, probably because shear forces in the marginal sinus are much weaker than those occurring in HEVs [83]. Although MAdCAM-1 is expressed in the marginal sinus lining cells, a function-blocking antibody against this molecule did not inhibit lymphocyte entry into the white pulp [84].

Tissue-Specific Trafficking of Antigen-Experienced Lymphocyte Subsets

Lymphocyte Subsets with Tissue Tropism to the Skin or Gut

Antigen stimulation in the secondary lymphoid tissues reprograms the trafficking properties of naïve T cells and B cells, enabling antigen-experienced effector and memory cells to migrate also to extralymphoid tissues, such as the skin and gastrointestinal tract. Tissue tropism of lymphocytes for the skin or intestine is strongly associated with the selective expression of cell surface adhesion receptors specific for endothelial adhesion molecules (fig. 5). The skin-tropic lymphocytes preferentially express functional E- and P-selectin ligands, such as P-selectin glycoprotein 1 (PSGL-1) posttranslationally mod-

ified with specific carbohydrates, which mediates lymphocyte rolling on postcapillary venules in inflamed skin [85, 86]. In humans, T cells with skin tropism express a unique marker, the cutaneous lymphocyte antigen (CLA), which is defined by the mAb HECA-452 [87, 88]. In contrast, gut-tropic lymphocytes express high levels of integrin $\alpha 4\beta 7$ [3], which mediates both the rolling and the firm adhesion of lymphocytes in intestinal venules by interacting with MAdCAM-1.

Recent studies revealed that tissue-selective chemokines are expressed in the skin and gut, and play important roles in the tissue-specific recruitment of effector/memory lymphocytes to the respective sites (fig. 5) [89, 90]. CCL17 (TARC), which is expressed by activated cutaneous endothelium, binds to the chemokine receptor CCR4, which is expressed at high levels by CLA⁺ skin-tropic lymphocytes, and CCL17 is reported to trigger the adhesion of rolling lymphocytes in cutaneous venules [91]. In addition, CCL27 (CTACK), which is mainly produced by keratinocytes in the skin and displayed on the surface of dermal endothelial cells, binds to the chemokine receptor CCR10, which is also specifically expressed by CLA⁺ skin-tropic lymphocytes [92, 93]. Signals through CCR4 and CCR10 appear to play overlapping or at least partially redundant roles in lymphocyte recruitment to the skin [94] (fig. 5).

In the gut, CCL25 (TECK) is selectively produced by epithelial cells in the small intestine [95] and presented on the intestinal venules [96]. In the small intestine, virtually all the T cells and B cells [95, 96] and at least a fraction of the IgA plasma cells [97] express the chemokine receptor CCR9, which binds CCL25, raising the possibility that CCL25 and CCR9 play critical roles in the trafficking of these lymphocytes into this tissue (fig. 5). A related chemokine, CCL28 (MEC), is produced by epithelial cells in the large and small intestines [98].

CCL28 is also expressed in the epithelial cells in other mucosal tissues, including the trachea, salivary gland, and mammary gland [98], and reciprocally, the CCL28 receptor CCR10 is expressed in nearly all IgA-secreting plasma cells in these tissues [99, 100]. These observations are compatible with the hypothesis that CCL28 and CCR10 help in targeting plasma cells secreting protective IgA to the mucosal surfaces in these tissues.

By contrast to the successful identification of adhesion receptors and chemokines controlling the tissue-specific lymphocyte trafficking to the skin or gut, relatively little is known about the mechanisms regulating lymphocyte trafficking to other nonlymphoid tissues.

While under inflammatory conditions certain adhesion molecules and chemoattractants, such as ICAM-1, ICAM-2 and VCAM-1, and chemokine ligands for CCR5 and CXCR3 have been implicated in the lymphocyte trafficking through extralymphoid organs, such as the liver, lung, and brain [89], tissue-specific determinants for lymphocyte recruitment in these sites have not yet been identified.

Induction of Tissue-Specific Trafficking Properties in Lymphocytes

How do tissue-specific lymphocyte subsets develop? Recent studies indicate that the tissue-specific trafficking properties of lymphocytes are induced during a lymphocyte activation process under the influence of the unique microenvironments of the secondary lymphoid tissues (fig. 5a). Campbell and Butcher [101] reported that within 2 days of immunization, cells responding in intestinal mesenteric LNs began to express high levels of the integrin $\alpha 4\beta 7$ and gained responsiveness to chemokine CCL25, while those activated in subcutaneous peripheral LNs upregulated ligands for P-selectin. These phenotypic changes occurred when antigen-specific lymphocytes remained sequestered within the secondary lymphoid tissues. Mora et al. [102] recently reported that the PP dendritic cells (DCs) can selectively imprint gut tropism on T cells. They showed that stimulation of CD8⁺ T cells by DCs from PPs, peripheral LNs, and spleen induced activation markers and effector activity in T cells to comparable levels, but only the PP DCs induced high levels of $\alpha 4\beta 7$ integrins, responsiveness to CCL25, and the ability to migrate into the small intestine. Thus, whereas the mechanisms by which antigen or antigen-pulsed DCs confer tissue specificity remain to be determined, these observations collectively suggest that lymphocytes are reprogrammed with trafficking properties upon antigen encounter in the secondary lymphoid organs, which enables them and their progeny to traffic to the extralymphoid tissue where the antigen is located.

Trafficking Properties of Effector and Memory T Cell Subsets

A number of studies have shown that helper T cell subsets (Th1 and Th2 cells) induced in vitro preferentially express a distinct set of chemokine receptors: IFN- γ -producing Th1 cells selectively express CXCR3 and CCR5, while IL-4-producing Th2 cells preferentially express CCR4, CCR3, and CCR8 [103]. However, a study

by Kim et al. [104] indicates that in vivo-activated Th1/Th2 cells show remarkable heterogeneity in their chemokine receptor expression. Studies by Nanki and Lipsky [105] showed that the expression of CXCR3, CXCR4, CCR3, CCR4, and CCR5 was not significantly different between Th1 and Th2 cells in the blood and in rheumatoid arthritis synovial tissues. These observations call into question the assumption that the expression patterns of chemokine receptors define these helper T cell populations [104]. In any case, given the requirement for lymphocytes to migrate to diverse tissue sites in the body, it may be advantageous for substantial heterogeneity to exist in the trafficking potential of functional lymphocyte subsets [104].

Memory T cells can be classified as either central memory (T_{CM}) cells or effector memory (T_{EM}) cells [106]: T_{CM} cells lack immediate effector function but can rapidly respond to antigen upon reexposure, whereas T_{EM} cells produce IL-4 and/or IFN- γ . It has been proposed that the expression of CCR7 represents a defining marker for non-polarized T_{CM} (CCR7⁺) and polarized T_{EM} cells (CCR7⁻) [106]. However, recent studies suggest that the majority of cytokine-producing CD4⁺ memory T cells that differentiate in vivo functionally express CCR7 and are able to recirculate through the secondary lymphoid tissues [107, 108]. Thus, the concept that CCR7 expression in memory T cell subsets predicts mutually exclusive cell populations (T_{CM} or T_{EM}) needs to be investigated further.

In addition to peptide chemokines, a lipid chemoattractant, LTB₄, also appears to be involved in the migration of Th1 and Th2 cells that express the LTB₄ receptor, BLT1 [109–111]. LTB₄ is one of the leukotrienes produced during mast cell degranulation [110] and has been shown to induce the chemotaxis of CD8⁺ T_{EM} cells in vitro and the rapid integrin-mediated arrest of the rolling of CD8⁺ T_{EM} cells and T_{CM} cells in the venules of the cremaster muscle [109]. Therefore, LTB₄ may play an important role in vivo in the initial recruitment to inflamed sites of not only myeloid leukocytes but also activated T cells.

Egress of Lymphocytes from Lymphoid Tissues

For an effective immune response to occur, lymphocytes should return to the circulation after surveying the secondary lymphoid tissues for antigens. Relatively little is known about this step of lymphocyte trafficking, but it is known that the transit time of lymphocytes within the secondary lymphoid tissues is altered during immune

responses. In an early phase of the immune response, there is a rapid ‘shutdown’ of lymphocytes exiting from the LNs [112], suggesting that the lymphocyte egress from LNs, i.e., entering the efferent lymph is an actively regulated process. An important observation in this area was made by Chiba et al. [113], who found that a novel immunosuppressant, FTY720, induces rapid lymphocyte sequestration in the LNs and PPs and a concomitant loss of these cells from the blood and lymph. A subsequent study by Mandala et al. [114] showed that FTY720 shares structural homology with sphingosine and that a phosphorylated form of FTY720 acts as an agonist on sphingosine-1-phosphate (S1P) receptors. They reported that the rapid sequestration of lymphocytes into LNs from the blood reflects the rapid inhibition of lymphocyte egress from these tissues into the lymph; after a single dose of FTY720, or other S1P receptor agonists, lymphocytes markedly accumulated immediately subjacent to the sinus-lining endothelium in mesenteric LNs. Recent studies by Matloubian et al. [115] showed that FTY720 treatment downregulated the S1P receptors on lymphocytes, which may contribute to the lymphocyte sequestration in LNs and PPs. These observations demonstrate the importance of sphingolipid mediators in lymphocyte trafficking and may explain the known immunosuppressive effect of FTY720, at least in part. We found that FTY720 induced severe lymphopenia in *aly/aly* mice (*aly*: lymphophasia), which systemically lack LNs and PPs [116], suggesting that the S1P pathway also regulates lymphocyte transit through organs other than LNs and PPs.

Remaining Enigmas

The identification of novel cell adhesion molecules, tissue-specific chemoattractants, and their receptors has revolutionized our understanding of tissue-specific lymphocyte recirculation and localization. However, there is a considerable amount of apparently contradictory data, which have often been neglected in review articles of this field.

Migration Efficiency of Naïve T Cells and B Cells

Although both naïve T and B cells recirculate among the secondary lymphoid tissues, conflicting data exist as to the efficiency of migration of these cells to the LNs. There are a few reports indicating that the migration of B cells into the LNs is less efficient than that of T cells, in mice [54, 117–120], and one study indicates that this is due to a lower expression of L-selectin on B cells com-

pared with T cells [120]. In addition, studies in mice and rats indicate that B cells recirculate more sluggishly than T cells [117, 121]. The time required to migrate from blood to lymph is 30 h for B cells and 15 h for T cells in rats [121]. However, studies in rats by Pabst, Westermann, and others [122, 123], who used unseparated cell populations and identified each subset immunohistologically subsequent to the migration, showed that both T and B cells enter various LNs with similar kinetics and in similar numbers, although T cells express L-selectin more abundantly than do B cells in rats [122]. Given these observations, they suggested that if B cells recirculate more slowly than T cells, it might be due to the difference in the retention time of B and T cells in lymphoid tissues rather than to the efficiency of their entry [122, 123].

Migration Preference of Naïve T Cells and B Cells

In some studies in mice, B cells have been reported to enter PPs preferentially, whereas T cells preferentially enter peripheral LNs [118]; this was determined by the presence of tissue-specific HEVs [118]. However, subsequent studies in rats showed the contradictory results that T and B cells entered peripheral LNs and PPs with a similar frequency and that there was no significant difference in the number of T and B cells that interact with HEVs [123, 124]. These findings suggest that, at least in rats, T and B cells enter LNs and PPs without preference. Thus, the extrapolation of results from one animal species to another has to be performed with caution.

Segmental Differences in HEV Functions

The use of intravital microscopy in mouse PPs suggested the interesting possibility that the sites of B and T cell firm adhesion in HEVs are geographically segregated. Warnock et al. [46] reported that T cells preferentially adhered to interfollicular HEVs that displayed high levels of CCL21, whereas B cells adhered mainly to follicular HEVs that expressed only low or undetectable levels of CCL21. On the basis of these observations, they proposed that T cell and B cell entry into lymphoid tissue is segmentally controlled at the level of HEVs, and that the differential display of chemokines that trigger firm lymphocyte adhesion by HEVs is responsible for this control [46]. However, these results are in sharp contrast to earlier studies in rats showing that T and B cells enter LNs via the same HEVs and that the sorting of T and B cells occurs after they have entered the LNs [123, 125, 126]. Our own data in mice also show that the majority of HEVs express both CCL21 and CXCL13 in both LNs and PPs [56]. Thus, although T-lymotropic and B-lymotropic

HEVs may exist to some extent, they may not represent the majority of HEVs. The issue of segmental differences in HEV functions requires further verification.

How Is Directional Cell Movement Regulated?

Lymphocyte trafficking is intrinsically linked with directional cell movement [127]. Although directional lymphocyte movement may be governed by mechanisms known as chemotaxis (migration along a gradient in solution) or haptotaxis (migration along a gradient bound to extracellular matrices or cell membranes), there has been no direct evidence for a graded deposition of positional signals, including chemokines or adhesive matrix components, in vivo [128, 129]. This may suggest that the formation of a gradient is not absolutely required for these molecules. In fact, a spatial concentration gradient of CXCL12 was not required to maintain the directed migration of T cells in vitro when it was presented by fibronectin [65], indicating that matrix-bound chemokines could stimulate T cells to promote directional migration in vitro. Clearly, further studies are needed to elucidate how positional signals are generated and maintained in vivo, and how they control directional lymphocyte motility.

Summary and Future Directions

Over the past several years, much progress has been made in our understanding of the molecular mechanisms of tissue-specific lymphocyte trafficking. Together with the differential expression of cell adhesion receptors for vascular addressins, the regulated expression of chemokine receptors seems to provide a plausible explanation for the tissue-specific trafficking of naïve and antigen-experienced lymphocytes. In addition, we are beginning to understand the trafficking properties of specific lymphocyte subsets, such as NKT cells [130], CD4⁺ regulatory T cells [131], plasma cells [97], marginal zone B cells [132], and B1-B cells [133].

Nevertheless, many fundamental questions remain to be answered. For instance, the physiological signals required for the induction and maintenance of the specific phenotype of HEVs are unknown. Although intact lymph flow is required for the expression of tissue-specific addressins in HEVs in vivo, the one or more lymph-borne factors that control the functions of HEVs remain to be identified. In addition, the molecular mechanisms underlying the directional lymphocyte transmigration observed in HEVs are also unclear. Although the sequential

engagement of distinct adhesion molecules, such as CD31, CD99, and JAMs, appears to be important in the transendothelial migration of monocytes, the precise requirement for adhesion molecules in the transmigration of lymphocytes across HEVs remains unknown. Finally, the mechanisms of the imprinting of tissue tropism on lymphocytes in secondary lymphoid tissues require further investigation. While tissue-specific antigen-presenting cells, such as DCs, may play a vital role in this process, signals for the induction of the tissue tropism in lymphocytes have not been identified. A detailed understanding of the cellular and molecular mechanisms that govern tis-

sue-specific lymphocyte trafficking should lead to the development of new approaches for manipulating immunological disorders and designing improved vaccines.

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