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Exploiting lymphatic vessels for immunomodulation: Rationale, opportunities, and challenges☆

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ABSTRACT

Lymphatic vessels are the primary route of communication from peripheral tissues to the immune system; as such, they represent an important component of local immunity. In addition to their transport functions, new immunomodulatory roles for lymphatic vessels and lymphatic endothelial cells have come to light in recent years, demonstrating that lymphatic vessels help shape immune responses in a variety of ways: promoting tolerance to self-antigens, archiving antigen for later presentation, dampening effector immune responses, and resolving inflammation, among others. In addition to these new biological insights, the growing field of immunoengineering has begun to explore therapeutic approaches to utilize or exploit the lymphatic system for immunotherapy.

Contents

Abbreviations: Ag, antigen; APC, antigen-presenting cell; DC, dendritic cell; DSS, dextran sodium sulfate; HEV, high endothelial venule; iBALT, induced bronchus-associated lymphoid tissue; i.d., intradermal; IDO, indoleamine-2,3-dioxygenase; i.m., intramuscular; i.n., intranodal; IPF, idiopathic pulmonary fibrosis; i.v., intravenous; LAM, lymphangioleiomyomatosis; LEC, lymphatic endothelial cell; LN, lymph node; LNSC, lymph node stromal cell; MPA, mycophenolic acid; MPLA, monophosphoryl lipid; NO, nitric oxide; NP, nanoparticle; OVA, ovalbumin; PEG, poly(ethylene glycol); PLGA, poly-(lactic-coglycolic-acid); PPS, poly(propylene sulfide); PTA, peripheral tissue antigen; S1P1, Sphingosine-1-phosphate receptor 1; s.c., subcutaneous; TAA, tumor-associated antigen; tdLN, tumor-draining LN; T_{Reg} cell, regulatory T cell; VEGF, vascular endothelial growth factor.

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1. Lymphatic vessels and their transport functions in immunology

Lymphatics drain extracellular fluid and its solutes, including antigens (Ag), as well as cells and particulates like exosomes from peripheral tissue to draining lymph nodes (LNs) and eventually into systemic circulation (Fig. 1). While traditionally considered as passive, exciting new insights in recent years have revealed multiple ways that the various transport functions of lymphatics may help shape immune responses. In this section we will give an overview of lymphatic transport from an immunology perspective and highlight new findings, including (i) how lymphatic endothelial cells (LECs) can actively modulate fluid and solute transport as well as store Ag, (ii) how antigen-presenting cells (APCs) and lymphocytes traffic within the lymphatic vasculature and potentially affect fluid drainage, and (iii) how the lymphatic-APC interface can modulate Ag distribution.

1.1. Lymphatic drainage of fluid and antigens

Drainage of fluid and Ags from interstitial spaces of tissues occurs mainly via the initial lymphatic capillaries. These blind-ended structures are non-muscular, have discontinuous basement membrane, and display overlapping cell-cell junctions anchored to the extracellular matrix by elastic filaments [1,2]. Consequently, lymphatic drainage has been considered passive (i.e. based on physical Starling forces), modulated by small, local pressure differences across the vessel wall. However, recent work from our lab has demonstrated that transcellular transport of fluid and solutes mediated through intracellular vesicles is an important component together with paracellular mechanisms, indicating that lymph formation can be actively regulated by LECs [3,4]. Backflow into the interstitium is prevented by a system of valves in initial lymphatics and contractile collecting lymphatics, the latter of which separate segments called lymphangions [1,2]. The collecting lymphatics transport lymph into the LNs through the subcapsular sinus, capsular sinusoids, then medullary sinusoids as well as reticular fibers; finally, the lymph leaves the LNs through the afferent lymphatics, and is eventually drained into the blood via the thoracic duct [5–7].

The free Ags carried by lymph are taken up by APCs in the LN. Ag uptake and presentation in the LN has distinct roles in activating immune responses compared to Ag picked up by peripheral DCs and brought to the LN. This is both because of differences in time to presentation – free Ag arrives within minutes, while DC-carried Ag can take several hours – and because of different functions of LN-resident vs. peripheral DCs [8–10]. Lymph-borne Ags (foreign or mutated self) that arrive together with inflammatory cytokines, danger signals, or other signals from the peripheral site, promote DC maturation in the LN and drive robust T cell education leading to effector T cell phenotypes [11]. Additionally, lymph-borne Ag can be taken up by subcapsular macrophages and B cells to mount humoral immunity [12,13].

Lymph is also rich in peripheral tissue Ags, which, in the absence of danger signals, can be taken up by immature dendritic cells (DCs) or LN stromal cells (LNSCs) and presented to autoreactive T cells for deletional tolerance [14,15]. Interestingly, work from the Santambrogio lab has demonstrated that lymph carries a large pool of tissue- and cell-derived peptides, including those derived from caspases and matrix metalloproteases, that are distinct from DC-presented self-Ag in terms of epitope repertoire [16–21]. In this way, lymphatic drainage in both steady-state and various inflammatory conditions is likely important for maintaining peripheral tolerance to self-Ags. Furthermore, regulatory T (T_{Reg}) cells reside in LNs, and their activation is thought to take place there, further leading to tolerance against peripheral tissue Ags [22].

That lymphatic drainage is important for both effector immunity and maintenance of peripheral tolerance through distinct mechanisms highlights the complex roles lymphatic vessels can have in regulating immunity. Using K14-VEGFR-3-Ig mice that lack dermal lymphatics [23], our lab showed that without lymphatic drainage of an intradermally (i.d.) delivered vaccine, B cell immunity was severely impaired, and T cell immunity was delayed (but could be initiated in the spleen). In contrast, tolerance to a foreign Ag could not be induced with a classical skin hypersensitivity assay, and older mice developed signs of autoimmunity to skin Ag [24]. Much earlier work using skin transplantation in rats demonstrated that local induction of tolerance required lymphatic drainage from the transplant [25,26]. More recently, using melanomas implanted i.d. into K14-VEGFR-3-Ig mice, we demonstrated that lymphatic vessels are essential for immune recognition and response to these melanomas, even though the resulting tumor immune infiltrate was highly suppressive [27]. Without dermal lymphatic drainage, tumors grew as if in an immune privileged site. Therefore, Ag transport by lymphatics is critical to initiating all types of immune responses.

1.2. Lymphatic transport of leukocytes

Extensive research has demonstrated that while immune cells enter tissue via blood, they use lymphatic vessels to exit tissue, and a number of key factors that help facilitate or control this trafficking have been identified. The most well-studied cell types are DCs and T cells, which we will briefly review here. For more extensive reviews we refer the reader to several excellent review articles [28–31].

Upon scavenging peripheral Ags, DCs home to and enter lymphatic vessels using the chemokine receptor CCR7, which is upregulated upon maturation [22,32–34] and allows chemotaxis towards its ligand CCL21, secreted by LECs (and upregulated upon inflammation) [35,36]. Blocking CCR7 signaling has been shown to severely compromise peripheral DC migration to LNs [22,30,32,37,38]. Two variants of CCL21 exist: a leucine-containing variant (CCL21-leu) that is constitutively expressed by LECs, and thus important for DC entry into peripheral lymphatics, and a serine-bearing variant (CCL21-ser) secreted by high endothelial venules (HEVs) and fibroblastic reticular cells along with the other known CCR7 ligand CCL19 [11,39–41], and thus primarily found in the LN. In mice lacking functional CCL19 and CCL21-ser, DCs migrated into peripheral lymphatic vessels but showed impaired trafficking into and within the LN stroma [39–41]. There are a vast number of receptors that aid in DC migration by facilitating transendothelial migration and promoting diapedesis that have been described in various review articles [11,30]. Interestingly, some of these molecules differentially modulate lymphatic trafficking of different DC subsets: for