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Review

Cellular traffic through afferent lymphatic vessels

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ABSTRACT

The lymphatic system has long been known to serve as a highway for migrating leukocytes from peripheral tissue to draining lymph nodes (dLNs) and back to circulation, thereby contributing to the induction of adaptive immunity and immunesurveillance. Lymphatic vessels (LVs) present in peripheral tissues upstream of a first dLN are generally referred to as afferent LVs. In contrast to migration through blood vessels (BVs), the detailed molecular and cellular requirements of cellular traffic through afferent LVs have only recently started to be unraveled. Progress in our ability to track the migration of lymph-borne cell populations, in combination with cutting-edge imaging technologies, nowadays allows the investigation and visualization of lymphatic migration of endogenous leukocytes, both at the population and at the single-cell level. These studies have revealed that leukocyte trafficking through afferent LVs generally follows a step-wise migration pattern, relying on the active interplay of numerous molecules. In this review, we will summarize and discuss current knowledge of cellular traffic through afferent LVs. We will first outline how the structure of the afferent LV network supports leukocyte migration and highlight important molecules involved in the migration of dendritic cells (DCs), T cells and neutrophils, *i.e.* the most prominent cell types trafficking through afferent LVs. Additionally, we will describe how tumor cells hijack the lymphatic system for their dissemination to draining LNs. Finally, we will summarize and discuss our current understanding of the functional significance as well as the therapeutic implications of cell traffic through afferent LVs.

1. Introduction

The lymphatic vascular system has important functions in tissue fluid homeostasis, transport of macromolecules and uptake of dietary fats from the intestine [1–3]. Moreover, LVs transport antigen-containing lymph and leukocytes from peripheral tissues to dLNs [2, 4], and from LNs back into the blood circulation. LVs present in peripheral tissues upstream of a first dLN are generally referred to as afferent LVs. Over the past years, increasing evidence revealed a crucial role for leukocyte trafficking through afferent LVs for the induction of adaptive immunity and general immune-surveillance [5, 6].

To date, the lymphatic system has been less well studied compared to the blood vascular system. However, lymphatic research has gained strong momentum over the past two decades, thanks to the discovery of lymphatic-specific markers, such as the lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) [7], the mucin type-1 protein Podoplanin [8], vascular endothelial growth factor receptor-3 (VEGFR-3) [9] and the lymphatic-specific transcription factor Prox-1 [10]. Despite their expression in other tissues and by other cell types, such as hepatic blood sinusoidal endothelial cells (LYVE-1) [11], kidney podocytes and lung alveolar type I cells (Podoplanin) [8] or skeletal muscles, neurons and retinal cells (Prox-1) [12], the use and combination of these makers nowadays enables the unambiguous molecular distinction between blood and lymphatic vasculature in tissues. Moreover, the generation of

Abbreviations: ACKR, atypical chemokine receptor; ALOX15, 15-Lipoxygenase-1; APC, antigen presenting cell; α-SMA, α-smooth muscle actin; BM, basal membrane; BV, blood vessel; CAM, cellular adhesion molecule; CFA, Complete Freund's Adjuvant; CLEVER-1, common lymphatic endothelial and vascular endothelial receptor-1; DC, dendritic cell; dLN, draining lymph node; ECM, extracellular matrix; HETE, hydroxy-eicosatetraenoic acid; HEVs, high endothelial venules; IVM, intravital microscopy; JAM, junctional adhesion molecule; LEC, lymphatic endothelial cell; LC, Langerhans cell; LN, lymph node; LV, lymphatic vessel; LYVE-1, lymphatic vessel endothelial hyaluronan receptor-1; MMR, macrophage mannose receptor; Prox-1, prospero-related homeobox-1; PLVAP, plasmalemma vesicle-associated protein; ROCK, rho-associated protein kinase; S1P, sphingosine-1-phospate; SLO, secondary lymphoid organ; SCS, subcapsular sinus; SMC, smooth muscle cell; T_{EM}, effector memory T cell; T_{reg}, regulatory T cell; T_{RCM}, recirculating memory T cell; T_{RM}, resident memory T cell; VEGF-C, vascular endothelial growth factor-C; VEGFR-3, vascular endothelial growth factor receptor-3

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lymphatic-specific conditional knock-outs [13–15] or fluorescent reporter mice [16, 17], together with technical advances like the isolation and cultivation of lymphatic endothelial cells (LECs) and time-lapse imaging performed in tissue explants or *in vivo*, has greatly enhanced our current understanding of leukocyte migration through afferent LVs. It has also become clear that not only leukocytes use LVs to reach dLNs, but that this pathway is also routinely hijacked by tumor cells. In fact, in many cancer types lymphatic involvement and the occurrence of LN metastasis has been shown to correlate with poor patient prognosis [18].

Vaccination is considered one of the crucial contributions to public health in the 20th century and highlights the importance of leukocyte migration through afferent LVs. Upon antigen recognition, following vaccine injection, antigen presenting DCs mature and start to migrate through afferent LVs to dLNs, where they induce a specific immune response, by presenting the antigen to T cells. Since their discovery approximately 40 years ago [19–21], it has become apparent that DC migration to dLNs is not only essential for priming the adaptive immune response in the context of vaccination and infection, but also for promoting and maintaining tolerance [4, 5, 22]. Besides DCs, different T cell subsets and neutrophils are also frequently found in afferent lymph, but the mechanisms and relevance of their migration is less well studied.

In this review, we will present and discuss current understanding of cellular migration through afferent LVs. We will first introduce the unique anatomy and morphology of the afferent lymphatic network and highlight the main leukocyte types commonly found in afferent lymph. Next, we will describe the stepwise migration of leukocytes from peripheral tissues to dLNs through afferent LVs and provide detailed insight of the molecules involved in their migration, as well as in the migration of tumor cells, which in part is guided by similar mechanisms. Finally, we will conclude with an overall discussion of the functional significance of cellular traffic through afferent LVs and its therapeutic implications.

2. Anatomical and morphological characteristics of the lymphatic vascular network

The lymphatic system is composed of central and peripheral secondary lymphoid organs (SLOs) and a highly dispersed network of LVs, which penetrates nearly all vascularized organs of the body [23, 24]. The afferent lymphatic network originates in peripheral tissues in the form of lymphatic capillaries, which sequentially merge into larger collecting vessels (Figs. 1A, 2). These collectors drain into and through one or more dLNs before converging into a single vessel, the thoracic duct. Ultimately, its content, called lymph, is returned to the blood circulation at the level of the subclavian veins [2, 24]. Considering that lymph is transported sequentially through a chain of LNs, an efferent LV leaving a LN, can be the afferent LV for the next downstream LN [25]. In this review, we will exclusively refer to afferent LVs as the initial peripheral vessels present in the tissue upstream of the first dLN.

The components of lymph (*i.e.* interstitial fluid, macromolecules and leukocytes) mainly enter LVs at the level of the lymphatic capillaries [26, 27]. Several distinct anatomical and morphological features support this process (Fig. 1A and B). The blind-ended lymphatic capillaries are formed by partially overlapping oakleaf-shaped LECs, which are connected *via* discontinuous button like cell-cell junctions. This gives rise to characteristic flaps, also termed primary valves, through which cells and fluid enter the lymphatic vessel lumen [27–29]. Capillary LECs are connected to the extracellular matrix (ECM) by anchoring filaments, which promote an opening of the flaps upon interstitial tissue fluid pressure increase [30, 31]. Furthermore, capillary LVs are surrounded by a thin and highly fenestrated basement membrane [30, 32].

Collecting LVs are uniquely adapted to ensure leakage-free transport of lymph (Fig. 1C). Collector LECs have an elongated morphology and are connected by continuous and tight zipper-like cell-cell junctions [28]. Collectors are enclosed by a continuous basement membrane and have smooth muscle cell (SMC) coverage [33]. Furthermore, collecting vessels contain intraluminal valves which prevent backflow of lymph upon vessel contraction and ensure unidirectional flow [34]. Eventually, afferent collecting LVs merge with the collagen-rich capsule of a dLN and lymph content is released into the subcapsular sinus (SCS). The inner layer of LECs of the SCS (floor LECs) regulates the entry of lymph-borne leukocytes into the parenchyma of the LN [4, 25, 35]. Moreover, macrophages are embedded in the SCS floor and sample the arriving lymph for soluble antigens [36]. From the SCS the lymph further percolates through the blind-ended cortical sinuses and the highly branched medullary sinuses. The latter eventually fuse to form the efferent LVs, which leave the LN in the area of the LN hilus [25, 37, 38]. The medullary sinuses are also lined by LECs interspersed with macrophages and have been shown to regulate the entry and exit of T cells into/from the LN parenchyma [25].

3. Cells present in afferent lymph

Early cannulation studies of afferent LVs conducted in sheep and healthy humans under homeostatic conditions revealed that T lymphocytes are the most common cell type in afferent lymph (80–90%) [39–42]. The majority of these cells represent $CD4^+$ effector memory T cells (T_{EMs}), while $CD8^+$ T cells are only found in small numbers. Functionally, $CD4^+$ T_{EM} are thought to migrate through LVs in order to recirculate from the periphery back to the blood circulation, in constant search of their antigen, thereby playing an important role in immune surveillance. In contrast, naïve T cells are only found in low numbers in cannulated lymph [43–47]. This is in line with the prevailing view that naïve T cells exclusively migrate between the blood and SLOs [48, 49]. In adoptive transfer studies, naïve lymphocytes injected into the skin arrived in dLNs, implying that these cells are nevertheless capable of migrating through afferent LVs [50, 51]. However, a more elegant experimental approach to investigate leukocyte migration *in vivo* is the use of transgenic mice, ubiquitously expressing a photo-convertible green-fluorescent protein, such as Kaede [52] or Kikume [53], in all cells. Upon exposure to violet light these proteins irreversibly switch their fluorescent spectrum to red. Therefore, upon irradiation of the skin, exposed photo-converted endogenous leukocytes can be tracked in a non-invasive manner *in vivo*. Photo-conversion experiments have recently revealed that regulatory T cells (T_{regs}) represent a prominent $CD4^+$ T cell subset migrating through afferent lymphatics in steady-state (approximately 20–25%) and in inflammation (up to 50%) [43, 54, 55]. Similarly, adoptive transfer studies recently reported that T_{regs} exit the skin *via* afferent LVs and migrate to dLNs [56, 57].

DCs are also frequently found in cannulated afferent lymph (5–15%) from sheep and humans [39–42]. While in sheep or human afferent lymph T cells greatly outnumber DCs (approximately 4-fold), comparable numbers of endogenous DCs and T cells arrived *via* afferent LVs in skin-dLNs of mice [43, 53]. This difference in composition is likely due to the fact that laboratory mice are housed in an almost pathogen-free, hyper-hygienic environment. Consequently, their immune system is mainly antigen-inexperienced, which translates into an overall smaller effector/memory T cell pool [58–60], possibly explaining the lowered T cell numbers migrating through afferent LVs in laboratory mice compared to in humans or sheep. Other immune cells such as monocytes, neutrophils, eosinophils, basophils or B cells are also routinely detected in steady-state afferent lymph, albeit at very low numbers [39, 40]. In inflammation, however, granulocyte numbers were shown to significantly increase [47, 61, 62].

4. Leukocyte migration through afferent lymphatic vessels

Despite the general knowledge of leukocyte migration through afferent LVs, the cellular and molecular mechanisms of this process are only now starting to be fully unraveled. In the last 20 years, cell-

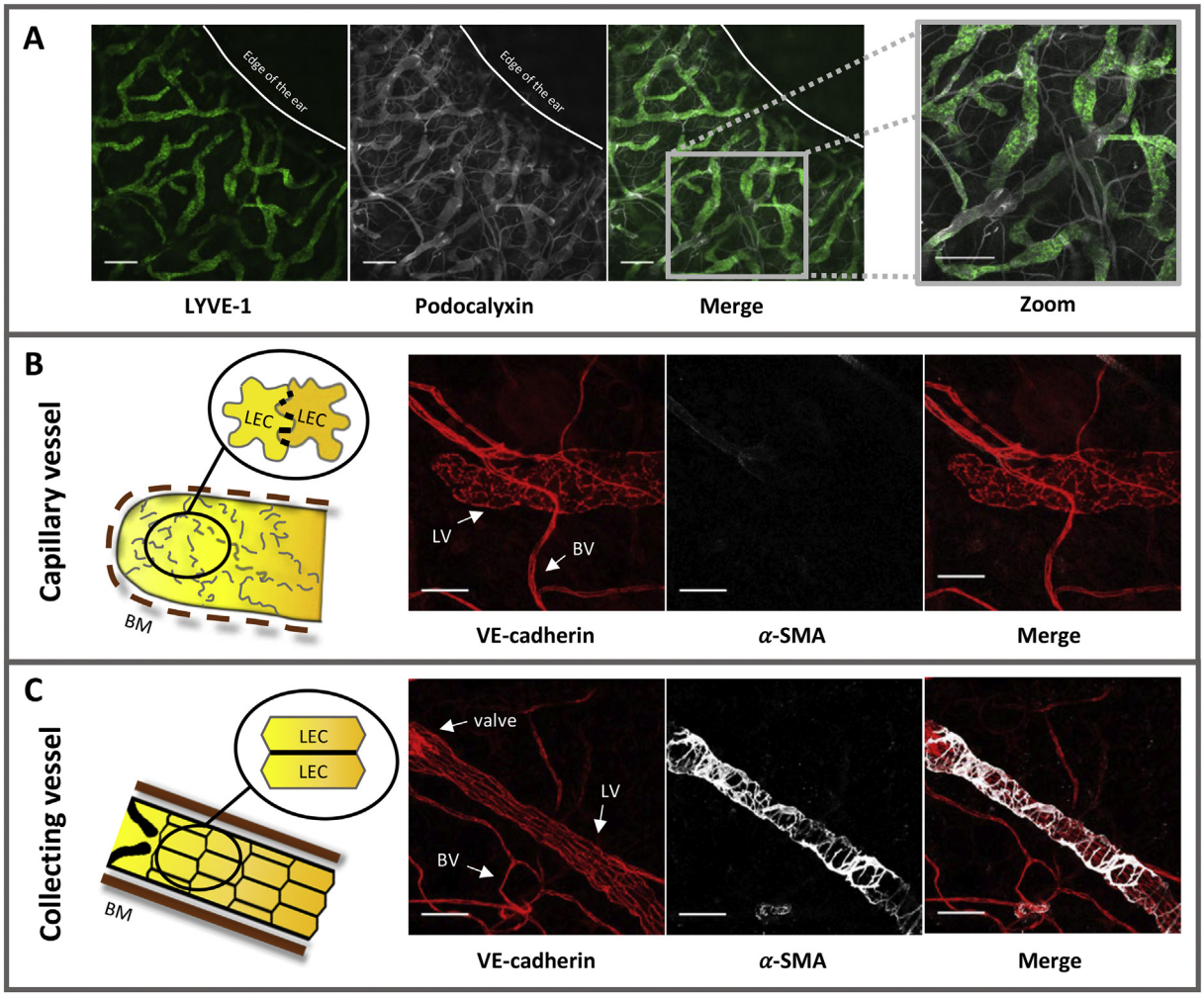

Fig. 1. Anatomical and morphological characteristics of afferent LVs.
A: Overview of the afferent lymphatic network in the mouse ear skin. Afferent LVs begin as blind-ended capillaries (LYVE-1$^+$, green) in peripheral tissues and subsequently merge into collecting vessels that eventually feed into a dLN. Staining for podocalyxin (grey), which marks the entire vasculature, reveals that LVs have a considerably larger diameter than BVs. The overlay of both stainings shows that (pre-)collecting vessels are LYVE-1$^-$ and contain valves (Zoom). Scale bar: 200 μm. B: The anatomy of lymphatic capillaries. Capillary LECs are oakleaf-shaped. Neighbouring cells partially overlap and are connected by discontinuous, button-like cell-cell junctions (thick black lines), generating open flaps, termed primary valves. Lymphatic capillaries lack SMC coverage, visualized by α-smooth muscle actin (α-SMA) staining, and are surrounded by a highly fenestrated basal membrane (BM). Scale bar: 50 μm. C: The anatomy of lymphatic collectors. Collector LECs have an elongated shape and are connected by tight and continuous zipper-like cell-cell junctions (black lines). Furthermore, they are surrounded by a continuous BM. Collecting LVs contain valves and are covered by SMCs, which mediate lymphatic contractility. Scale bar: 50 μm.

tracking studies revealed that leukocytes rely on specific molecules to migrate from peripheral tissues to dLNs. More recently, time-lapse imaging performed in tissue explants or *in vivo* intravital microscopy (IVM) has allowed us to visualize and study leukocyte migration in real-time and with cellular resolution. These experiments have revealed that the migration of T cells, DCs and neutrophils, which represent the main leukocyte subsets present in afferent lymph, follows a stepwise migration pattern: In a first step, leukocytes actively migrate and squeeze through the dense interstitial space, guided by chemotactic cues, in order to reach afferent LVs (Fig. 2A) [29, 63]. They then transmigrate, preferentially at the site of blind-ended lymphatic capillaries, through the primary flaps formed by LECs, into the LVs (Fig. 2B) [27]. Once inside, leukocytes remain attached to the luminal side of the vessel and continue crawling on the surface of LECs that line the lymphatic capillary wall (Fig. 2C) [64–68]. Intriguingly, immune cells within capillaries frequently patrol back and forth or even remain arrested for long time periods (Movies 1 and 2). For DCs, this migration was shown to occur semi-directedly towards dLNs [69]. Although DCs frequently migrated in the opposite direction of lymph flow, their migration was still reported to be overall directed towards the downstream dLN. Once leukocytes reach the downstream, contracting lymphatic collectors, they frequently detach from the lymphatic endothelium and become passively transported by lymph flow (Fig. 2D). The reason why passive transport by flow is only supported in collectors likely lies in the low lymph flow within capillaries (ranging from 1 to 30 μm/s [70, 71]), which is several orders of magnitude lower than peak flow measured in large contracting collectors [72, 73] or in BV capillaries [74]. In addition, it is likely that differences in the expression of adhesion molecules and chemokines in lymphatic capillaries *versus* collectors further contribute to the transition from crawling to flowing. In line with this assumption, we recently found that the adhesion molecule ICAM-1 [66] and chemokine CCL21 [69] are expressed at higher levels by LECs in lymphatic capillaries than in collectors.

Once in the dLN, leukocytes exit lymphatic collectors and translocate across the lymphatic sinuses into the LN parenchyma (Fig. 2E). Intravital imaging of the popliteal LN has revealed that arriving DCs entered the T cell zone across the SCS, while T cells preferentially penetrated the LN at the level of the medullary sinuses [35]. However,

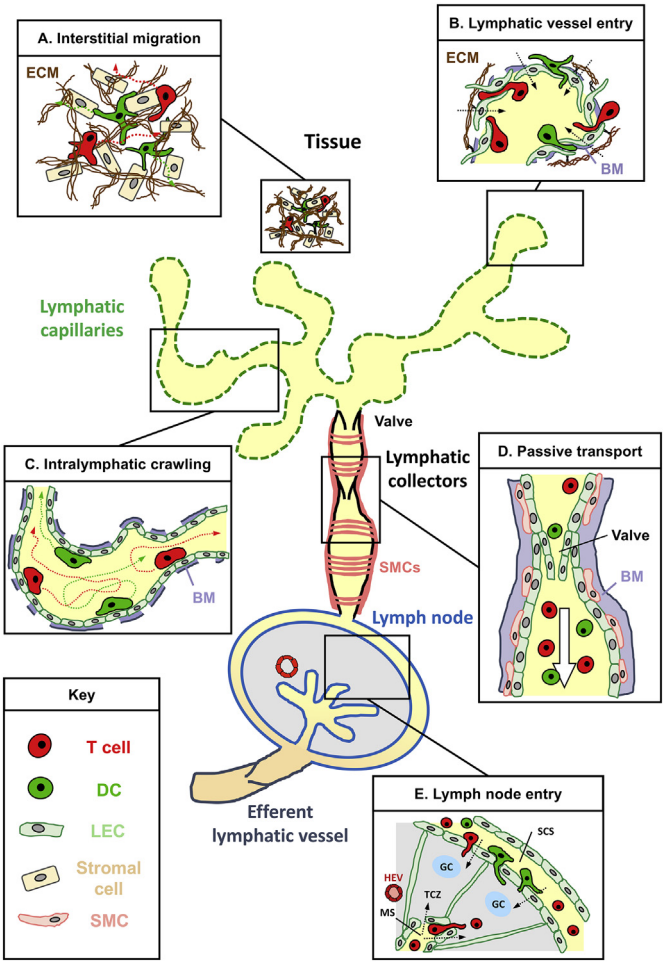

Fig. 2. Leukocyte migration from peripheral tissue *via* afferent LVs to dLNs. The LV tree consists of lymphatic capillaries, which merge into lymphatic collectors and connect to dLNs. The different steps in leukocyte migration from peripheral tissues *via* afferent LVs to dLNs are depicted in Boxes A-E. A: Interstitial leukocyte migration. Immune cells such as DCs (green) and T cells (red) squeeze and migrate through the dense interstitium consisting of stromal cells (beige) and extracellular matrix (ECM) components (*i.e.* collagen & fibronectin fibres - brown). They approach blind-ended lymphatic capillaries guided by chemotactic cues (*e.g.* CCL21). B: Lymphatic vessel entry. Leukocytes actively transmigrate through the primary flaps formed by LECs (light green) while entering into lymphatic capillaries. The lymphatic capillaries are surrounded by a fenestrated BM (purple), which is thought to facilitate leukocyte entry. C: Intralymphatic crawling within lymphatic capillaries. Once transmigrated, leukocytes remain attached to the intraluminal side of LECs (light green) lining the lymphatic capillary wall and actively crawl on their surface in a semi-directed manner. D: Passive transport in lymphatic collectors. Lymph flow and lower expression of certain adhesion molecules and chemokines in contracting lymphatic collectors, located downstream of the capillaries, likely contribute to leukocyte detachment and passive propagation. Lymphatic collectors contain valves, which ensure unidirectional lymph flow towards the dLN. SMCs (pink), which are able to contract, surround the lymphatic collectors and contribute to the elevated flow conditions compared to lymphatic capillaries. Moreover, lymphatic collectors are surrounded by a thick, continuous BM (purple). E: Lymph node entry. Leukocytes arrive in the dLN at the site of the SCS and actively transmigrate into the LN parenchyma. T cells were shown to also penetrate into the LN parenchyma from the medullary sinus (MS). GC (light blue): Germinal center. TCZ: T cell zone. HEV: High endothelial venule.

other studies suggested that lymphocytes may also directly cross the SCS: lymphocytes injected into the murine footpad could be detected in parenchymal areas underneath the SCS already 4 h after injection [75, 76].

In the following sections, we will highlight in greater detail the most important molecular requirements involved in lymphatic migration for each individual cell type commonly present in afferent lymph. Furthermore, we will summarize current knowledge of molecules involved in tumor cell migration/dissemination through afferent LVs.

5. Molecular mechanisms of DC migration through afferent lymphatic vessels

5.1. Role of CCR7 and its ligands

The undoubtedly best-studied molecules involved in DC migration are the chemokine receptor CCR7, which is upregulated on maturing DCs [77], and its two ligands, chemokines CCL21 and CCL19. Genetic deletion of CCR7 [78, 79] or antibody-mediated blockade of CCL21 [80] profoundly reduces DC migration to dLNs in mice. CCL21 is constitutively expressed by LECs of afferent LVs [81–83], with higher expression in capillaries than in collectors [69]. CCL21 comprises a highly positively charged C-terminus, which confers its immobilization on heparan sulfate-containing glycosaminoglycans (HS-GAGs) present on the LEC surface and in the ECM [84, 85]. Consequently, CCL21 forms a haptotactic interstitial gradient, which radiates from the LVs up to 90 μm into the tissue. This peri-lymphatic CCL21 gradient was recently visualized by whole-mount immunofluorescence and functionally shown to influence the directionality of DC movement in relationship to DC proximity to LVs in murine skin explants by time-lapse imaging [63].

In mice CCL21 is encoded by two genes, yielding two functional proteins that differ in one amino acid: CCL21-Leu and CCL21-Ser. Observation of naturally occurring mutant mice ($plt^{-/-}$: paucity of lymph node T cells), which have an impaired expression of CCL21-Ser and CCL19 revealed that CCL21-Leu is mainly produced by peripheral LVs, whereas CCL21-Ser is mainly produced by SLOs [86, 87]. Consequently, DC migration into the LVs is intact in $plt^{-/-}$ mice, whereas the entry of DCs from the SCS into LNs is compromised [88]. In agreement with this finding, absence of CCR7 on DCs was shown to abolish DC transmigration across the SCS into the LN parenchyma [35]. At the level of the SCS, the atypical chemokine receptor 4 (ACKR4), a scavenging receptor for CCL21 and CCL19, was also shown to play a major role in CCL21-guided DC entry into the LN parenchyma. ACKR4 is expressed by the ceiling but not the floor LECs of the SCS. Due to its scavenging activity, ACKR4 creates a chemokine gradient across the sinus floor that enables DCs to enter into the CCL21-rich paracortex of the LN [89].

In general, the spatio-temporal availability of CCL21 is thought to strongly influence gradient formation and hence DC migration. Using IVM, our group recently showed that the downstream-directed DC migration in lymphatic capillaries is CCL21/CCR7-dependent [69]. Performing imaging in murine skin explants and in flow chambers, we showed that low flow, as present in lymphatic capillaries, forms an intralymphatic downstream-oriented CCL21 gradient, which promotes DC trafficking within afferent lymphatics [69]. Besides flow, inflammatory cytokines and DC interactions with LECs were also shown to impact the extracellular distribution of CCL21: Immunohistology of tissue whole-mounts and of *in vitro* cultivated dermal LECs revealed that a substantial amount of CCL21 is stored intracellularly in the trans-Golgi network [63, 82, 90]. An *in vitro* study by Schumann et al. showed that DCs can proteolytically cleave off the positively charged C-terminal moiety of CCL21, resulting in the formation of a soluble gradient [91]. Moreover, the interaction of DCs with capillary LECs was shown to induce Ca^{2+} signaling in LECs, resulting in secretion of CCL21 from its intra-Golgi depots [90]. Increased CCL21 levels facilitated the *in vitro* transmigration of DCs and served as a strong stimulus for subsequently transmigrating leukocytes [90]. In addition, TNF-α was shown to increase CCL21 expression in murine tissues [92], by inducing its release from intralymphatic stores [82].

In contrast to CCL21, CCL19 (the second CCR7 ligand) is not

Table 1
Summary of molecules involved in DC migration through afferent LVs.

Molecule	Comments	References
CCR7/CCL21/CCL19	CCR7 gets upregulated on mature DCs. Depletion of CCR7 and blockade or deletion of CCL21 prevents DC migration from the skin to dLNs. CCL21 is expressed by LECs and forms interstitial and intralymphatic gradients.	[77] [78–80, 88] [63, 69, 82, 83, 85–88, 91]
ACKR4	ACKR4 on SCS ceiling LECs scavenges CCL21, contributing to CCL21 gradient formation across the SCS. ACKR4 on keratinocytes scavenges CCL19 in the epidermis.	[89] [96]
CXCL12/CXCR4	$CXCR4^+$ cells migrate in $CCR7^{-/-}$ mice and CXCR4 blockade reduces DC migration to dLNs.	[101]
CX_3CL1/CX_3CL1R	Blockade or deletion of CX_3CL1 reduces inflammation-induced DC migration from skin to dLNs.	[100]
$S1P/S1P_1/S1P_3$	Mature DCs upregulate S1P receptors. $S1P_1$ regulates dermal DC migration to dLNs. $S1P_3$ regulates migration of DCs from the intestine.	[15, 102, 103]
Integrin ligands (ICAM-1/VCAM-1)	Integrin ligands are expressed at low levels by LECs in steady-state, but get upregulated in inflammation. DC migration to dLNs is unaffected by depletion of integrins in steady-state. DC migration to dLNs during inflammation is integrin-dependent.	[83, 99, 105] [29] [99, 104, 105]
Rho-associated protein kinase (ROCK)	ROCK induces integrin de-adhesion of DCs. Blockade of ROCK reduces DC migration to dLNs in inflammation.	[64]
L1CAM	Mice lacking L1CAM expression in endothelial cells display reduced DC migration to dLNs.	[106]
JAM-A/JAM-C	Genetic deletion of JAM-A on endothelial cells or systemic treatment of mice with a JAM-C blocking antibody enhanced DC migration, possibly due to increased LV permeability.	[107, 108, 183]
LYVE-1	LYVE-1 supports docking of DCs to LECs of the capillary LVs.	[109]
Podoplanin/Clec-2	Clec-2-deficient DCs display reduced crawling on podoplanin positive vessels and an overall reduction in DC migration to dLNs.	[184]
Semaphorin3a (Sema3a)	Sema3a contributes to actomyosin-mediated nuclear contraction. Depletion of Sema3a reduces DC entry into LVs and migration to dLNs.	[185]
Metalloproteases (MMP)	Blocking MMP-2 and MMP-9 reduces the migration of LCs and dermal DCs to dLNs.	[186, 187]
Prostaglandin-Receptors	Treatment with prostaglandin E2 enhances DC migration to dLNs by modulating CCR7 signaling and MMP-9 expression.	[165, 187]
CCR8/CCL1	CCR8 is expressed on monocyte-derived DCs and regulates their migration to dLNs in inflammation.	[188, 189]
Leukotriene B4 Leukotriene C4	DCs upregulate CCR7 and CCL19 upon LTB4 or LTC4 stimuli and egress from skin to dLNs.	[166, 190]

expressed by LECs but is produced by activated DCs, fibroblastic reticular cells and high endothelial venules (HEVs) in SLOs [84, 93, 94]. FITC-painting experiments in $CCL19^{-/-}$ mice revealed no defect in DC migration through dermal LVs [95]. However, an involvement of CCL19 in DC migration to dLNs was recently implicated by the analysis of ACKR4-deficient mice, which displayed a defect in the migration of epidermal Langerhans cells (LCs) [96]. Besides its expression in SCS ceiling LECs, ACKR4 is abundantly expressed by keratinocytes, where it appears to sequester CCL19 produced by activated LCs. Intriguingly, defective LC migration in $ACKR4^{-/-}$ mice was rescued upon simultaneous deletion of CCL19 [96]. The likely explanation for this phenomenon is that constant signaling of CCL19 can desensitize epidermal DCs and LCs, making them insensitive for CCL21 [96, 97].

5.2. Role of other chemotactic cues

Despite the strong dependence of DC migration on CCR7 [78, 79], several other chemotactic cues influencing DC migration to dLNs have been described. LECs upregulate various chemokines during tissue inflammation [83, 98, 99]. CX_3CL1 (fraktalkine), for example, was shown to promote transmigration of DCs *in vitro* and to impact inflammation-induced DC migration *in vivo* [100]. Similarly, inflamed LECs also express the chemokine CXCL12 [46, 83, 101], which interacts with DC-expressed CXCR4 [101]. *In vivo* trafficking studies performed in the presence of the CXCR4 inhibitor AMD3100 resulted in significantly reduced DC migration [101]. Surprisingly, genetic CCR7 deletion profoundly reduces DC migration by approximately 90% [78, 79, 83], but deletion or blockade of CX_3CL1 or CXCR4 nevertheless resulted in an approximately 50% reduction of migration [100, 101]. This somewhat puzzling observation might point towards a spatially separated or sequential mode of action of the different chemokines. Besides chemokines, the well-known chemoattractant sphingosine-1-phosphate (S1P), which is produced by LECs in peripheral tissues and in LNs [15], reportedly also mediates DC migration. Maturing DCs upregulate the S1P receptors $S1P_1$ and $S1P_3$ [102, 103]. Mice treated with the S1P analog FTY720 or deficient in $S1P_1$ (but not $S1P_3$) displayed reduced migration of DCs from the skin to dLNs [102]. In contrast, DC migration from the intestine to dLNs was shown to depend on $S1P_3$ [103].

5.3. Role of adhesion molecules

Besides chemotactic cues, the role of cellular adhesion molecules in DC migration towards and into LVs has been intensively studied. Already in 2001, FITC-painting and adoptive transfer of DCs by footpad injection into $ICAM\text{-}1^{-/-}$ mice revealed a reduced migration of DCs and LCs to dLNs under inflammatory conditions [104]. Similarly,

Table 2
Summary of molecules involved in T cell migration through afferent LVs.

Molecule	Comments	References
CCR7	CCR7 deficiency in mice or on adoptively transferred T cells results in reduced migration to dLNs. T cell emigration from skin to dLNs depends on CCR7 function during acute but to a lesser extent during chronic inflammation.	[50, 110–112] [46]
S1P/$S1P_1$	T cells treated with FTY720 or S1P migrate less to dLNs. S1P produced by LECs promotes T cell survival.	[46, 51] [117]
CD44/macrophage mannose receptor (MMR)	Lack of either CD44 on T cells or MMR on LECs reduces T cell migration to dLNs.	[118]
Common lymphatic endothelial and vascular endothelial receptor-1 (CLEVER-1)	Antibody-mediated blockade of CLEVER-1 decreases $CD4^+$ and $CD8^+$ T cell migration to dLNs.	[120, 121]
ICAM-1/VCAM-1	T cell migration to dLNs is reduced upon ICAM-1 or VCAM-1 blockade.	[66]
Lymphotoxin (LT)	Reduced migration of LT-alpha deficient or LT-beta receptor Fc-fusion protein treated T_{regs} to dLNs.	[57]
MECA-32 (PLVAP)	PLVAP expressed by LN LECs forms diaphragms at the entry into the LN conduits and serves as a physical sieve that regulates entry of soluble antigen and of lymphocytes into the LN parenchyma.	[75]
Macrophage scavenger receptor 1 (MSR-1)	MSR-1 deficient mice have more lymphocytes entering into LN parenchyma *via* the SCS.	[76]

antibody-based blockade of VCAM-1, ICAM-1 or LFA-1 was shown to diminish inflammation-induced DC migration [99, 105]. Surprisingly, adoptively transferred pan-integrin$^{-/-}$ DCs, which lack all integrins including the ICAM-1 and VCAM-1 ligands LFA-1 and Mac-1, were found to migrate normally within the tissue and into lymphatic capillaries in steady-state and to arrive in normal numbers in dLNs [29]. These findings are likely explained by the fact that LECs express very little ICAM-1 and VCAM-1 in steady-state, whereas these molecules become upregulated in inflammation [83, 99, 105]. Matching the reports above, the rho-associated protein kinase (ROCK), which induces actomyosin-mediated nuclear contraction and de-adhesion from integrin ligands, was shown to contribute to *in vivo* migration of DCs under inflammatory conditions [64]. Collectively, these studies established the concept of integrin-independent DC migration in steady-state, but integrin-dependency under inflammatory conditions.

Besides integrins and their ligands, several other adhesion molecules were shown to contribute to DC migration: For example, mice with conditional ablation of the cellular adhesion molecule (CAM) L1 on endothelial cells displayed a reduction in inflammation-induced DC migration [106]. On the other hand, genetic or pharmaceutical blockade of junctional adhesion molecule (JAM)-A and JAM-C was shown to modulate DC migration to dLNs [107, 108]. Moreover, *in vivo* studies recently identified a novel role for the LEC-specific hyaluronan receptor LYVE-1 in DC migration. Specifically, LYVE-1 was shown to mediate DC entry into lymphatic capillaries [109]. In addition, a number of other LEC- and DC-expressed molecules have been shown to contribute to DC migration, as indicated in Table 1.

6. Molecules involved in T cell migration through afferent lymphatic vessels

In agreement with cannulation studies, the majority of endogenous T cells exiting tissues in mice are $CD4^+$ cells displaying a T_{EM}-like phenotype [110]. Similar to DCs, the best-known molecule involved in T cell migration *via* afferent LVs is CCR7. In mice, transferred CCR7-deficient T cells failed to arrive in dLNs in steady-state [50] or to exit non-lymphoid tissues through afferent lymphatics in a model of immunization-induced airway inflammation [111]. Moreover, in CCR7$^{-/-}$ mice endogenous lymphocytes reportedly accumulated in various mucosal epithelial tissues [112]. Murine $CD4^+$ T_{regs} were also shown to depend on CCR7 signaling to efficiently migrate from skin to dLNs under homeostatic conditions [113] and during an alloimmune response [56]. In a mouse model of Complete Freund's Adjuvant (CFA)-induced inflammation, T cell emigration was found to greatly increase in inflammation and to depend on G protein-coupled receptor signaling [46]. Surprisingly, however, CCR7 was mostly required during acute but to a lesser extent during chronic inflammation, implying that alternative chemotactic mediators become relevant during chronic inflammation [46].

Interestingly, a unique subset of endogenous recirculating memory $CD4^+$ T cell (T_{RCM}) present in the skin of mice was recently identified. These cells entered afferent LVs and migrated to dLNs in a CCR7-dependent manner [110]. T_{RCMs} reportedly are CCR7$^{int/+}$, CD62L^{int}, $CD69^-$, $CD103^{+/-}$, $CCR4^{+/-}$ and *E*-selectin ligands$^+$, a phenotype that distinguishes them from classical $CCR7^-$, $CD103^+$ and $CD69^+$ tissue-resident memory T cells (T_{RM}) [114, 115]. Of note, CD69 is a molecule known to destabilize and down-regulate $S1P_1$ and, consequently, to induce retention of lymphocytes in LNs [116]. In mice, the $S1P_1$ ligand S1P was shown to be mainly produced by LECs [15]. Moreover, in mouse models, adoptive transfer of $CD4^+$ T cells in presence of the functional $S1P_1$ receptor antagonist FTY720, which induces down-regulation of $S1P_1$, resulted in reduced skin exit of $CD4^+$ T cells to dLNs [46, 51]. Overall, this indicates that, in concert with CCR7, CD69 expression levels on peripheral T cells determine T cell tissue retention or exit into afferent LVs. Intriguingly, LEC-derived S1P was recently shown to promote T cell survival in mice [117], implying that the high levels of S1P within afferent LVs provide a suitable pro-survival environment for migratory T cells.

In addition to chemotactic signaling, adhesion molecules have also been shown to regulate lymphatic T cell migration. For instance, macrophage mannose receptor (MMR), which is expressed by LECs, was identified as a ligand of CD44 on T cells [118]. CD44 is a marker for T_{EM} and reportedly is expressed by most T cells present in afferent lymphatics [45, 110]. Lack of either CD44 expression by T cells or of MMR on LECs reduced T cell homing from skin to dLNs in mice [118, 119]. Furthermore, inhibition of common lymphatic endothelial and vascular endothelial receptor-1 (CLEVER-1), a glycoprotein receptor on LECs, was shown to reduce *in vitro* transendothelial migration and to significantly decrease skin exit of $CD4^+$ and $CD8^+$ T cell to dLNs *in vivo* [120, 121]. Under inflammatory conditions, murine T cell migration to dLNs was shown to depend on the LEC-expressed integrin ligands ICAM-1 and VCAM-1 [66].

More molecules that have also been described to regulate T cell migration through afferent LVs are summarized in Table 2.

7. Polymorphonuclear cell migration through afferent lymphatic vessels

Cannulation studies in humans and in sheep also detected low numbers of neutrophils, monocytes, basophils, eosinophils and B cells in afferent lymph [39–42, 122]. Of these cell types, the best studied one, migrating through afferent lymphatics, are neutrophils. Neutrophils become recruited to infected tissues where they establish the primary frontline of defense against invading pathogens. In addition to several other effector functions, neutrophils display high phagocytic activity and may also function as non-professional antigen presenting cells (APCs) [123, 124].

Immunization studies in mice using FITC-labeled ovalbumin revealed that neutrophils are the first type of APC to carry antigen to dLNs [124]. In recent years, studies involving IVM or the use of photoconvertible mice have shed more light on neutrophil migration through afferent LVs. One study using Kaede mice in a bacterial infection context found that neutrophil migration from the skin to dLNs is dependent on CXCR4 and CD11b, but not on CCR7 [125]. Conversely, studies using TNF-α- and/or CFA-induced inflammation models in the mouse ear skin [126] or cremaster muscle [67], reported a strong CD11b- and CCR7-dependence of neutrophil migration. A possible explanation for these seemingly opposing findings regarding CCR7 might lie in the different vascular beds/tissues investigated (ear skin *vs.* cremaster muscle) and the diverse inflammatory mediators applied (inactivated bacteria *vs.* soluble antigen).

In line with observations made for DCs and T cells (Fig. 2), IVM performed in the infected ear skin [125] and in the inflamed cremaster muscle [67] revealed that neutrophils actively crawl and patrol in lymphatic capillaries. Interestingly, comparison of DCs and neutrophils by IVM indicated that the migratory speed of neutrophils (5–13 μm/min [67, 125]) is considerably higher than that of DCs (5–8 μm/min [64, 68, 69]). However, this fact alone does not explain why neutrophils reportedly are the first APC-type arriving in dLNs [125, 127, 128]. Of additional importance might be that neutrophils enter inflamed tissues in an already semi-mature state [129] and were shown to rapidly upregulate CCR7 from premade intracellular stores [67]. Conversely, upon contact with a pathogen, tissue-resident DCs first need to undergo maturation, which involves transcriptional reprogramming and *de-novo* CCR7 synthesis, before they start migrating to the dLN. Intriguingly, a recent study suggested that neutrophils might also employ a completely different transmigratory mechanism when entering into LVs. *In vitro*, secretion of metalloproteinases and of the arachidonic acid metabolite 12(*S*)-HETE (hydroxy-eicosatetraenoic acid) by neutrophils triggered a partial disintegration of human LEC-LEC junctions and LEC retraction, thereby creating hotspots for the transmigration and passage of neutrophils across the LEC monolayer [130].

In addition, various other molecules reportedly influence the migration of neutrophils and are mentioned in Table 3.

Table 3
Summary of molecules involved in neutrophil migration through afferent LVs.

Molecule	Comments	References
CCR7	CCR7 is dispensable for neutrophil migration in the context of bacterial infections in mouse ear skin.	[125]
	Neutrophil migration is CCR7 dependent in TNF-α- or CFA-induced inflammation in the ear skin and the cremaster muscle.	[67, 126]
CXCR4	Inhibition of CXCR4 reduces migration of neutrophils from infected ear skin to dLNs.	[125]
CD11b	Blocking the integrin and complement receptor CD11b reduces neutrophil migration to dLNs.	[125, 130]
ICAM-1	Blocking ICAM-1 prevents neutrophil entry into afferent LVs in bacterial- and TNF-α-induced inflammation.	[125, 130]
CXCL8	CXCL8 promotes neutrophil transmigration through human LECs *in vitro*.	[130]
CXCL1 CXCL2 CXCL3 CXCL5 CXCL6	Several chemokines have been shown to be expressed by activated lymphatic endothelium and could possibly interact with CXCR1/2 on neutrophils. Neutralizing CXCL2 and CXCL5, however, had no effect in transmigration of neutrophils through human LEC monolayers *in vitro*.	[83, 98] [130]

8. Molecular mechanisms involved in tumor cell migration through afferent lymphatic vessels

Tumor dissemination *via* lymphatics leads to regional LN metastasis and strongly correlates with disease progression [131–133]. Tumor cells are known to release vascular growth factors like vascular endothelial growth factor (VEGF)-C or VEGF-D to stimulate proliferation of peripheral LVs [134]. Tumor-induced lymphangiogenesis provides an increased lymphatic density in vicinity or within the tumor, thereby promoting lymphatic tumor invasion [135]. Lymphatic pumping and lymph flow were also found to increase in tumor-draining LVs [136, 137], which could additionally promote tumor spread. In general, primary tumors growing in close proximity of LVs may infiltrate them passively, due to physical destruction caused by their massive growth. However, increasing evidence suggests that tumor spread to dLNs also involves active and directed tumor cell migration towards LVs.

Tumor cells may migrate either collectively, in small cohesive groups, or as single cells [138]. For collective tumor cell migration, IVM in mice revealed that tumor cells migrate towards and into LVs in bulks, thereby facilitating their spread to dLNs [139]. Intriguingly, a recent study suggested that migrating groups of human breast cancer cells are able to specifically disrupt the cellular integrity of the lymphatic endothelium *in vitro* by inducing large circular discontinuities in LEC monolayers [140]. This process reportedly was regulated by 12(*S*)-HETE, a product of 15-lipoxygenase-1 (ALOX15). In mice, pharmacological inhibition or sh-RNA-mediated knockdown of ALOX15 inhibited the formation of these circular discontinuities in LECs, and ALOX15-deficient tumor xenografts metastasized less to dLNs *in vivo* [140].

Individual tumor cell migration was shown to be surprisingly reminiscent of the amoeboid migration pattern of leukocytes [141]. In fact, recent findings indicate that tumor cells utilize the same chemotactic cues as leukocytes for migration towards LVs [142, 143]. For instance, many cancers, including breast cancer and melanoma, upregulate the chemokine receptors CCR7 and CXCR4 [144–147]. Expression of both receptors was shown to correlate with LN metastasis in patients and has been suggested as a prognostic marker for LN involvement [148–153]. Both ligands CCL21 (for CCR7) and CXCL12 (for CXCR4) are expressed by tumor-associated LVs [101, 152, 154, 155], implying that the lymphatic system could play an active part in driving directed tumor cell migration to dLNs. Indeed, *in vitro* chemotaxis experiments revealed that murine malignant melanoma cells released VEGF-C to directly attract LECs, which in turn produced CCL21 and consequently induced directed tumor migration [155, 156]. Similarly, injection of recombinant VEGF-C into the mouse skin was shown to increase CCL21 expression in LECs [156]. In mice, cancer cells were described to sense chemotactic signals produced by LECs, leading to directed migration and growth towards and into LVs [157]. Moreover, in several murine tumors, overexpression of CCR7 resulted in increased metastasis to dLNs [150, 158, 159], and CCL21 neutralizing antibodies were found to decrease LN metastasis of CCR7-expressing melanoma cells [158]. Similarly, antibody-mediated blockade of CXCR4 reduced LN metastasis in a murine breast cancer model [144]. These findings are supported by several correlation studies on patient material that reveal the relationship between CCR7 and CXCR4 expression and

Table 4
Summary of molecules involved in tumor cell migration through afferent LVs.

Molecule	Comments	References
CCR7/CCL21	CCR7 overexpressing tumor cells have increased rates of LN metastasis.	[144, 158]
	Tumor cells produce VEGF-C, which in turn increases CCL21 expression by LECs *in vitro* and *in vivo* and stimulates directed migration of tumor cells towards LECs.	[155–157]
	CCL21 stimulates tumor adhesion, migration, invasion and actin polymerization.	[144, 149, 155, 191–193]
CXCR4	Blockade of CXCR4 leads to reduced LN metastasis.	[144]
CCR8/CCL1	CCR8-CCL1 interaction promotes exit of CCR8-expressing tumor cells from collecting LVs and entry into LNs *via* the SCS. CCR8 blockade reduces LN metastasis.	[194]
CXCR3	CXCR3 expression on tumor cells correlates with increased LN metastasis.	[195]
12(*S*)-HETE/ALOX15	Reduced metastasis to dLNs of ALOX15-deficient tumor cells and upon pharmacological inhibition or sh-RNA mediated knockout.	[140]

disease progression in multiple cancers (*e.g.* [147–153, 160]).

A summary of different stimuli regulating tumor cell invasion of LVs is provided in Table 4.

9. Conclusion and outlook

Research on cellular traffic through afferent LVs has recently made great progress thanks to technical advances like the generation of lymphatic-specific knockouts, the use of photo-convertible mice to study endogenous cell trafficking or novel imaging approaches such as IVM. The latter experiments have revealed that leukocyte migration through afferent LVs represents a more complex process than previously assumed, involving the dynamic interplay of numerous molecules and distinct cellular migration steps. Besides leukocytes, tumor cells also make use of afferent LVs as routes for their dissemination to dLNs and beyond. The importance of lymphatic migration of DCs for the induction of protective immune responses and also for the maintenance of tolerance has been well established. By contrast, the relevance of T cell or neutrophil trafficking remains less well understood. Experiments in mice suggest that T cell emigration through afferent LVs may help to regulate T cell composition in the tissue and, consequently, the strength of the inflammatory response: While antigen-specific T_{EMs} recruited to the inflamed tissue enable the specific resolution of the infection, the concomitant exit of non-specific T_{EMs} from the tissues *via* the lymphatics likely helps to prevent overshooting inflammatory responses, due to bystander T cell activation [161, 162]. On the other hand, T_{RCM} are believed to contribute to immunesurveillance, by constantly migrating through peripheral tissues, in search of cognate antigen [110]. However, intense research has recently established the importance of T_{RM} in triggering rapid recall responses in the tissue during recurrent infections [163, 164]. In the future, the full significance of T cell residence *versus* recirculation in immune protection will need to be further addressed. Equally intriguing are recent observations of T_{regs} as a major portion of T cells exiting from inflamed tissues. The current understanding is that T_{regs} that have emigrated from tissues *via* afferent LVs have a more suppressive phenotype than T_{regs} that have entered inflammation-draining LNs from blood [43, 54–56]. How exactly T_{regs} contribute to suppressing overshooting inflammatory responses in tissues and dLNs, and potentially also to maintaining tolerance towards self-antigens, will require further investigation. With regards to neutrophils, several reports have shown that they can act as APCs in dLNs, but the overall relevance of their early migration and APC function [125, 127, 128] on the development of the adaptive immune response is still unclear at this point. It is also important to mention that thus far the mechanisms regulating cellular traffic through afferent LVs have mainly been investigated in mice. Although many molecules implicated in trafficking in these murine studies have also been detected in human tissues, they have most of the time only been investigated in *in vitro* studies with human cells.

Nevertheless, there is increasing interest in investigating and developing novel therapeutic approaches to modulate leukocyte migration through afferent LVs. This particularly concerns DC migration or tumor cell metastasis, for which the functional significance, *i.e.* in the induction of adaptive immunity or in tumor dissemination *via* LVs, is well established. Considerable efforts have been made to enhance DC-based vaccines used for the treatment of cancer, *e.g.* by optimising *in vitro* maturation protocols to enhance CCR7 expression/responsiveness [97, 165–168]. An alternative approach has been preconditioning of the skin with inflammatory mediators to enhance migration of adoptively transferred DCs [92, 169–171]. However, in the context of transplant rejection, therapeutic strategies to inhibit DC migration are also being investigated, for example as potential treatment of corneal allograft rejection. In the latter, a possible complication is the formation of LVs into the normally avascular cornea promoting the migration of corneal APCs to dLNs, where they trigger alloimmunity. Prolonged allograft survival could be achieved in preclinical models by inhibiting transplant-induced lymphangiogenesis [172, 173] or by blocking CCR7 [174].

In the context of tumor cell metastasis, a plethora of studies investigating different human cancer types have revealed a positive correlation between CCR7 or CXCR4 expression and tumor progression [148–153, 175], and preclinical mouse studies using CCR7 or CXCR4 inhibitors have reported reduction in LN metastasis [147, 160, 175]. In the case of CXCR4 antagonism, this has been demonstrated to not only reduce tumor cell invasiveness and metastasis, but also to reduce tumor growth and therapeutic resistance [175, 176]. Several CXCR4 inhibitors are currently under clinical investigation for the treatment of cancer [176, 177]. At the same time, a second receptor of CXCL12 was also recently identified (2005) and is increasingly investigated: ACKR3 (formerly known as CXCR7) is an atypical chemokine receptor with scavenging activity for CXCL12 and is expressed on many cancer cells and on cells of the tumor stroma [176, 178]. Since many small molecule CXCR4 inhibitors also display activity towards CXCR7 [175, 176], the exact contribution of these two receptors to tumor cell metastasis will require more detailed analysis. Interestingly, ACKR3 was recently also found to be expressed on afferent LVs and to impact lymphangiogenic processes during development [179].

A general observation concerning current approaches to modulate cellular traffic through afferent LVs is that the majority of molecules investigated are not specific for this particular trafficking step, but also involved in other migratory processes: *e.g.* ICAM-1, VCAM-1, CXCL12 are important for leukocyte extravasation from blood vascular capillaries [180], S1P/$S1P_1$ and CCL21/CCR7 for lymphocyte entry into/exit from LNs [4, 25, 181] and CXCL12/CXCR4 also regulate the release of hematopoietic stem cells from the bone marrow [182]. Future approaches will therefore also lie in the identification and therapeutic

investigation of molecules that are more specific for the trafficking of a particular cell type through afferent LVs.

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References

[1] K. Alitalo, The lymphatic vasculature in disease, Nat. Med. 17 (11) (2011) 1371–1380.
[2] S. Schulte-Merker, A. Sabine, T.V. Petrova, Lymphatic vascular morphogenesis in development, physiology, and disease, J. Cell Biol. 193 (4) (2011) 607–618.
[3] D. Kerjaschki, The lymphatic vasculature revisited, J. Clin. Invest. (2014) 874–877. American Society for Clinical Investigation.
[4] R. Forster, A. Braun, T. Worbs, Lymph node homing of T cells and dendritic cells via afferent lymphatics, Trends Immunol. 33 (6) (2012) 271–280.
[5] H. Ueno, et al., Dendritic cell subsets in health and disease, Immunol. Rev. 219 (2007) 118–142.
[6] K. Palucka, J. Banchereau, Dendritic-cell-based therapeutic cancer vaccines, Immunity 39 (1) (2013) 38–48.
[7] S. Banerji, et al., LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan, J. Cell Biol. (1999) 789–801. The Rockefeller University Press.
[8] S. Breiteneder-Geleff, et al., Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium, Am. J. Pathol. 154 (2) (1999) 385–394.
[9] A. Kaipainen, et al., Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development, Proc. Natl. Acad. Sci. U. S. A. (1995) 3566–3570 National Academy of Sciences.
[10] J.T. Wigle, G. Oliver, Prox1 function is required for the development of the murine lymphatic system, Cell (1999) 769–778.
[11] C. Mouta Carreira, et al., LYVE-1 is not restricted to the lymph vessels: expression in normal liver blood sinusoids and down-regulation in human liver cancer and cirrhosis, Cancer Res. 61 (22) (2001) 8079–8084.
[12] G. Oliver, et al., Prox 1, a prospero-related homeobox gene expressed during mouse development, Mech. Dev. 44 (1) (1993) 3–16.
[13] E. Bazigou, et al., Genes regulating lymphangiogenesis control venous valve formation and maintenance in mice, J. Clin. Invest. (2011) 2984–2992. American Society for Clinical Investigation.
[14] I. Martinez-Corral, et al., Vegfr3-CreER (T2) mouse, a new genetic tool for targeting the lymphatic system, Angiogenesis, Springer, Netherlands, 2016, pp. 433–445.
[15] T.H. Pham, et al., Lymphatic endothelial cell sphingosine kinase activity is required for lymphocyte egress and lymphatic patterning, J. Exp. Med. 207 (1) (2009) 17–27.
[16] I. Choi, et al., Visualization of lymphatic vessels by Prox1-promoter directed GFP reporter in a bacterial artificial chromosome-based transgenic mouse, Blood (2011) 362–365. American Society of Hematology.
[17] R. Hägerling, et al., Intravital two-photon microscopy of lymphatic vessel development and function using a transgenic Prox1 promoter-directed mOrange2 reporter mouse, Biochem. Soc. Trans. (2011) 1674–1681 Portland Press Limited.
[18] A. Alitalo, M. Detmar, Interaction of tumor cells and lymphatic vessels in cancer progression, Oncogene, 4499–4508 Nature Publishing Group., 2012.
[19] R.M. Steinman, Z.A. Cohn, Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution, J. Exp. Med. 137 (1973) 1142–1162.
[20] R.M. Steinman, Identification of a novel cell type in peripheral lymphoid organs of mice: II. Functional properties in vitro, J. Exp. Med. (1974) 380–397.
[21] R.M. Steinman, Identification of a novel cell type in peripheral lymphoid organs of mice: III. Functional properties in vivo, J. Exp. Med. (1974) 1431–1445.
[22] C. Audiger, et al., The importance of dendritic cells in maintaining immune tolerance, J. Immunol. (2017) 2223–2231.
[23] G. Oliver, K. Alitalo, The lymphatic vasculature: Recent progress and paradigms, Annu. Rev. Cell Dev. Biol. 457–483 (2005) Annual Reviews.
[24] L.N. Cueni, M. Detmar, The Lymphatic System in Health and Disease, in Lymphatic Research and Biology, Mary Ann Liebert, Inc, USA, 2008, pp. 109–122 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801–5215.
[25] J.-P. Girard, C. Moussion, R. Förster, HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes, Nat. Rev. Immunol. (2012) 762–773 Nature Publishing Group.
[26] K. Aukland, R.K. Reed, Physiological Reviews, Interstitial-lymphatic mechanisms in the control of extracellular fluid volume American Physiological Society Bethesda, MD, 1993, pp. 1–78.
[27] H. Pflicke, M. Sixt, Preformed portals facilitate dendritic cell entry into afferent lymphatic vessels, J. Exp. Med. 206 (13) (2009) 2925–2935.
[28] P. Baluk, et al., Functionally specialized junctions between endothelial cells of lymphatic vessels, J. Exp. Med. 204 (10) (2007) 2349–2362.
[29] T. Lammermann, et al., Rapid leukocyte migration by integrin-independent flowing and squeezing, Nature 453 (7191) (2008) 51–55.
[30] L.V. Leak, J.F. Burke, Fine structure of the lymphatic capillary and the adjoining connective tissue area, Am. J. Anat. (1966) 785–809 Wiley Subscription Services, Inc., A Wiley Company.
[31] T. Tammela, K. Alitalo, Lymphangiogenesis: Molecular mechanisms and future promise, Cell 140 (4) (2010) 460–476.
[32] G.W. Schmid-Schönbein, Mechanisms causing initial lymphatics to expand and compress to promote lymph flow, Arch. Histol. Cytol. (1990) 107–114.
[33] T. Makinen, et al., PDZ interaction site in ephrinB2 is required for the remodeling of lymphatic vasculature, Genes Amp. Develop. (2005) 397–410.
[34] E. Bazigou, et al., Integrin-α9 Is Required for Fibronectin Matrix Assembly during Lymphatic Valve Morphogenesis, in Developmental Cell, Elsevier Ltd, 2009, pp. 175–186.
[35] A. Braun, et al., Afferent lymph-derived T cells and DCs use different chemokine receptor CCR7-dependent routes for entry into the lymph node and intranodal migration, Nat. Immunol. 12 (9) (2011) 879–887.
[36] Y.R. Carrasco, F.D. Batista, B cells acquire particulate antigen in a macrophage-rich area at the boundary between the follicle and the subcapsular sinus of the lymph node, Immunity (2007) 160–171.
[37] U.H. von Andrian, T.R. Mempel, Homing and cellular traffic in lymph nodes, Nat. Rev. Immunol. (2003) 867–878 Nature Publishing Group.
[38] C.L. Willard-Mack, Normal structure, function, and histology of lymph nodes, Toxicol. Pathol. (2006) 409–424 SAGE Publications.
[39] J.G. Hall, B. Morris, The output of cells in lymph from the popliteal node of sheep, Q J. Exp. Physiol. Cogn. Med. Sci. 47 (1962) 360–369.
[40] J.B. Smith, G.H. McIntosh, B. Morris, The traffic of cells through tissues: a study of peripheral lymph in sheep, J. Anat. 107 (Pt 1) (1970) 87–100.
[41] J. Sokolowski, E. Jakobsen, J.V. Johannessen, Cells in peripheral leg lymph of normal men, Lymphology 11 (4) (1978) 202–207.
[42] W.L. Olszewski, et al., Immune cell traffic from blood through the normal human skin to lymphatics, Clin. Dermatol. 13 (5) (1995) 473–483.
[43] M. Tomura, et al., Activated regulatory T cells are the major T cell type emigrating from the skin during a cutaneous immune response in mice, J. Clin. Invest. 120 (3) (2010) 883–893.
[44] C.R. Mackay, W.L. Marston, L. Dudler, Naive and memory T cells show distinct pathways of lymphocyte recirculation, J. Exp. Med. 171 (1990) 801–817.
[45] N. Yawalkar, et al., Human afferent lymph from normal skin contains an increased number of mainly memory/effector CD4(+) T cells expressing activation, adhesion and co-stimulatory molecules, Eur. J. Immunol. 30 (2) (2000) 491–497.
[46] M.N. Brown, et al., Chemoattractant Receptors and Lymphocyte Egress from Extralymphoid Tissue: Changing Requirements during the Course of Inflammation, J. Immunol. 185 (8) (2010) 4873–4882.
[47] J.B. Smith, G.H. McIntosh, B. Morris, The migration of cells through chronically inflamed tissues, J. Pathol. 100 (1) (1970) 21–29.
[48] D. Masopust, J.M. Schenkel, The integration of T cell migration, differentiation and function, Nat. Rev. Immunol. 13 (5) (2013) 309–320.
[49] U.H. von Andrian, C.R. Mackay, T-cell function and migration. Two sides of the same coin, N. Engl. J. Med. 343 (14) (2000) 1020–1034.
[50] G.F. Debes, et al., Chemokine receptor CCR7 required for T lymphocyte exit from peripheral tissues, Nat. Immunol. 6 (9) (2005) 889–894.
[51] L.G. Ledgerwood, et al., The sphingosine 1-phosphate receptor 1 causes tissue retention by inhibiting the entry of peripheral tissue T lymphocytes into afferent lymphatics, Nat. Immunol. 9 (1) (2008) 42–53.
[52] M. Tomura, et al., Monitoring cellular movement in vivo with photoconvertible fluorescence protein "Kaede" transgenic mice, Proc. Natl. Acad. Sci. U. S. A. 105 (31) (2008) 10871–10876.
[53] M. Tomura, et al., Tracking and quantification of dendritic cell migration and antigen trafficking between the skin and lymph nodes, Sci. Rep. 4 (2014) 6030.
[54] R. Ikebuchi, et al., A rare subset of skin-tropic regulatory T cells expressing Il10/Gzmb inhibits the cutaneous immune response, Sci. Rep. 6 (2016) 35002.
[55] Y. Nakanishi, et al., Regulatory T cells with superior immunosuppressive capacity emigrate from the inflamed colon to draining lymph nodes, Mucosal Immunol. 11 (2017) 437–448.
[56] N. Zhang, et al., Regulatory T cells sequentially migrate from inflamed tissues to draining lymph nodes to suppress the alloimmune response, Immunity 30 (3) (2009) 458–469.
[57] C.C. Brinkman, et al., Treg engage lymphotoxin beta receptor for afferent lymphatic transendothelial migration, Nat. Commun. 7 (2016) 12021.
[58] S.P. Rosshart, et al., Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance, Cell 171 (5) (2017) 1015–1028 e13.
[59] S. Abolins, et al., The comparative immunology of wild and laboratory mice, Mus musculus domesticus, Nat. Commun. 8 (2017) 14811.
[60] L.K. Beura, et al., Normalizing the environment recapitulates adult human immune traits in laboratory mice, Nature 532 (7600) (2016) 512–516.
[61] R.N. Cahill, H. Frost, Z. Trnka, The effects of antigen on the migration of recirculating lymphocytes through single lymph nodes, J. Exp. Med. 143 (4) (1976) 870–888.
[62] D. Haig, et al., The cytokine response of afferent lymph following orf virus re-infection of sheep, Vet. Dermatol. 7 (1) (1996) 11–20.
[63] M. Weber, et al., Interstitial dendritic cell guidance by haptotactic chemokine gradients, Science 339 (6117) (2013) 328–332.
[64] M. Nitschke, et al., Differential requirement for ROCK in dendritic cell migration

within lymphatic capillaries in steady-state and inflammation, Blood 120 (11) (2012) 2249–2258.
[65] D. Sen, et al., Selective and site-specific mobilization of dermal dendritic cells and Langerhans cells by Th1- and Th2-polarizing adjuvants, Proc. Natl. Acad. Sci. U. S. A. 107 (18) (2010) 8334–8339.
[66] A. Teijeira, et al., T cell migration from inflamed skin to draining lymph nodes requires intralymphatic crawling supported by ICAM-1/LFA-1 interactions, Cell Rep. 18 (4) (2017) 857–865.
[67] S. Arokiasamy, et al., Endogenous TNFα orchestrates the trafficking of neutrophils into and within lymphatic vessels during acute inflammation, Sci. Rep. (2017) 44189 Nature Publishing Group.
[68] O. Tal, et al., DC mobilization from the skin requires docking to immobilized CCL21 on lymphatic endothelium and intralymphatic crawling, J. Exp. Med. (2011) 2141–2153 Rockefeller University Press.
[69] E. Russo, et al., Intralymphatic CCL21 promotes tissue egress of dendritic cells through afferent lymphatic vessels, CellReports (2016) 1723–1734.
[70] D.A. Berk, et al., Transport in lymphatic capillaries. II. Microscopic velocity measurement with fluorescence photobleaching, Am. J. Phys. 270 (1 Pt 2) (1996) H330–H337.
[71] M.A. Swartz, D.A. Berk, R.K. Jain, Transport in lymphatic capillaries. I. Macroscopic measurements using residence time distribution theory, Am. J. Phys. 270 (1 Pt 2) (1996) H324–H329.
[72] J.B. Dixon, et al., Measuring microlymphatic flow using fast video microscopy, J. Biomed. Opt. 10 (6) (2005) 064016.
[73] J.B. Dixon, et al., Lymph flow, shear stress, and lymphocyte velocity in rat mesenteric prenodal lymphatics, Microcirculation 13 (7) (2006) 597–610.
[74] A.S. Popel, P.C. Johnson, Microcirculation and Hemorheology, Annu. Rev. Fluid Mech. 37 (2005) 43–69.
[75] P. Rantakari, et al., The endothelial protein PLVAP in lymphatics controls the entry of lymphocytes and antigens into lymph nodes, Nat. Immunol. 16 (4) (2015) 386–396.
[76] E.K.I. Iftakhar, et al., Gene-expression profiling of different arms of lymphatic vasculature identifies candidates for manipulation of cell traffic, Proc. Natl. Acad. Sci. U. S. A. 113 (38) (2016) 10643–10648.
[77] F. Sallusto, et al., Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation, Eur. J. Immunol. 28 (9) (1998) 2760–2769.
[78] R. Forster, et al., CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs, Cell 99 (1) (1999) 23–33.
[79] L. Ohl, et al., CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions, Immunity 21 (2) (2004) 279–288.
[80] H. Saeki, et al., Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes, J. Immunol. 162 (5) (1999) 2472–2475.
[81] H. Nakano, M.D. Gunn, Gene duplications at the chemokine locus on mouse chromosome 4: multiple strain-specific haplotypes and the deletion of secondary lymphoid-organ chemokine and EBI-1 ligand chemokine genes in the plt mutation, J. Immunol. (2001) 361–369.
[82] L.A. Johnson, D.G. Jackson, Inflammation-induced secretion of CCL21 in lymphatic endothelium is a key regulator of integrin-mediated dendritic cell transmigration, Int. Immunol. 22 (10) (2010) 839–849.
[83] B. Vigl, et al., Tissue inflammation modulates gene expression of lymphatic endothelial cells and dendritic cell migration in a stimulus-dependent manner, Blood 118 (1) (2011) 205–215.
[84] J.L. de Paz, et al., Profiling heparin-chemokine interactions using synthetic tools, ACS Chem. Biol. 2 (11) (2007) 735–744.
[85] X. Bao, et al., Endothelial heparan sulfate controls chemokine presentation in recruitment of lymphocytes and dendritic cells to lymph nodes, Immunity (2010) 817–829.
[86] S.A. Luther, et al., Coexpression of the chemokines ELC and SLC by T zone stromal cells and deletion of the ELC gene in the plt/plt mouse, Proc. Natl. Acad. Sci. U. S. A. 97 (23) (2000) 12694–12699.
[87] G. Vassileva, et al., The reduced expression of 6Ckine in the plt mouse results from the deletion of one of two 6Ckine genes, J. Exp. Med. 190 (8) (1999) 1183–1188.
[88] M.D. Gunn, et al., Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell locavlization, J. Exp. Med. 189 (1999) 451–460.
[89] M.H. Ulvmar, et al., The atypical chemokine receptor CCRL1 shapes functional CCL21 gradients in lymph nodes, Nat. Publ. Group (2014) 623–630.
[90] K. Vaahtomeri, et al., Locally Triggered Release of the Chemokine CCL21 Promotes Dendritic Cell Transmigration across Lymphatic Endothelia, CellReports (2017) 902–909.
[91] K. Schumann, et al., Immobilized chemokine fields and soluble chemokine gradients cooperatively shape migration patterns of dendritic cells, Immunity 32 (5) (2010) 703–713.
[92] A. MartIn-Fontecha, et al., Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming, J. Exp. Med. 198 (4) (2003) 615–621.
[93] S.A. Luther, et al., Differing activities of homeostatic chemokines CCL19, CCL21, and CXCL12 in lymphocyte and dendritic cell recruitment and lymphoid neogenesis, J. Immunol. (2002) 424–433.
[94] A. Link, et al., Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells, Nat. Immunol. (2007) 1255–1265 Nature Publishing Group.
[95] M.R. Britschgi, S. Favre, S.A. Luther, CCL21 is sufficient to mediate DC migration, maturation and function in the absence of CCL19, Eur. J. Immunol. 40 (5) (2010) 1266–1271.
[96] S.A. Bryce, et al., ACKR4 on stromal cells scavenges CCL19 to enable CCR7-dependent trafficking of APCs from inflamed skin to lymph nodes, J. Immunol. (2016) 3341–3353. American Association of Immunologists.
[97] M. Hansen, et al., Autocrine CCL19 blocks dendritic cell migration toward weak gradients of CCL21, Cytotherapy (2016) 1187–1196.
[98] Y. Sawa, et al., Leukocyte adhesion molecule and chemokine production through lipoteichoic acid recognition by toll-like receptor 2 in cultured human lymphatic endothelium, Cell Tissue Res. (2008) 237–252.
[99] L.A. Johnson, et al., An inflammation-induced mechanism for leukocyte transmigration across lymphatic vessel endothelium, J. Exp. Med. 203 (12) (2006) 2763–2777.
[100] L.A. Johnson, D.G. Jackson, The chemokine CX3CL1 promotes trafficking of dendritic cells through inflamed lymphatics, J. Cell Sci. 126 (22) (2013) 5259–5270.
[101] K. Kabashima, et al., CXCL12-CXCR4 engagement is required for migration of cutaneous dendritic cells, Am. J. Pathol. 171 (4) (2007) 1249–1257.
[102] N. Czeloth, et al., Sphingosine-1-phosphate mediates migration of mature dendritic cells, J. Immunol. 175 (5) (2005) 2960–2967.
[103] A. Rathinasamy, et al., The origin and maturity of dendritic cells determine the pattern of sphingosine 1-phosphate receptors expressed and required for efficient migration, J. Immunol. 185 (7) (2010) 4072–4081.
[104] H. Xu, et al., The role of ICAM-1 molecule in the migration of Langerhans cells in the skin and regional lymph node, Eur. J. Immunol. 31 (10) (2001) 3085–3093.
[105] A. Teijeira, et al., Lymphatic endothelium forms integrin-engaging 3D structures during DC transit across inflamed lymphatic vessels, J. Investig. Dermatol. 133 (9) (2013) 2276–2285.
[106] L. Maddaluno, et al., The adhesion molecule L1 regulates transendothelial migration and trafficking of dendritic cells, J. Exp. Med. 206 (3) (2009) 623–635.
[107] R. Ballet, et al., Blocking junctional adhesion molecule C enhances dendritic cell migration and boosts the immune responses against Leishmania major, PLoS Pathog. (2014) e1004550.
[108] M.R. Cera, et al., Increased DC trafficking to lymph nodes and contact hypersensitivity in junctional adhesion molecule-A-deficient mice, J. Clin. Investig. (2004) 729–738. American Society for Clinical Investigation.
[109] L.A. Johnson, et al., Dendritic cells enter lymph vessels by hyaluronan-mediated docking to the endothelial receptor LYVE-1, Nat. Publ. Group (2017) 762–770.
[110] S.K. Bromley, et al., Recirculating memory T cells are a unique subset of CD4+ T cells with a distinct phenotype and migratory pattern, J. Immunol. 190 (3) (2013) 970–976.
[111] S.K. Bromley, S.Y. Thomas, A.D. Luster, Chemokine receptor CCR7 guides T cell exit from peripheral tissues and entry into afferent lymphatics, Nat. Immunol. 6 (9) (2005) 895–901.
[112] U.E. Hopken, et al., CCR7 deficiency causes ectopic lymphoid neogenesis and disturbed mucosal tissue integrity, Blood 109 (3) (2007) 886–895.
[113] A. Menning, et al., Distinctive role of CCR7 in migration and functional activity of naive- and effector/memory-like Treg subsets, Eur. J. Immunol. 37 (6) (2007) 1575–1583.
[114] R.A. Clark, et al., The vast majority of CLA+ T cells are resident in normal skin, J. Immunol. 176 (7) (2006) 4431–4439.
[115] T. Gebhardt, et al., Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus, Nat. Immunol. 10 (5) (2009) 524–530.
[116] L.R. Shiow, et al., CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs, Nature 440 (7083) (2006) 540–544.
[117] A. Mendoza, et al., Lymphatic endothelial S1P promotes mitochondrial function and survival in naive T cells, Nature 546 (7656) (2017) 158–161.
[118] M. Salmi, et al., CD44 binds to macrophage mannose receptor on lymphatic endothelium and supports lymphocyte migration via afferent lymphatics, Circ. Res. 112 (12) (2013) 1577–1582.
[119] F. Marttila-Ichihara, et al., Macrophage mannose receptor on lymphatics controls cell trafficking, Blood 112 (1) (2008) 64–72.
[120] M. Salmi, et al., CLEVER-1 mediates lymphocyte transmigration through vascular and lymphatic endothelium, Blood 104 (13) (2004) 3849–3857.
[121] M. Karikoski, et al., Clever-1/Stabilin-1 regulates lymphocyte migration within lymphatics and leukocyte entrance to sites of inflammation, Eur. J. Immunol. 39 (12) (2009) 3477–3487.
[122] C. Jakubzick, et al., Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes, Immunity (2013) 599–610.
[123] P. Kruger, et al., Neutrophils: Between host defence, immune modulation, and tissue injury, PLoS Pathog. (2015) e1004651 Public Library of Science.
[124] B.A. Maletto, et al., Presence of neutrophil-bearing antigen in lymphoid organs of immune mice, Blood (2006) 3094–3102. American Society of Hematology.
[125] H.R. Hampton, et al., Microbe-dependent lymphatic migration of neutrophils modulates lymphocyte proliferation in lymph nodes, Nat. Commun. 6 (2015) 7139.
[126] C. Beauvillain, et al., CCR7 is involved in the migration of neutrophils to lymph nodes, Blood (2011) 1196–1204. American Society of Hematology.
[127] V. Abadie, et al., Neutrophils rapidly migrate via lymphatics after Mycobacterium bovis BCG intradermal vaccination and shuttle live bacilli to the draining lymph nodes, Blood 106 (5) (2005) 1843–1850.
[128] S. Calabro, et al., Vaccine adjuvants alum and MF59 induce rapid recruitment of neutrophils and monocytes that participate in antigen transport to draining lymph nodes, Vaccine (2011) 1812–1823.

[129] E. Kolaczkowska, P. Kubes, Neutrophil recruitment and function in health and inflammation, Nat. Rev. Immunol. (2013) 159–175 Nature Publishing Group.
[130] D.A. Rigby, et al., Neutrophils rapidly transit inflamed lymphatic vessel endothelium via integrin-dependent proteolysis and lipoxin-induced junctional retraction, J. Leukoc. Biol. (2015) 897–912.
[131] Y. Chen, et al., A meta-analysis of the relationship between lymphatic microvessel density and clinicopathological parameters in breast cancer, Bull. Cancer 100 (3) (2013) 1–10.
[132] M. Yu, et al., Intratumoral vessel density as prognostic factors in head and neck squamous cell carcinoma: a meta-analysis of literature, Head Neck 36 (4) (2014) 596–602.
[133] I. Pastushenko, et al., Blood microvessel density, lymphatic microvessel density and lymphatic invasion in predicting melanoma metastases: systematic review and meta-analysis, Br. J. Dermatol. 170 (1) (2014) 66–77.
[134] Y. He, T. Karpanen, K. Alitalo, Role of lymphangiogenic factors in tumor metastasis, Biochim. Biophys. Acta 1654 (1) (2004) 3–12.
[135] S. Karaman, M. Detmar, Mechanisms of lymphatic metastasis, J. Clin. Invest. 124 (3) (2014) 922–928.
[136] S.T. Proulx, et al., Quantitative imaging of lymphatic function with liposomal indocyanine green, Cancer Res. 70 (18) (2010) 7053–7062.
[137] M.I. Harrell, B.M. Iritani, A. Ruddell, Tumor-induced sentinel lymph node lymphangiogenesis and increased lymph flow precede melanoma metastasis, Am. J. Pathol. 170 (2) (2007) 774–786.
[138] N.V. Krakhmal, et al., Cancer invasion: patterns and mechanisms, Acta Nat. 7 (2) (2015) 17–28.
[139] S. Giampieri, et al., Localized and reversible TGFbeta signalling switches breast cancer cells from cohesive to single cell motility, Nat. Cell Biol. 11 (11) (2009) 1287–1296.
[140] D. Kerjaschki, et al., Lipoxygenase mediates invasion of intrametastatic lymphatic vessels and propagates lymph node metastasis of human mammary carcinoma xenografts in mouse, J. Clin. Invest. 121 (5) (2011) 2000–2012.
[141] C.D. Madsen, E. Sahai, Cancer dissemination–lessons from leukocytes, Dev. Cell 19 (1) (2010) 13–26.
[142] A. Ben-Baruch, Organ selectivity in metastasis: regulation by chemokines and their receptors, Clin. Exp. Metastasis 25 (4) (2008) 345–356.
[143] S. Das, M. Skobe, Lymphatic vessel activation in cancer, Ann. N. Y. Acad. Sci. 1131 (2008) 235–241.
[144] A. Muller, et al., Involvement of chemokine receptors in breast cancer metastasis, Nature 410 (6824) (2001) 50–56.
[145] D. Raman, et al., Role of chemokines in tumor growth, Cancer Lett. 256 (2) (2007) 137–165.
[146] P. Houshmand, A. Zlotnik, Therapeutic applications in the chemokine superfamily, Curr. Opin. Chem. Biol. 7 (4) (2003) 457–460.
[147] A. Zlotnik, A.M. Burkhardt, B. Homey, Homeostatic chemokine receptors and organ-specific metastasis, Nat. Rev. Immunol. 11 (9) (2011) 597–606.
[148] S. Ishigami, et al., Prognostic value of CCR7 expression in gastric cancer, Hepato-Gastroenterology 54 (76) (2007) 1025–1028.
[149] K. Mashino, et al., Expression of chemokine receptor CCR7 is associated with lymph node metastasis of gastric carcinoma, Cancer Res. 62 (10) (2002) 2937–2941.
[150] T. Irino, et al., CC-chemokine receptor CCR7: a key molecule for lymph node metastasis in esophageal squamous cell carcinoma, BMC Cancer 14 (2014) 291.
[151] N. Cabioglu, et al., CCR7 and CXCR4 as novel biomarkers predicting axillary lymph node metastasis in T1 breast cancer, Clin. Cancer Res. 11 (16) (2005) 5686–5693.
[152] S. Hirakawa, et al., Nodal lymphangiogenesis and metastasis: role of tumor-induced lymphatic vessel activation in extramammary Paget's disease, Am. J. Pathol. 175 (5) (2009) 2235–2248.
[153] J. Kodama, et al., Association of CXCR4 and CCR7 chemokine receptor expression and lymph node metastasis in human cervical cancer, Ann. Oncol. 18 (1) (2007) 70–76.
[154] M. Kim, et al., CXCR4 signaling regulates metastasis of chemoresistant melanoma cells by a lymphatic metastatic niche, Cancer Res. 70 (24) (2010) 10411–10421.
[155] J.D. Shields, et al., Chemokine-mediated migration of melanoma cells towards lymphatics–a mechanism contributing to metastasis, Oncogene 26 (21) (2007) 2997–3005.
[156] A. Issa, et al., Vascular endothelial growth factor-C and C-C chemokine receptor 7 in tumor cell-lymphatic cross-talk promote invasive phenotype, Cancer Res. 69 (1) (2009) 349–357.
[157] M.S. Emmett, et al., CCR7 mediates directed growth of melanomas towards lymphatics, Microcirculation 18 (3) (2011) 172–182.
[158] H.E. Wiley, et al., Expression of CC chemokine receptor-7 and regional lymph node metastasis of B16 murine melanoma, J. Natl. Cancer Inst. 93 (21) (2001) 1638–1643.
[159] J. Sperveslage, et al., Lack of CCR7 expression is rate limiting for lymphatic spread of pancreatic ductal adenocarcinoma, Int. J. Cancer 131 (4) (2012) E371–E381.
[160] P.J. Sarvaiya, et al., Chemokines in tumor progression and metastasis, Oncotarget 4 (12) (2013) 2171–2185.
[161] D. Gomez, et al., Effector T cell egress via afferent lymph modulates local tissue inflammation, J. Immunol. 195 (8) (2015) 3531–3536.
[162] E.N. McNamee, et al., Chemokine receptor CCR7 regulates the intestinal TH1/TH17/Treg balance during Crohn's-like murine ileitis, J. Leukoc. Biol. 97 (6) (2015) 1011–1022.
[163] S.N. Mueller, A. Zaid, F.R. Carbone, Tissue-resident T cells: dynamic players in skin immunity, Front. Immunol. 5 (2014) 332.
[164] S.N. Mueller, L.K. MacKay, Tissue-resident memory T cells: local specialists in immune defence, Nat. Rev. Immunol. 16 (2) (2016) 79–89.
[165] E. Scandella, et al., Prostaglandin E2 is a key factor for CCR7 surface expression and migration of monocyte-derived dendritic cells, Blood 100 (4) (2002) 1354–1361.
[166] A. Del Prete, et al., Regulation of dendritic cell migration and adaptive immune response by leukotriene B4 receptors: a role for LTB4 in up-regulation of CCR7 expression and function, Blood 626-631 (2007) American Society of Hematology.
[167] H. Jonuleit, et al., Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions, Eur. J. Immunol. 27 (12) (1997) 3135–3142.
[168] G.A. Pizzurro, et al., Cytokine-enhanced maturation and migration to the lymph nodes of a human dying melanoma cell-loaded dendritic cell vaccine, Cancer Immunol. Immunother. (2015) 1393–1406 Springer Berlin Heidelberg.
[169] R.M. Prins, et al., The TLR-7 agonist, imiquimod, enhances dendritic cell survival and promotes tumor antigen-specific T cell priming: relation to central nervous system antitumor immunity, J. Immunol. 176 (1) (2006) 157–164.
[170] C.H. Tripp, et al., Conditioning of the injection site with CpG enhances the migration of adoptively transferred dendritic cells and endogenous CD8+ T-cell responses, J. Immunother. (2010) 115–125.
[171] E.H.J.G. Aarntzen, et al., Targeting of 111In-labeled dendritic cell human vaccines improved by reducing number of cells, Clin. Cancer Res. (2013) 1525–1533.
[172] L. Chen, et al., Vascular endothelial growth factor receptor-3 mediates induction of corneal alloimmunity, Nat. Med. 10 (8) (2004) 813–815.
[173] T. Dietrich, et al., Cutting edge: lymphatic vessels, not blood vessels, primarily mediate immune rejections after transplantation, J. Immunol. 184 (2) (2010) 535–539.
[174] D. Hos, et al., Blockade of CCR7 leads to decreased dendritic cell migration to draining lymph nodes and promotes graft survival in low-risk corneal transplantation, Exp. Eye Res. (2016) 1–6.
[175] S. Chatterjee, B. Behnam Azad, S. Nimmagadda, The intricate role of CXCR4 in cancer, Adv. Cancer Res. (2014) 31–82 Elsevier.
[176] S. Scala, Molecular pathways: targeting the CXCR4-CXCL12 Axis—untapped potential in the tumor microenvironment, Clin. Cancer Res. (2015) 4278–4285.
[177] B. Debnath, et al., Small molecule inhibitors of CXCR4, Theranostics 3 (1) (2013) 47–75.
[178] C. Freitas, et al., The relevance of the chemokine receptor ACKR3/CXCR7 on CXCL12-mediated effects in cancers with a focus on virus-related cancers, Cytokine Growth Factor Rev. 25 (3) (2014) 307–316.
[179] K.R. Klein, et al., Decoy receptor CXCR7 modulates adrenomedullin-mediated cardiac and lymphatic vascular development, Dev. Cell (2014) 528–540.
[180] S. Nourshargh, R. Alon, Leukocyte migration into inflamed tissues, Immunity (2014) 694–707.
[181] C. Moussion, J.P. Girard, Dendritic cells control lymphocyte entry to lymph nodes through high endothelial venules, Nature 479 (7374) (2011) 542–546.
[182] J.F. Dipersio, et al., Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma, J. Clin. Oncol. (2009) 4767–4773.
[183] M. Murakami, et al., Inactivation of junctional adhesion molecule-A enhances antitumoral immune response by promoting dendritic cell and T lymphocyte infiltration, Cancer Res. (2010) 1759–1765.
[184] S.E. Acton, et al., Podoplanin-rich stromal networks induce dendritic cell motility via activation of the C-type lectin receptor CLEC-2, Immunity (2012) 276–289.
[185] H. Takamatsu, et al., Semaphorins guide the entry of dendritic cells into the lymphatics by activating myosin II, Nat. Immunol. 11 (7) (2010) 594–600.
[186] G. Ratzinger, et al., Matrix metalloproteinases 9 and 2 are necessary for the migration of Langerhans cells and dermal dendritic cells from human and murine skin, J. Immunol. 168 (9) (2002) 4361–4371.
[187] J.H. Yen, T. Khayrullina, D. Ganea, PGE2-induced metalloproteinase-9 is essential for dendritic cell migration, Blood 111 (1) (2008) 260–270.
[188] C. Qu, et al., Role of CCR8 and other chemokine pathways in the migration of monocyte-derived dendritic cells to lymph nodes, J. Exp. Med. 200 (10) (2004) 1231–1241.
[189] C. Jakubzick, et al., Modulation of dendritic cell trafficking to and from the airways, J. Immunol. (2006) 3578–3584.
[190] D.F. Robbiani, et al., The leukotriene C(4) transporter MRP1 regulates CCL19 (MIP-3beta, ELC)-dependent mobilization of dendritic cells to lymph nodes, Cell 103 (5) (2000) 757–768.
[191] J.D. Shields, et al., Autologous chemotaxis as a mechanism of tumor cell homing to lymphatics via interstitial flow and autocrine CCR7 signaling, Cancer Cell 11 (6) (2007) 526–538.
[192] K. Koizumi, et al., CCL21 promotes the migration and adhesion of highly lymph node metastatic human non-small cell lung cancer Lu-99 in vitro, Oncol. Rep. 17 (6) (2007) 1511–1516.
[193] H. Takeuchi, et al., CCL21 chemokine regulates chemokine receptor CCR7 bearing malignant melanoma cells, Clin. Cancer Res. 10 (7) (2004) 2351–2358.
[194] S. Das, et al., Tumor cell entry into the lymph node is controlled by CCL1 chemokine expressed by lymph node lymphatic sinuses, J. Exp. Med. 210 (8) (2013) 1509–1528.
[195] K. Kawada, et al., Chemokine receptor CXCR3 promotes colon cancer metastasis to lymph nodes, Oncogene 26 (32) (2007) 4679–4688.
[196] R.L. Lindquist, et al., Visualizing dendritic cell networks in vivo, Nat. Immunol. 5 (12) (2004) 1243–1250.