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ORIGINAL PAPER

Lymphatic vessels: new targets for the treatment of inflammatory diseases

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Abstract The lymphatic system plays an important role in the physiological control of the tissue fluid balance and in the initiation of immune responses. Recent studies have shown that lymphangiogenesis, the growth of new lymphatic vessels and/or the expansion of existing lymphatic vessels, is a characteristic feature of acute inflammatory reactions and of chronic inflammatory diseases. In these conditions, lymphatic vessel expansion occurs at the tissue level but also within the draining lymph nodes. Surprisingly, activation of lymphatic vessel function by delivery of vascular endothelial growth factor-C exerts anti-inflammatory effects in several models of cutaneous and joint inflammation. These effects are likely mediated by enhanced drainage of extravasated fluid and inflammatory cells, but also by lymphatic vessel-mediated modulation of immune responses. Although some of the underlying mechanisms are just beginning to be identified, lymphatic vessels have emerged as important targets for the development of new therapeutic strategies to treat inflammatory conditions. In this context, it is of great interest that some of the currently used anti-inflammatory drugs also potently activate lymphatic vessels.

Keywords Lymphangiogenesis · Inflammation · Psoriasis · Arthritis · Endothelial cells

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Introduction

The lymphatic system is closely connected to inflammatory processes and immune functions. One of its major functions is the transport of immune cells and soluble antigens from the periphery to the lymph nodes, which are the primary sites for activation of immune responses. Correspondingly, lymphatic vessels can be found in most organs and their network is particularly dense in the skin and near mucosal surfaces, the main entry sites for foreign material and pathogens into the body [1, 2]. It is thus surprising that lymphatic vessels have been thought for decades to merely represent a passive drainage system without any active role in disease processes. However, recent findings indicate that lymphatic vessels are dynamic structures that sensibly react to inflammatory stimuli and that are activated, both at the level of the peripheral inflamed tissue and within the draining lymph nodes. In this review, we highlight the capacity of lymphatic vessels to regulate inflammation resolution and to modulate immune responses and tolerance, with a focus on therapeutic approaches to target lymphatic vessels for the treatment of inflammatory conditions.

Molecular markers of lymphatic endothelium and their modulation in inflammation

The dramatic progress in our understanding of the active role of lymphatic vessels in development, physiology and disease is due to the identification of lymphatic endothelial cell (LEC)-specific markers [3], in particular vascular endothelial growth factor receptor (VEGFR)-3, Prox1, LYVE-1 and podoplanin. These LEC markers allow for the specific staining of LECs in tissue sections, their specific

isolation in vitro and ex vivo and, subsequently, the characterization of differentially expressed genes and proteins in LECs versus blood vascular endothelial cells (BECs) [4–7].

VEGFR-3 is a receptor tyrosine kinase for the lymphangiogenic growth factors VEGF-C and -D and is crucial for growth, survival and migration of LECs [8, 9]. During embryonic development, VEGFR-3 is expressed on both blood vessels and on lymphatic vessels (LVs), whereas it is mostly expressed by LECs in healthy adult tissue [10]. Some VEGFR-3 expression has been found on blood capillaries in the spleen, kidney, and glands of the endocrine system [11]. Prox1 is required for the development of the lymphatic system [12] and plays a major role as master transcription factor responsible for LEC differentiation—ectopic Prox1 expression partially reprograms cultured BECs to a LEC-like phenotype [7, 13]. LYVE-1, a homolog of the CD44 glycoprotein, is a hyaluronan receptor of yet unclarified function that is predominantly expressed by LECs in adult tissues [14, 15]. LYVE-1 expression is high in lymphatic capillaries but very low in collecting LVs [16]. LYVE-1 is only rarely seen on blood vessels, such as sinusoidal endothelial [14, 17]. Podoplanin is a membrane glycoprotein that is expressed by LECs but not BECs [18] and that plays a major role in normal lymphatic development [19]. While its expression is high on LECs of lymphatic capillaries, some LECs in lymphatic pre-collectors show low podoplanin expression [20].

Although these markers allow the distinction of LECs from BECs in most tissues, some non-endothelial expression has been observed. For instance, VEGFR-3 has been found to be expressed by corneal DCs [21] and epithelium [22], and Prox1 is expressed in liver, pancreas [23] and the brain [24, 25]. Moreover, strong LYVE-1 expression has been observed on a subset of macrophages in various tissues, which makes it essential to distinguish them from LVs on tissue sections [26–28]. Podoplanin is also expressed in the choroid plexus of the brain [29, 30], alveolar type I epithelial cells in the lung [29, 31], ciliary epithelia of the intestine [29], stromal cells of lymphoid organs such as the spleen [32] and LNs [33], possibly leukocyte subsets including macrophages [34, 35] and Th17 cells [36], nerve fibers and bile duct associated mesenchymal cells in the liver [37]. Therefore, it is recommended to use combinations of two different lymphatic markers or a combination of a lymphatic marker with a pan-endothelial marker such as CD31 [38] for the unambiguous detection of lymphatic vessels.

Despite their usefulness for the identification of lymphatic vessels in normal tissues, the expression of these lymphatic markers can be strongly modulated during tumorigenesis [39] and inflammation, and these changes are likely stimulus- and species-specific. In general, the expression of most LEC markers appears to be down-regulated on lymphatic vessels during inflammation, whereas upregulation of LEC markers by inflammatory stimuli has been observed in BECs, at least under culture conditions [40]. Importantly, LYVE-1 was found to be down-regulated on LECs during chronic airway inflammation in vivo [41] and also on cultured LECs upon inflammatory stimuli [42]. Lower mRNA levels of VEGFR-3, LYVE-1 and Prox1 and reduced podoplanin protein expression were found on cutaneous LECs during contact hypersensitivity-induced inflammation in mice but not by complete Freund‘s adjuvant-induced inflammation [43]. Our own studies in acute skin inflammation models recently also revealed inflammation-mediated down-regulation of VEGFR-3 on LECs [44] whereas up-regulation of VEGFR-3 and Prox-1 on LECs was found in a peritonitis model, possibly mediated by nuclear factor-kappaB activation [45]. Together, these studies highlight the stimulus-dependent response of LECs with regard to marker expression, and they indicate that at least two different LEC markers should be used to investigate the response of lymphatic vessels in inflammation.

Lymphatic vessels expansion in inflammation

Expansion of the lymphatic vascular network has been observed in several inflammatory conditions, both in experimental mouse models and in human inflammatory diseases (reviewed in [46]). This expansion includes dilation of pre-existing vessels (lymphatic hyperplasia) as well as induction of lymphatic sprouting (lymphangiogenesis) and the growth of new lymphatic vessels, resulting in an increased lymphatic vascular density. Our laboratory identified enlargement of lymphatic vessels in the skin of patients suffering from psoriasis, a chronic inflammatory skin disease, as well as in a mouse model of psoriasis [47]. In this model, transgenic mice with skin-specific overexpression of VEGF-A develop a chronic, psoriasis-like skin inflammation after treatment with the contact sensitizer oxazolone, associated with angiogenesis and with lymphatic vessel expansion (Fig. 1 a, b). Similarly, lymphatic expansion has been observed in clinical samples and mouse models of arthritis [48–50], atopic dermatitis [51] and inflammatory bowel disease [52, 53].

It is currently a matter of debate whether the inflammation (or growth factor)-induced lymphatic vessel expansion might be reversible or not. After adenoviral delivery of VEGF-A to mouse ears, expanded and functionally impaired lymphatic vessels persisted for many months whereas angiogenic blood vessels regressed [54], and chronic airway inflammation-induced lymphatic hyperplasia persisted for an extended period of time even

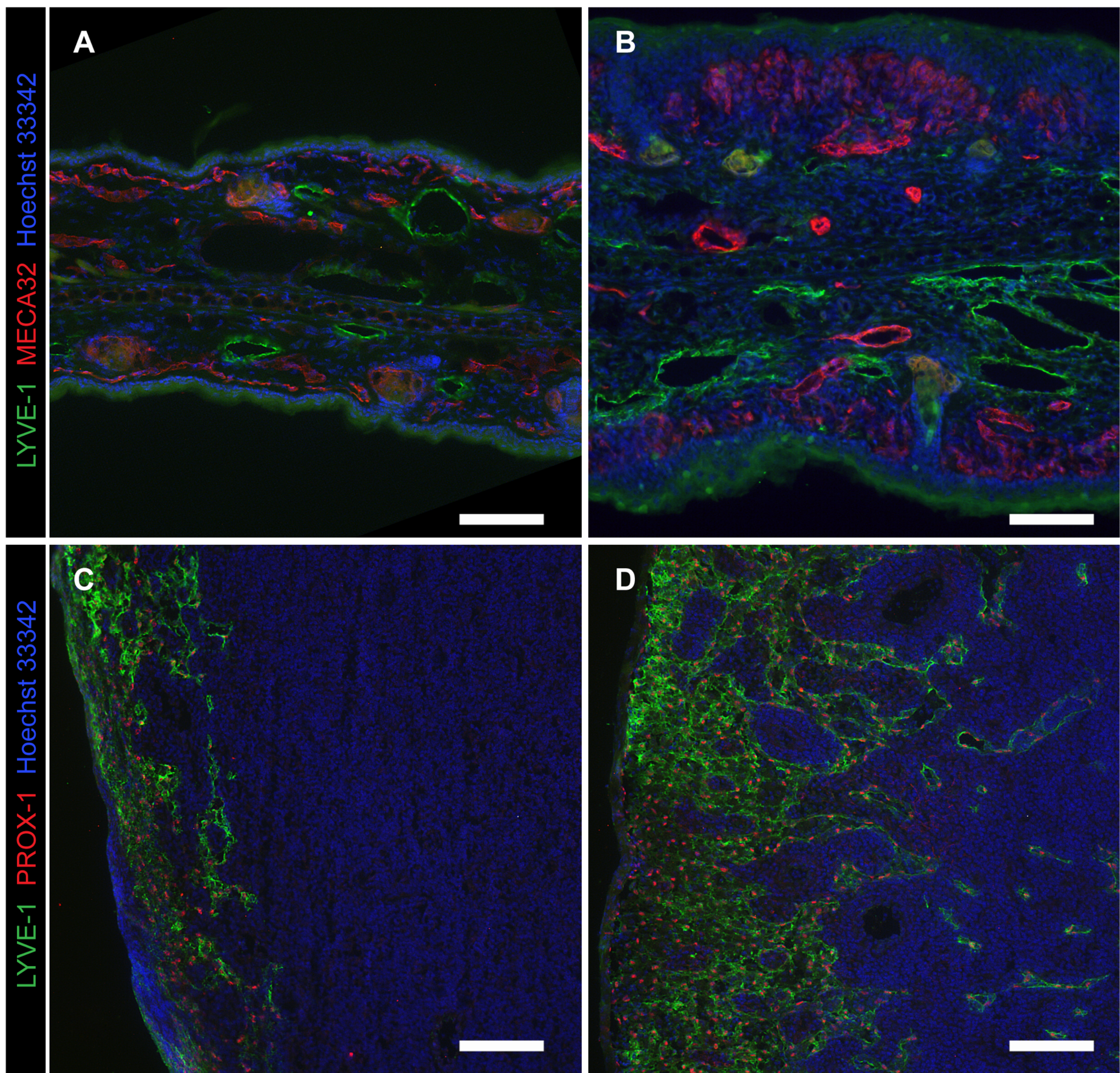

Fig. 1 Microscopic images of LYVE-1+ lymphatic vessels (*green*) and Meca32-positive blood vessels (*red*) in healthy mouse ear skin **a** and in chronically inflamed ear skin of the K14-VEGF-A mouse psoriasis model **b**. Note the tissue swelling and the enlargement and of lymphatic vessels in inflammation. **c** Lymphatic vasculature stained for LYVE-1 (*green*) and Prox-1 (*red*) in the auricular lymph node of a healthy mouse. **d** Expansion of the lymphatic vascular network in the auricular lymph node at day 7 after induction of ear skin inflammation. Nuclei are stained in *blue*. *Bars* = 100 µm. (Color figure online)

after inflammatory resolution [41]. In contrast, inflammation-induced lymphatic expansion regressed after several months in an experimental skin inflammation model [55] and in suture-induced inflammation in the mouse cornea where lymphatic vessels expanded faster, however, upon re-challenge [56]. Clearly, further research is warranted to investigate the dynamics of lymphatic networks expansion in different types of inflammation.

Lymph node lymphangiogenesis

In 2005, studies from our laboratory revealed that solid tumors can induce lymphangiogenesis within their draining lymph nodes ("lymph node lymphangiogenesis"), even before they actually metastasize to these lymph nodes [57]. Shortly thereafter, it became clear that lymph node (LN) lymphangiogenesis also occurs in those lymph nodes that

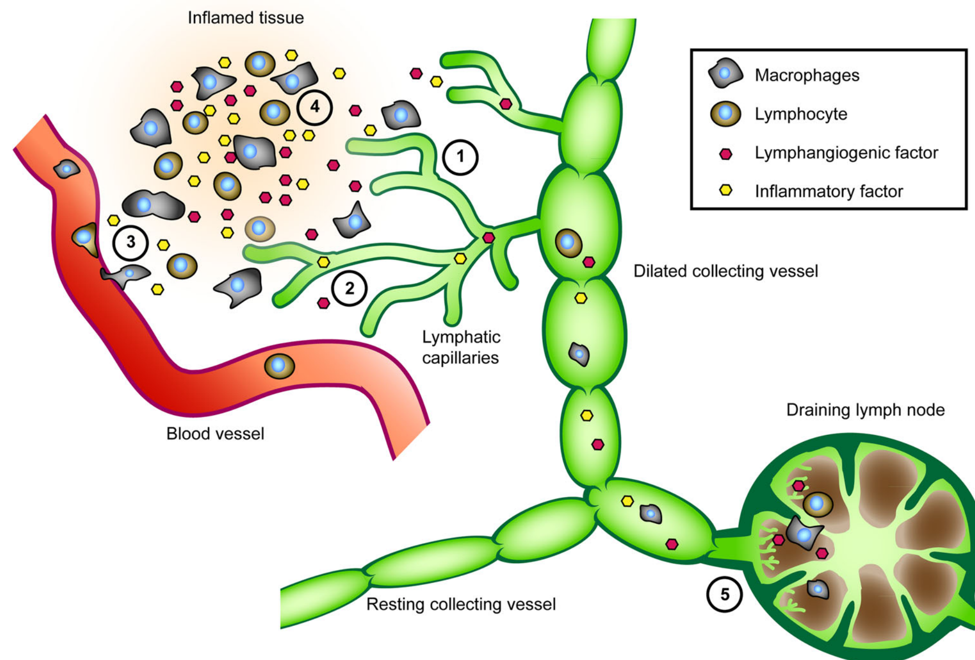

Fig. 2 Schematic overview of the lymphangiogenic process during inflammation. Lymphangiogenic factors produced in the inflamed stroma directly act on local lymphatic vessels and induce sprouting and vessel dilation *1*. Inflammatory factors may directly stimulate lymphatic vessel expansion *2*. Inflammatory factors also act on local blood vessels and mediate the recruitment of macrophages which express lymphangiogenic growth factors such as VEGF-C *3*. Inflammatory factors may directly induce transcription of lymphangiogenic factors in immune cells and other stromal cells in the inflamed tissue *4*. Lymph node lymphangiogenesis is induced by factors drained from the site of inflammation and also by factors produced locally by lymph node resident cells *5*

drain inflammatory sites, in particular inflamed skin [58, 59] (Fig. 1 C, D). This lymphatic expansion may be mediated by VEGF family members and other lymphangiogenic factors released by cells within the LN, such as B cells [58] or macrophages [60], as well as by factors released at the site of inflammation and subsequently drained to the LN [59]. The relative contribution of locally produced versus peripherally produced mediators towards the expansion of lymphatic vessels within LNs appears to depend on the experimental model. It is of interest that interferon-gamma producing T lymphocytes have been found to inhibit LN lymphangiogenesis [61] and that inflammatory LN lymphangiogenesis was reversible in a skin inflammation model [55].

Mediators of inflammatory lymphatic vessel expansion

VEGF-C represents the best characterized inducer of inflammatory lymphangiogenesis, acting via binding to VEGFR-3 and neuropilin-2 [9] and—after proteolytic cleavage—to VEGFR-2 that is also expressed by lymphatic endothelial cells [62]. Whereas the effects of the lymphangiogenic factor VEGF-D in inflammation are at present less well studied, VEGF-A has emerged as a major activator of inflammatory lymphatic vessel expansion within the peripheral inflamed tissue as well as within the draining lymph nodes [47, 54, 58, 59, 62, 63]. These effects are likely mediated both directly, via activation of VEGFR-2 on LECs, and indirectly via recruitment of inflammatory cells and activation of VEGF-C production by blood vessels. Macrophages appear to represent the major source of VEGFs in inflammation [60], but other inflammatory and resident cells likely contribute as well [58]. The major stimuli of VEGF production in inflammation are inflammatory mediators and hypoxic tissue conditions [64].

Several other growth factors have been implicated in the mediation of lymphangiogenesis, including angiopoietins 1 and 2 [65, 66], fibroblast growth factors [67, 68], hepatocyte growth factor [69], platelet-derived growth factors [70], and insulin-like growth factors [71]. Their relative contribution to inflammatory lymphatic vessel expansion remains at present unclear (Fig. 2).

There is increasing evidence that macrophages play a major role in lymphangiogenesis in general, and in particular in inflammatory lymphangiogenesis, via secretion of lymphangiogenic factors [60, 72, 73]. Thus, a large number of mediators that recruit macrophages/myeloid-derived cells to inflamed tissues have been implicated in the indirect promotion of lymphatic vessel expansion including IL-

1β [74, 75], IL-4 [51], and CSF-1 [76]. In addition, inflammatory mediators were found to directly stimulate the production of VEGF-C by resident cells such as synoviocytes [77]. Some inflammatory factors likely stimulate LECs directly. However, due to the pleiotropic nature of these mediators, these effects are difficult to investigate in vivo. Consequently, direct effects of inflammatory factors on lymphangiogenesis have mostly been described in vitro, using proliferation, migration, or tube formation assays with cultured LECs. Such assays cannot reproduce the whole complexity and cellular interactions during inflammatory lymphangiogenesis in vivo, and it is therefore not surprising that results obtained in vitro do not always coincide with observations made in vivo. One example is IL-1β, which has been found to stimulate lymphangiogenesis in vivo but to inhibit LEC tube formation in vitro [74, 78]. A detailed list of inflammatory cytokines and chemokines and their known activities on lymphatic endothelial cells in vitro and lymphangiogenesis in vivo is provided in Table 1.

Lymphatic drainage function in inflammation

It has been a topic of great dispute whether and how the lymphatic drainage function is affected in acute and chronic inflammatory conditions. Recently developed in vivo imaging techniques and newly developed dyes and tracers have helped to investigate this issue. There is strong evidence that inflammation-draining lymphatic vessels are hyperpermeable and drain less well, as found in a mouse psoriasis model with transgenic VEGF-A overexpression in the skin [47] and after acute or chronic UVB irradiation of the skin [79]. Whereas LPS (lipopolysaccharide)-induced peritonitis was associated with impaired lymphatic drainage in the diaphragm [73], acute skin inflammation induced by LPS was associated with increased lymphatic flow [60]. In the K/BxN mouse model of arthritis, which is dependent on a T cell mediated immune response against an autoantigen, an initial increase in lymphatic flow during the acute phase was followed by an increase in lymphangiogenesis but a decrease in lymphatic flow during the chronic phase [80]. Similarly, in the TNF-α transgenic model, in which arthritis develops independently of a specific immune response, lymphatic drainage from the inflamed paws was reported to be reduced in the chronic disease phase of the disease [81]. However, the same group reported increased drainage from popliteal lymph nodes in the same model [82]. Thus, there is a need for the standardization and quantification of methods applied for measuring lymph flow in vivo.

VEGF-A appears to be a mediator of the lymphatic dysfunction in inflammation. VEGF-A expression is consistently increased in inflammatory dieseases, and adenoviral delivery of VEGF-A to the skin resulted in enlarged, functionally abnormal LVs with delayed lymphatic clearance [54]. K14-VEGF-A transgenic mice, which over-express VEGF-A in the skin, develop a psoriasis-like inflammation with enlarged and leaky LVs [47, 83]. In UVB-irradiation studies, VEGF-A overexpression resulted in increased skin inflammation and lymphatic dysfunction, whereas blockade of VEGF-A signaling prevented UVB-induced LV enlargement and hyperpermeability, edema formation and inflammation [79, 84]. Our own recent studies indicate that lymphatic vessel drainage function might be less affected during acute skin inflammation but becomes severly impaired under chronic inflammatory conditions [44]. Overall, the increased blood vascular permeability in chronically inflamed tissues, often mediated by VEGF-A, appears to be associated with decreased lymphatic fluid drainage, often also mediated by VEGF-A, promoting edema formation and reduced drainage of inflammatory mediators from the infamed site.

Activation of lymphatic vessel function inhibits inflammation

It has been a matter of debate whether lymphatic vessels contribute to inflammatory processes—via transport of immune cells towards the lymph nodes and secretion of inflammatory chemokines—or whether they might contribute to inflammation resolution—via drainage of inflammatory cells, mediators and fluids away from the inflamed tissue. Our laboratory has recently found that activation of lymphatic vessels via transgenic overexpression of VEGF-C or VEGF-D in the skin leads not only to an expanded lymphatic network with enhanced fluid drainage, but also to a potent inhibition of acute and chronic skin inflammation [44, 63]. In accordance with these findings, viral delivery of VEGF-C increased lymphangiogenesis and lymphatic flow, and also reduced the severity of joint lesions in a model of chronic inflammatory arthritis [81]. Importantly, intradermal injection of the VEGFR-3 specific mutant VEGF-C156S inhibited UVB irradiation-induced lymphatic impairment, edema formation and inflammation in the skin [85], indicating potential therapeutic applications. In agreement with these therapeutic results, blockade of VEGFR-3 resulted in functionally impaired LVs with decreased drainage, enhanced edema, increased inflammatory cell infiltration and prolonged inflammation in models of UVB irradiation-induced skin inflammation, psoriasis, chronic airway inflammation, chronic inflammatory arthritis and bacterial pathogen-induced acute inflammation [41, 60, 63, 82, 86], as well as in prolonged inflammation in experimental inflammatory

Table 1 Pro-inflammatory factors involved in lymphangiogenesis and lymphatic hyperplasia

Factor	Model	Effect	Mode of action	References
TLR ligands				
LPS	Intraperitoneal/intradermal injection	Lymphangiogenesis (+)	Recruitment of macrophages	[60, 72, 73]
TNF family				
TNF-α	Chronic *M.pulmonis* infection	Lymphangiogenesis (+)	Recruitment of inflammatory cells, induction of VEGF-C	[115]
	In vitro stimulation of primary synoviocytes	Lymphangiogenic factors (+)	Induction of VEGF-C in synoviocytes	[77]
	In vitro LEC tube formation	Tube formation (−)	Direct effect	[78]
Interleukins				
IL-1β	IL-1β over-expression in trachea	Lymphangiogenesis (+)	Recruitment of macrophages	[74]
	Corneal lymphangiogenesis	Lymphangiogenesis (+)	Recruitment of macrophages	[75]
	In vitro LEC tube formation	Tube formation (−)	Direct effect	[78]
IL-4	IL-4 driven atopic dermatitis	Lymphangiogenesis (+)	Recruitment of macrophages	[51]
IL-6	In vitro stimulation of OSCC cells	Lymphangiogenic factors (+)	Induction of VEGF-C in OSCC cells	[112]
	In vitro LEC tube formation	Tube formation (+)	Direct effect	[116]
IL-17	In vitro stimulation of NSCLC cells	Lymphangiogenic factors (+)	Induction of VEGF-C in NSCLC cells	[117]
	Corneal lymphangiogenesis	Lymphangiogenesis (+)	Induction of VEGF-C and –D in the cornea	[118]
Colony stimulating factors				
CSF-1 (M-CSF)	Tumor lymphangiogenesis	Lymphangiogenesis (+)	Recruitment of macrophages	[76]
CSF-2 (GM-CSF)	In vitro LEC tube formation	Tube formation (+)	Direct effect	[116]
Interferons				
INF-γ	LPS/ConA induced skin inflammation In vitro LEC sprouting and	Lymphangiogenesis (−) Sprouting (−)	Direct effect	[61]
	tube formation	Tube formation (−)	Direct effect	[78]
Chemokines				
CCL19	Diffuse alveolar damage	Lymphangiogenesis (+)	Recruitment of macrophages	[119]
CXCL8 (IL-8)	In vitro LEC tube formation	Tube formation (+)	Direct effect	[100]
	In vitro LEC tube formation, Matrigel plug assay, cornea micropocket assay	Lymphangiogenesis, tube formation (+)	Direct effect	[101]
CXCL12 (SDF-1)	In vitro LEC tube formation, matrigel plug assay	Lymphangiogenesis, tube formation (+)	Direct effect	[120]
Other factors				
Prostaglandin E2 (PGE2)	In vitro stimulation of lung adenocarcinoma cells	Lymphangiogenic factors (+)	Induction of VEGF-C in lung adenocarcinoma cells	[121]
	Matrigel plug assay	Lymphangiogenesis (+)	Induction of VEGF-C and -D	[122]
Nitric oxide (NO)	In vitro LEC tube formation, UVB induced inflammation	Tube formation (+), hyperplasia (+)	Direct effect	[123]
	In vitro LEC proliferation	Proliferation (+), hyperplasia (+)	Direct effect	[124]

OSCC oral squamous cell carcinoma, *NSCLC* non-small-cell lung cancer

bowel disease [87]. Overall, these studies clearly indicate that promotion of lymphatic drainage by an expanded functional lymphatic network might represent a new therapeutic strategy to treat inflammatory diseases.

Modulation of inflammation and immune responses by lymphatic vessels

In addition to the increased drainage function, activation of lymphatic vessels by VEGF-C and other mediators might also induce additional anti-inflammatory mechanisms [88]. While it is beyond the scope of this review to provide a detailed discussion of the various effects of LECs on the immune system, it is important to note that LECs are not only involved in dendritic cell (DC) and lymphocyte trafficking from the periphery to the lymph nodes, but also in regulating lymphocyte egress from lymph nodes, immune cell maturation and tolerance.

Upon inflammation, the chemokine-scavenging receptor D6, an essential regulator of inflammatory leukocyte interactions with LECs, is up-regulated on LECs. D6 binds all inflammatory CC-chemokines but not homeostatic chemokines such as CCL21, followed by internalization and degradation, which prevents inappropriate adhesion of inflammatory leukocytes and immature DCs to LECs [89]. D6 is also expressed by mucosal LECs where it controls intestinal inflammation [90]. Furthermore, LECs are the sole source of sphingosine-1-phosphate (S1P) in the LN [88], which is essential to release activated lymphocytes from LNs, while egress of naïve lymphocytes is transiently blocked during inflammation [91]. Importantly, during prolonged inflammation, VEGF-A-induced LN lymphangiogenesis was recently found to restore lymphocyte egress from LNs independently of their activation status [92].

LECs may also directly modulate the maturation of immune cells. Recent studies found that LN stromal cell populations, among them LECs, limit DC-induced proliferation of T cells to control the expansion of activated T cells within the LN [93, 94]. Moreover, LECs seem to suppress DC maturation and function in inflamed tissue [95]. This suppression was active only in the absence of pathogen-derived signals and might thus provide a relatively unspecific peripheral tolerance mechanism during inflammation.

Diverse LN stromal cell types, among them LECs, can act as antigen presenting cells. They express a characteristic set of peripheral tissue antigens, controlled by an unknown, autoimmune regulator (Aire)-independent mechanism [96]. LN LECs present peripheral tissue antigens on MHC-I, leading to deletion of self-reactive CD8 T cells [96, 97]. This LEC-mediated deletion of self-reactive T cells requires two interdependent pathways: a lack of costimulation and provision of the inhibitory molecule PD-L1. Lack of costimulation leads to an up-regulation of PD-1 on CD8 T cells, further enhancing inhibition via the PD-L1/PD-1 pathway. Confirming the importance of both pathways, peripheral tissue antigen presentation by LECs together with PD-L1 blockade or exogenous costimulation lead to the development of autoimmune disease [98]. In contrast to DCs, LECs up-regulate the inhibitory molecule PD-L1 upon TLR3 stimulation, suggesting a tolerogenic function of LECs even during exposure to danger signals [97].

Lymphatic vasculature as a new therapeutic target

Much progress has been made with regard to inhibiting tumor-associated lymphangiogenesis and metastasis by targeting the VEGF-C/VEGF-D/VEGFR-3 axis. The currently evaluated drugs include anti-VEGFR-3 antibodies, anti-VEGF-C or -D antibodies, as well as antibodies against neuropilin-2, and at least one of them, a monoclonal antibody against VEGFR-3 (IMC-3C5, ImClone Systems) has recently entered clinical testing [99]. In contrast, with regard to inflammatory diseases, lymphatic vessel function should most likely be therapeutically enhanced, based on the findings discussed above. While the direct intradermal injection of VEGF-C protein has shown efficacy in preclinical skin inflammation models [63, 85], this approach does not seem feasible for the treatment of human diseases. Similar restrictions might apply to the reported use of viral vectors to deliver VEGF-C [2], cell based therapies [100] and application of interleukin-8 [101], since sufficient site-specific delivery of these agents represents a major challenge, in addition to safety aspects.

It is of interest, however, that several established anti-inflammatory drugs have been recently found to also affect lymphangiogenesis. In particular, all-trans-retinoic acid was identified to play a major role during embryonic lymphatic development, and 9-cis retinoic acid was shown to promote lymphangiogenesis and lymphatic vessel regeneration in vivo [102, 103]. Given the clinical use of retinoids for the treatment of inflammatory diseases such as psoriasis and psoriatic arthritis, one might speculate that their pro-lymphangiogenic activity might contribute to the therapeutic effects in these diseases.

Most non-steroidal antiinflammatory drugs (NSAIDs) inhibit COX-1 and/or COX-2 enzymes which mediate the biosynthesis of prostaglandins including prostaglandin E2. Recently, it was found that prostaglandin E2 promotes lymphangiogenesis by up-regulation of VEGF-C and -D (Table 1) and that COX inhibitors reduce tumor-induced lymphangiogenesis [104] and secondary lymphedema [105]. Moreover, NSAIDs inhibited VEGF-D mediated

dilation of tumor draining collecting lymphatics [106]. At present, however, more research is needed to investigate how these findings might be related to anti-inflammatory efficacy. Similarly, glucocorticoid treatment reduced lymphangiogenesis in a model of chronic infection with *M. pulmonis* [107], in cornea inflammation [108, 109] and in tumor xenografts [110]. However, glucocorticoids might also have direct activating effects on lymphatic endothelial cells (our own unpublished results), and hydrocortisone is routinely added to cultures of LECs. Therefore, future studies are needed to directly investigate the effects of anti-inflammatory drugs on lymphatic vessel function, with a particular focus on fluid drainage and immune cell interactions.

Anakinra, a recombinant version of the endogeneous IL-1 scavenger IL-1Ra, and tocilizumab, an IL-6R blocking antibody, have also been found to affect lymphangiogenesis [111, 112]. Both drugs have been approved for the treatment of rheumatoid arthritis but their effects on lymphangiogenesis in inflamed joints need to be investigated. Importantly, the TNF-α neutralizing antibody infliximab has been reported to increase lymphangiogenesis in treated mice suffering from inflammatory arthritis [113]. Since infliximab is also used for other auto-immune diseases such as psoriasis and colitis, it will be of great interest to investigate whether infliximab treatment has similar effects in those conditions as well, and whether the anti-inflammatory activity of infliximab may be at least in part dependent on its pro-lymphangiogenic activity.

Outlook

The active role of lymphatic vessels in inflammation is well established today and lymphatic vessels have become the target for the development of new therapeutic strategies to treat inflammatory diseases. Thus, we have recently initiated a screening program, using a three-dimensional LEC sprouting assay as a read-out [114], to identify new activators of lymphatic vessel function. Using phenotypic screens of small molecule chemical libraries as well as natural extract libraries, we identified a natural extract and defined molecular compounds that promote lymphatic vessel drainage in vivo (unpublished data). It will be of great interest to investigate whether these compounds might also affect lymphatic vessels under inflammatory conditions. However, there are currently many unanswered questions with regard to the functional role of lymphatic vessels in inflammation. Can we specifically promote certain functions of lymphatic vessels such as fluid drainage or anti-inflammation, without major effects on lymphangiogenesis? Can we promote inflammatory lymphatic vessel pumping and reduce lymphatic hyperpermeability at the same time? Is the expansion of lymphatic vessels within inflamed tissues and their draining lymph nodes reversible in human diseases? What is the detailed functional role of lymph node lymphangiogenesis with regard to inflammation and tolerance? What are the effects of currently used anti-inflammatory drugs on inflammation-associated lymphangiogenesis and/or lymphatic drainage function? Thus, future studies are needed to better understand the molecular mediators and pathomechanisms regulating lymphatic vessel activation in inflammation.

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Conflict of interest The authors declare no competing financial interests.

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