Emerging roles of lymphatic endothelium in regulating adaptive immunity

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Emerging research on the roles of stromal cells in modulating adaptive immune responses has included a new focus on lymphatic endothelial cells (LECs). LECs are presumably the first cells that come into direct contact with peripheral antigens, cytokines, danger signals, and immune cells travelling from peripheral tissues to lymph nodes. LECs can modulate dendritic cell function, present antigens to T cells on MHC class I and MHC class II molecules, and express immunomodulatory cytokines and receptors, which suggests that their roles in adaptive immunity are far more extensive than previously realized. This Review summarizes the emergent evidence that LECs are important in maintaining peripheral tolerance, limiting and resolving effector T cell responses, and modulating leukocyte function.

Introduction

Our appreciation for the varied functions of lymphatic vessels has evolved from different scientific disciplines. Traditionally, microcirculatory physiologists studied the essential transport functions of lymphatic vessels in removing fluid, molecules, and cells after leaking from blood vessels in the periphery and before returning them to the blood circulation. Lymph indicated the state of the local interstitial fluid it drained, with Starling forces dictating fluid and solute balance (1–3). Immunologists recognized the importance of lymphatic vessels as channels for leukocyte trafficking from peripheral sites to their draining LNs (4–6), and as conduits for soluble antigens that can be taken up directly by LN-resident B cells and immature DCs (7–10), which help regulate the kinetics of antigen presentation.

More connections between these two different perspectives have emerged in recent years. Lymphatic endothelial cells (LECs) themselves have been shown to play active roles in controlling their transport functions and in directly communicating with immune cells to modulate their immediate and downstream functions. Indeed, a growing body of evidence is demonstrating how LECs help shape both innate and adaptive immune responses through (a) expression of multiple cytokines, adhesion molecules, and inhibitory receptors; (b) scavenging and processing antigens for direct presentation to T cells or modulating the activity of professional APCs; and (c) actively regulating fluid and solute transport functions in response to inflammatory signals. These new ideas, in turn, reveal a paradigm whereby the transport and immune functions of lymphatic vessels, which were previously considered separately, are in fact intimately coupled.

In this Review, we highlight these connections to reveal new roles of LECs, along with their transport functions, in modulating adaptive immune responses. While we particularly focus on LEC interactions with DCs and T cells, we also highlight features that support immune regulation, including the structure and function of lymphatic vessels and the compartmentalization of the LN stroma, which help control the manner in which LECs can interface with immune cells. Ongoing research in this area is essential to understanding how inflammatory lymphangiogenesis affects both cancer progression as well as chronic inflammation that leads to autoimmunity.

Transport and trafficking functions of the lymphatic system

The lymphatic vessels and secondary lymphoid organs (SLOs) are arranged in a manner that optimizes interactions among antigens, APCs, and innate and adaptive effector cells. SLOs include the spleen, LNs, and Peyer’s patches, but this Review focuses on LNs, since LEC/T cell interactions are best described in this compartment.

Lymph flow transports soluble antigens. The LNs serve as hubs of antigen presentation, where lymphocytes are primed or tolerized against antigens presented by APCs. Lymph fluid drained from the periphery via afferent lymphatic vessels bathes LNs with soluble foreign antigens as well as tissue-specific self-antigens and any other molecules present in the local periphery, such as cytokines from inflamed tissues or tumors (10). Because lymph originates as interstitial fluid surrounding the cells of peripheral tissues, it is enriched in peptides that are processed extracellularly, including those resulting from local catabolism, ECM degradation, apoptosis, and tissue remodeling (9, 11). While tissue-resident DCs take up and process antigens for presentation on MHC molecules, often resulting in DC maturation and migration to LNs (12), free antigens can rapidly drain to the LN via the subcapsular sinus, where large antigens and opsonized material may be directly taken up by subcapsular macrophages (Figure 1). Smaller antigens are channeled deeper into the B and T cell zones via intricate conduit systems. In the paracortex, conduits are formed by fibroblastic reticular cells (FRCs) wrapped around bundled collagen fibers (13, 14), while in the B cell zone, follicular DCs help form channels for perfusion.

The importance of the B cell conduit system has been demonstrated using two-photon microscopy. For example, Roozendaal and colleagues using two-photon microscopy. They observed that after intradermal injection, lymph-borne antigen rapidly entered the draining LN and either was taken up by subcapsular macrophages (large antigens) or bathed the B cell zone via conduits (small antigens), where antigen-specific B cells efficiently internalized the antigen (14). In the paracortex, immature LN-resident DCs can also take up and present lymph-borne antigen (first wave) prior to the arrival...
of antigen-loaded tissue-resident DCs, which further activates T cells in a second wave. These differential presentation kinetics have been suggested to fine-tune immune responses, as it is likely that the first wave primes the draining LN for the arrival of the second wave, driving adaptive immune responses (7).

This notion that antigen presentation kinetics help prime as well as control immune responses has profound implications for the importance of lymphatic flow in adaptive immunity. A recent study demonstrated that the absence of soluble antigen transport severely impacts the education of B and T cells in LNs (15). Using K14–VEGFR-3-Ig transgenic mice, which lack dermal lymphatic capillaries but possess intact LNs and otherwise normal lymphatic vasculature, it was shown that intradermal vaccination of these mice led to a drastically reduced antibody response, as expected. However, T cell activation, although delayed, remained robust even though DC trafficking from the injection site to the draining LN was nearly absent. Whereas T cell activation took place primarily in the draining LNs in WT mice, this process was found to occur in the spleens of the K14–VEGFR-3-Ig mice. Interestingly, the lack of lymphatic drainage from skin led to autoimmunity in aged mice, and young mice could not be tolerized to exogenous antigen using a classic skin tolerance test (15). Therefore, blocked lymphatic flow prevents soluble antigen transport to the LNs, leading to repercussions in the generation of inflammatory responses against foreign antigens and in the maintenance of peripheral tolerance (16).

Lymphatic recruitment of DCs and other migratory cells. We will briefly discuss how LECs facilitate the transport of leukocytes from the periphery, since this is the topic of several excellent recent
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<tr>
<td>CCL1</td>
<td>Recruitment of monocytes, NK cells, T cells, DCs and B cells via CCR8</td>
<td>Primary human LECs, human melanoma tissues</td>
<td>Increased by treatment with LPS, IL-1β, or TNF-α</td>
<td>55</td>
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<td>CCL2 (MCP-1)</td>
<td>Recruitment of monocytes, DCs, basophils, and memory T cells via CCR1, CCR2, and CCR4</td>
<td>Primary human LECs, murine LECs in vivo</td>
<td>Increased upon contact hypersensitivity</td>
<td>23, 28, 31,</td>
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<td>CCL5 (RANTES)</td>
<td>Recruitment of T cells and leukocytes via CCR1, CCR3, CCR4, and CCR5</td>
<td>Primary human neonatal dermal LECs</td>
<td>Increased following TLR3 engagement</td>
<td>28, 31, 91</td>
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<td>CCL7 (MCP-3)</td>
<td>Recruitment of monocytes and regulation of macrophages</td>
<td>Murine ear lymphatics</td>
<td>Increased upon contact hypersensitivity</td>
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<td>CCL8 (MCP-2)</td>
<td>Activation of a range of immune cells via CCR1, CCR2, CCR3, and CCR5</td>
<td>Murine ear lymphatics</td>
<td>Increased upon contact hypersensitivity</td>
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<td>CCL20 (MIP-3α)</td>
<td>Recruitment of DCs, memory T cells, and B cells via CCR6</td>
<td>Primary and cultured human LECs, murine LECs in vivo</td>
<td>Increased following TLR stimulation</td>
<td>26, 28, 31,</td>
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<td>CCL21</td>
<td>Recruitment of DCs, macrophages, naive T cells, and regulatory T cells via CCR7</td>
<td>Demonstrated in various mouse models and in human tissues</td>
<td>Considerable baseline expression on resting LECs</td>
<td>23, 26, 33,</td>
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<td>CXCL1</td>
<td>Recruits neutrophils via CXCR1 and CXCR2</td>
<td>Murine LECs in vivo</td>
<td>Increased upon contact hypersensitivity</td>
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<td>CXCL3</td>
<td>Recruitment of neutrophils via CXCR2</td>
<td>Cultured human dermal LECs</td>
<td>Increased following TLR2 stimulation</td>
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<td>CXCL5</td>
<td>Recruitment of neutrophils via CXCR2</td>
<td>Cultured human dermal LECs, murine LECs in vivo</td>
<td>Increased following contact hypersensitivity and TLR engagement</td>
<td>23, 28, 31,</td>
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<td>CXCL6</td>
<td>Recruitment of neutrophils via CXCR1 and CXCR2</td>
<td>Cultured human dermal LECs</td>
<td>Increased following TLR2 stimulation</td>
<td>28, 31</td>
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<tr>
<td>CXCL8</td>
<td>Recruitment of neutrophils via CXCR1 and CXCR2</td>
<td>Cultured human dermal LECs</td>
<td>Increased following TLR stimulation</td>
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<td>CXCL9</td>
<td>Recruitment of T cells via CXCR3</td>
<td>Cultured human dermal LECs, murine LECs in vivo</td>
<td>Increased following TLR stimulation and upon contact hypersensitivity</td>
<td>23, 26, 31,</td>
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<td>CXCL10 (IP-10)</td>
<td>Recruitment and adhesion of T cells via CXCR3</td>
<td>Primary and cultured human LECs, murine LECs in vivo</td>
<td>Upregulated by LPS/IFN-γ, increased upon contact hypersensitivity</td>
<td>23, 26, 32,</td>
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<tr>
<td>CXCL11</td>
<td>Recruitment and adhesion of T cells via CXCR3</td>
<td>Cultured human dermal LECs</td>
<td>Increased following TLR3 engagement</td>
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<td>IDO</td>
<td>Tryptophan-depleting enzyme; inhibits T cell activation and proliferation</td>
<td>Primary human LECs, murine tumor-draining LNs</td>
<td>Upregulated following IFN-γ treatment</td>
<td>32, 93, 94</td>
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<td>IL-1β</td>
<td>Multiple immunostimulatory effects</td>
<td>Cultured human dermal LECs</td>
<td>Increased following TLR stimulation</td>
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<tr>
<td>IL-6</td>
<td>Signals via CD126 and CD130 to mediate acute inflammatory responses</td>
<td>Primary and cultured human LECs</td>
<td>Increased following TLR stimulation</td>
<td>29, 91</td>
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<td>IL-7</td>
<td>Engages CD127 on naive and memory T cells, leading to their proliferation and activation in STAT5-dependent manner</td>
<td>Murine primary LECs</td>
<td>Increased in various in vivo inflammation models</td>
<td>26, 63, 79, 80</td>
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<tr>
<td>IL-8</td>
<td>Recruits neutrophils and promotes angiogenesis</td>
<td>Primary and cultured human LECs</td>
<td>Upregulated following LPS treatment</td>
<td>29</td>
</tr>
<tr>
<td>Lipocalin-2 (LCN2)</td>
<td>Sequesters iron, limits bacterial growth (involved in innate immunity)</td>
<td>Murine primary LECs</td>
<td>Increased with LPS/OVA priming and adoptive transfer of OT-1 CD8+ cells</td>
<td>26</td>
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<tr>
<td>iNOS</td>
<td>NO catalyst, inhibits T cell proliferation</td>
<td>Murine LECs engineered to express OVA in vivo</td>
<td>Upregulated following IFN-γ treatment</td>
<td>63</td>
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<td>TGF-β</td>
<td>Multiple immunoregulatory effects</td>
<td>Primary human neonatal foreskin LECs, primary murine LECs</td>
<td>Considerable baseline expression on resting LECs</td>
<td>22, 26</td>
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<td>TRAIL (TNFSF10)</td>
<td>Induces apoptosis in target cells</td>
<td>Primary murine LECs</td>
<td>Considerable baseline expression on resting LECs</td>
<td>26</td>
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reviews (19–21). LECs both attract and facilitate transmigration of immune cells through a number of signaling axes, many of which have been identified in LEC gene expression studies (22–26). In addition to identifying multiple cytokines and receptors expressed by LECs under steady-state conditions (22), these studies also identified genes that are upregulated during localized inflammatory processes such as in models of contact hypersensitivity (23), tumor drainage (25), and inflammation (26). These studies and others indicate that LECs are capable of expressing and actively signaling through a variety of cytokines and adhesion receptor–ligand interactions important in immunity (Tables 1, 2, and 3, and refs. 17, 27–30).

LEC receptors sensing inflammatory or danger signals

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<tr>
<td>CD120 (TNFR1)</td>
<td>Heterodimer that responds to TNF-α by activating the NF-κB pathways downstream</td>
<td>Murine primary LECs</td>
<td>Considerable baseline expression on resting LECs</td>
<td>26</td>
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<tr>
<td>CD206</td>
<td>Macrophage mannose receptor, mediates binding and uptake of multivalent mannoseylated motifs</td>
<td>Healthy human tissues and LNs</td>
<td>Considerable baseline expression on resting LECs</td>
<td>95</td>
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<tr>
<td>IFNAR1</td>
<td>Responds to IFN-α and IFN-β by dimerizing with IFNAR2 to activate multiple Jak/STAT pathways</td>
<td>Murine primary LECs</td>
<td>Considerable baseline expression on resting LECs</td>
<td>26</td>
</tr>
<tr>
<td>IFNAR2</td>
<td>Responds to IFN-α and IFN-β by dimerizing with IFNAR2 to activate multiple Jak/STAT pathways</td>
<td>Murine primary LECs</td>
<td>Considerable baseline expression on resting LECs</td>
<td>26</td>
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<tr>
<td>IFNGR1</td>
<td>Responds to IFN-γ by dimerizing with IFNGR2 to activate the STAT1 pathway</td>
<td>Murine primary LECs</td>
<td>Considerable baseline expression on resting LECs</td>
<td>26</td>
</tr>
<tr>
<td>IFNGR2</td>
<td>Responds to IFN-γ by dimerizing with IFNGR1 to activate the STAT1 pathway</td>
<td>Murine primary LECs</td>
<td>Considerable baseline expression on resting LECs</td>
<td>26</td>
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<td>TLR1</td>
<td>Recognizes peptidoglycan and lipoproteins from gram-positive bacteria</td>
<td>Cultured human dermal and lung LECs</td>
<td>Considerable baseline expression on resting LECs</td>
<td>91</td>
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<tr>
<td>TLR2</td>
<td>Recognizes gram-positive bacteria and yeast</td>
<td>Cultured human dermal and lung LECs</td>
<td>Considerable baseline expression on resting LECs</td>
<td>31, 91</td>
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<td>TLR3</td>
<td>Recognizes double-stranded RNA from viral infections</td>
<td>Cultured human dermal and lung LECs</td>
<td>Considerable baseline expression on resting LECs</td>
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<tr>
<td>TLR4</td>
<td>Recognizes LPS (found in most gram-negative bacteria)</td>
<td>Human intestinal tissue, cultured neonatal dermal microvascular LECs</td>
<td></td>
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<tr>
<td>TLR5</td>
<td>Recognizes flagellin in bacterial flagella</td>
<td>Cultured human dermal and lung LECs</td>
<td>Considerable baseline expression on resting LECs</td>
<td>91</td>
</tr>
<tr>
<td>TLR6</td>
<td>Recognizes bacterial lipoproteins in concert with TLR2</td>
<td>Cultured human dermal and lung LECs</td>
<td>Considerable baseline expression on resting LECs</td>
<td>91</td>
</tr>
<tr>
<td>TLR9</td>
<td>Recognizes unmethylated CpG sequences in pathogenic DNA; expressed intracellularly</td>
<td>Cultured human dermal and lung LECs</td>
<td>Considerable baseline expression on resting LECs</td>
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Other chemokines secreted by LECs recruit T and B lymphocytes, which enter the LNs in large numbers to be educated by APCs and to mount effective adaptive immune responses. The recruitment of naive T cells into LNs is controlled by LECs and FRs via CCL21 and CCL19 (another ligand of CCR7), which are present throughout the cortical zone and eventually transcytosed into the lumen of high endothelial venules (HEVs) (40–42). CCL21 also enhances the affinity of lymphocyte function–associated antigen 1 (LFA-1) on circulating naive T cells for ICAM-1 on blood endothelial cells (BECs), thereby promoting migration (42, 43).

LEC can also modulate DC and macrophage trafficking through expression of the chemokine-scavenging decoy receptor D6, which is upregulated in the context of inflammation and tumor drainage (44–47). It has been suggested that D6 helps regulate the extracellular concentrations of its ligands CCL2 and CCL5 (48), as mice deficient for D6 develop exacerbated inflammatory responses (49) and exhibit congestion of the lymphatic vessels by macrophages. Thus, by regulating extracellular CCL2, D6 may help to ensure that lymphatic conduits are uncongested to facilitate DC trafficking (47).

Many adhesion molecules are expressed by LECs to facilitate leukocyte transmigration across LECs. ICAM-1 is important for DC and T cell adhesion and synergizes with CCL21 to promote lymphocyte binding and migration (50). DCs can also transmi-
grate into the lymphatic vessels using the plexin-A1/neuropilin-1 (Pxa1/Nrp1) receptor complex to engage semaphorin-3A (Sema3A) expressed on LECs (51). These are also upregulated upon inflammatory stimuli (refs. 17, 27–30, and Table 2). For example, elevated local interstitial fluid stresses result in increased fluid flow into the lymphatics, triggering augmented lymphatic secretion of CCL21 and upregulation of ICAM-1 and E-selectin (17). CCR7 signaling may in turn enhance the affinity of LFA-1 on DCs for ICAM-1, suggesting that this may be a synergistic mechanism to facilitate DC homing to LNs following antigenic challenge (43). In this way, antigenic challenge can rapidly lead to changes in lymphatic phenotype that result in enhanced recruitment of DCs and their migration to the LNs, comprising the early stages of an effective antigen-specific, adaptive immune response (Figure 2).

Interestingly, in contrast to K14–VEGFR-3–Ig mice that lack dermal lymphatic capillaries and demonstrate impaired DC migration, Chy mice, which lack the majority of dermal lymphatic capillaries, have rare patches of these vessels in their back skin that express higher levels of CCL21. In these mice, DC migration to LNs draining the back skin appeared normal despite severely impaired lymphatic drainage, indicating that lymphatic density governs fluid and antigen drainage rather than DC migration (52). This has implications for understanding the role of local lymphangiogenesis, where lymphatic vessels expand and become hyperplastic, which occurs in chronic inflammation.

Thus, LECs actively regulate leukocyte trafficking by modulating expression of chemokines and adhesion molecules according to the local state of inflammation in the tissue. LECs can integrate multiple signals, including complement activation products and increased flow as well as most danger signals via TLR signaling (Table 2), allowing them to modulate their differential regulation of leukocyte trafficking or delivery of antigens and local tissue cytokines (17).

LEC regulators entry of immune cells into LNs. Upon arrival at the LNs, migratory DCs exit afferent lymphatic vessels into the subcapsular sinus (53). At this junction, LECs lining the subcapsular sinus express not only CCR7 ligands, but also CCL1, which binds CCR8 on DCs (5, 54, 55). In this way, LECs may act as gatekeeper cells, selecting CCR8+ cells for entry into the LNs (55). DCs then traverse the intranodal sinuses in order to gain access to the T cell zone, enabling them to influence downstream adaptive immune responses. The T cell zone is rich with FRCs, which help guide and modulate DC–T cell interactions (56).

In addition to lining the subcapsular sinus, LECs also infiltrate into the cortical and medullary sinuses (Figure 1) to presumably direct antigen and leukocytes into the T cell zone. Although T cells generally enter LNs via HEVs (19, 57), afferent lymph-derived T cells may also enter the parenchyma via medullary sinuses (58). LECs direct lymphocyte trafficking to the medullary sinuses and eventually guide egress of T cells from the LN. As such, T cells frequently encounter LECs throughout their migration through the LN.

Collectively, these data establish that LECs not only express trafficking molecules that affect migration of immune cells, but also possess the appropriate machinery to modulate DC and T cell function.
T cell tolerance is not unique to LECs and FRCs. Liver sinusoidal observations prove to hold true in humans, LEC-induced tolerance is consistent with previous data demonstrating the loss of tolerance in SLOs (71). LEC expression of PTAs, including Ty369, was found to be independent of Aire, whereas PTA expression by double-negative (DN) LNSCs (podoplanin-/CD31-) and CD45+ cells was strongly Aire dependent (60). Thus, it remains unknown what drives PTA expression in LECs and FRCs, but both subsets express Deaf1 (60), a transcriptional regulator that induces PTA expression in pancreatic LN (72), suggesting a possible role for this factor in mediating tolerance induced by LECs and FRCs.

There are multiple potential pathways by which peripheral tolerance may be induced. For example, lack of appropriate co-stimulation or engagement of inhibitory receptors during CD8+ T cell activation results in anergy or deletion. As such, the relative expression of co-stimulatory and inhibitory receptors on APCs influences the outcome of T cell activation. In the steady state, expression of most co-stimulatory receptors by LECs is low (32, 62), while multiple inhibitory receptors are expressed at high levels (62). In a model of LEC-induced tolerance of Ty369-specific CD8+ T cells, lack of co-stimulation through CD137 (4-1BB) led to upregulation of PD-1 and inhibition of CD25 (IL-2Rα) on CD8+ T cells, rendering them resistant to IL-2-mediated pro-survival signaling. Deletion was mediated by PD-L1 expressed on LECs, since blockade of this receptor prevented deletion of Ty369-specific CD8+ T cells, resulting in autoimmunity (62). This observation was consistent with previous data demonstrating the loss of LNSC-induced CD8+ tolerance against intestinal PTAs induced by PD-L1 blockade, which led to autoimmune enteritis (73).

In addition to their role in inducing tolerance of autoreactive CD8+ T cells, we recently demonstrated that LECs can scavenge and cross-present foreign antigen to naive CD8+ T cells (59). In this model, B16 F10 melanomas expressed OVA as a foreign antigen, and a second B16 F10 cell line also expressed VEGF-C to enhance tumor and LN lymphangiogenesis. LECs in the tumor-draining LN cross-presented OVA to CD8+ T cells, leading to deletion of OVA-specific CD8+ T cells. Moreover, VEGF-C–induced lymphangiogenesis further promoted tolerance, and could even protect the tumor against CD8+ T cell immunity (59).

The interactions between LECs and CD4+ T cells are less well described. In the steady state, LECs express low basal levels of MHC class II molecules (26, 32, 74), and IFN-γ induces MHC class II upregulation (32). Although LECs do not express conventional co-stimulatory molecules such as CD80 and CD86, they express LFA-1 (CD58), which can bind CD2 to provide co-stimulation to T cells. Despite the presence of these molecules, LECs failed to induce CD4+ T cell proliferation or cytokine production in an allogenic co-culture model (32). Thus, while it is now clear that LECs can take up and cross-present exogenous antigens on MHC class I to CD8+ T cells (59), it remains to be seen whether this scavenging activity can lead to antigen presentation on MHC class II and subsequent CD4+ T cell activation.

**Modulation of T cell activation by lymphatics**

**LEC present antigen for T cell regulation.** LECs express MHC class I (59–61) and MHC class II (26, 62) molecules and can directly induce T cell tolerance as well as suppress DC-mediated T cell activation. A variety of immunoregulatory factors are expressed by LECs that enable these functions. For example, LECs secrete TGF-β, indoleamine-2,3-dioxygenase (IDO) and nitric oxide, all of which are immunosuppressive (22, 26, 32, 63). T cell activation is also affected by the relative balance of co-stimulatory and inhibitory receptors on the surface of the activating APC. LECs express high levels of the inhibitory receptor PD-L1 (62) and suboptimal levels of co-stimulatory molecules such as CD80, CD86, and ICOSL (Table 3 and refs. 26, 62).

These expression patterns suggest that LECs may act as APCs. Indeed, several studies have now shown that LECs, as well as other LN stromal cell (LNSC) subsets such as FRCs, can modulate T cell function through direct presentation of endogenous and exogenous antigens (Figure 3). In murine models, LNSCs express various peripheral tissue antigens (PTAs), which they can present to naive CD8+ T cells, thus leading to activation and tolerance (61, 64, 65) due to the absence of co-stimulatory molecules and high levels of PD-L1. Different LNSC subsets, including LECs, FRCs, and BECs, have distinct patterns of PTA expression (60, 66), suggesting different roles in modulating responses to these antigens. However, the antigen-presenting capacity of LNSCs seems to be attributable primarily to LECs and FRCs (66). Notably, LECs were the only subset to express the PTA tyrosinase epitope Ty369, a major immunotherapy target for melanoma (60, 66). If these observations prove to hold true in humans, LEC-induced tolerance of Ty369-specific CD8+ T cells could have a direct impact on the clinical efficacy of anti-melanoma immunotherapies.

The observation that LECs and FRCs can tolerate CD8+ T cells led to a paradigm shift in models of peripheral tolerance induction, as conventional wisdom attributed suppression of autoreactive CD8+ T cells to activation by quiescent DCs (67). However, the ability of non-hematopoietic cells to induce peripheral CD8+ T cell tolerance is not unique to LECs and FRCs. Liver sinusoidal endothelial cells (LSECs) are considered critical for tolerance to food antigens, as they mount CD8+ deletional tolerance against exogenous antigens coming directly from the gut (68, 69). Tolerance induction by stromal cells is reminiscent of central tolerance induced in the thymus by stromal medullary thymic epithelial cells (mTECs), a process that is dependent on PTA expression driven by the autoimmune regulator Aire (70). Due to the similarities between LNSCs and mTECs, the dependence of Aire in LNSC-mediated induction of CD8+ T cell tolerance was investigated. Using a PTA expressed under the Aire promoter, extrathymic Aire-expressing cells (eTACs) were implicated in inducing CD8+ T cell tolerance in SLOs (71). LEC expression of PTAs, including Ty369, was found to be independent of Aire, whereas PTA expression by double-negative (DN) LNSCs (podoplanin+/CD31-) and CD45+ cells was strongly Aire dependent (60). Thus, it remains unknown what drives PTA expression in LECs and FRCs, but both subsets express Deaf1 (60), a transcriptional regulator that induces PTA expression in pancreatic LN (72), suggesting a possible role for this factor in mediating tolerance induced by LECs and FRCs.

Figure 2

LEC-CD8+ T cell interaction. Tissue resident DCs (red) take up antigens in peripheral tissues, home to nearby draining lymphatic vessels via CCL21-driven chemotaxis, and engage ICAM-1, VCAM, and Sema3A for transmigration. Under inflammatory conditions, LECs can increase their expression of adhesion molecules and chemokines that further promote the lymphatic recruitment of DCs and other cell subsets, including macrophages via CCL2. LEC expression of decoy receptors such as D6 helps limit local chemokine concentrations and shape gradients.
LEC regulation of T cell homeostasis

The size, diversity, and distribution of the mature peripheral T cell pool is tightly regulated through homeostatic pathways affecting migration, proliferation, survival, and apoptosis of activated cells. For example, despite continuous thymic production of mature T cells, total T cell numbers remain relatively steady, suggesting that peripheral T cells are continually lost without compromising the diversity of T cells in circulation (76). As major producers of homeostatic factors, LECs are emerging as important players in regulating the T cell pool, although this notion merits further study.

LEC regulate T cell homeostasis through production of IL-7. IL-7 is a key homeostatic cytokine produced primarily by non-hematopoietic stromal cells. It binds the IL-7Rα chain (CD127) in combination with the common γ-chain (CD132) expressed on developing T, B, and NK lymphocytes, mature T cells, and certain subsets of DCs, macrophages, and innate lymphocytes. In LNs, IL-7 provides survival signals to naive and memory T cells, which respond by upregulating anti-apoptotic factors and downregulating IL-7Rα. Disruptions in IL-7 signaling cause imbalances in T cell numbers, such that loss of IL-7 signaling leads to lymphopenia (77).

LNSCs produce high levels of IL-7, which is critical for naive T cell survival (78). Although FRCs were proposed to be the major source of this cytokine due to their presence in T cell zones of the LN and expression of high levels of IL7 mRNA (78), recent studies demonstrated that LECs produce the most IL-7 in the LN (79–81). Indeed, LECs can support survival of naive T cells in vitro in an IL-7–dependent manner (79, 80). Importantly, IL-7 expression by LECs is not restricted to the LN; IL-7+ LECs have been observed in multiple tissues, including the lung, skin, and gut (80, 81), suggesting that LECs may play an important role in the homeostasis of other cells that express functional IL-7 receptors.

Furthermore, T cells may regulate IL-7 production by LECs and FRCs in a negative regulatory loop. Specifically, blockade of lymphocyte entry into LNs induced elevated IL-7 expression in the LN (80), and similarly, LN IL-7 is upregulated during HIV-associated lymphopenia (82). Since T cells may negatively regulate LN lymphangiogenesis (83), it is possible that increased IL-7 is the result of increased numbers of IL-7+ stromal cells in the absence of T cell regulation. IL-7-expression by embryonic LECs during LN development acts on lymphoid tissue inducer (LI) cells, promoting their survival and enabling them to drive LN organization (79). In line with this, IL-7+ LECs and FRCs were shown to preferentially expand during LN remodeling after destruction by an inflammatory viral infection (79).

LEC regulate T cell egress from LNs. As mentioned above, naive T cells traffic to LNs in a CCR7- and CD62L-dependent manner. These signals also provide cues for T cell retention within the LN but are countered by binding of sphingosine-1-phosphate (S1P) to S1P receptor 1 (SIPR1), which triggers migration of lymphocytes into cortical sinuses. However, if a naive T cell becomes activated by an APC bearing its cognate antigen while in the LN, SIPR1 is
downregulated, promoting retention of differentiating T cells in the LN. Following clonal expansion, activated effector and memory T cells downregulate CCR7 and CD62L and upregulate S1PR1, promoting migration to cortical sinuses (84). Within the cortical sinuses, fluid flow also promotes egress of T cells into efferent lymphatic vessels (85).

While the kinetics of LN egress differs between naive and activated T cells, both are mediated by LECS. Preliminary evidence of this role was provided by studies that demonstrated, that although hematopoietic cells in the periphery produce S1P, radio-resistant cells were responsible for S1P production in the LN cortical sinuses and efferent lymphatics, implicating a non-hematopoietic cellular source of S1P (86). Further studies utilized mice with deficiencies in S1P production by LYVE-1⁺ lymphatics to establish LECS as the source of S1P in these tissues. In the absence of S1P production by LECS, lymphocyte egress from LNs did not occur (87).

During inflammation, lymphocyte egress is transiently shut down but returns to steady-state levels in cases of prolonged inflammation. This restoration in homeostasis is reliant on LECS, as preferential lymphangiogenic expansion of the cortical and medullary sinuses facilitates lymphocyte egress during the late stages of inflammation (88).

Collectively, these studies demonstrate the pivotal role of LECS in controlling the homeostasis of the T cell pool. This is accomplished by coordinated regulation of T cell survival though IL-7 signaling and by guiding the dissemination of T cells through production of S1P. Future studies may uncover additional pathways by which LECS and T cells interact under normal and diseased states.

Clinical significance and future directions for the field

From a clinical standpoint, an appreciation for interactions between LECS in controlling the lymphatic endothelium in this suppression, which is further exacerbated by lymphangiogenesis in the tumor context (59). Investigating anti-lymphangiogenic therapies may be one way to counter this suppression, thereby improving the efficacy of anti-cancer immunotherapy. HIV presents another example of the potential of therapeutics that target the lymphatic endothelium. In chronic HIV infection, the LN suffers structural damage from inflammation and collagen deposition (90). It is likely that damage to the LN stroma interferes with the regulation of T cell homeostasis by LECS and exacerbates the lymphopenia that is characteristic of progressive HIV disease. In this case, it might be necessary to develop interventions that maintain LN architecture and promote functional interactions between T cells and LNSCs.

In summary, recent studies of lymphatic phenotype and function have demonstrated that LECS are capable of directly shaping the adaptive immune response through influencing immune cell trafficking, promoting T cell tolerance, and mediating T cell homeostasis. Furthermore, activated T cells appear to regulate LECS expansion and function in settings of inflammation.

It remains to be seen whether LECS can present antigen to CD4⁺ T cells and what type of response is initiated under those circumstances. In addition, there is a paucity of information on the interactions between LECS and B cells that can modulate immunity. These interactions need to be further delineated to better understand the dynamics of humoral immunity during health and disease. Future development of immunotherapies should consider the pivotal role of lymphatics in shaping immunity and regulating homeostasis of innate and adaptive immune subsets.

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