Cell. Mol. Life Sci. (2012) 69:897–914
DOI 10.1007/s00018-011-0848-6

Cellular and Molecular Life Sciences

REVIEW

Macrophages are important mediators of either tumor- or inflammation-induced lymphangiogenesis

Rui-Cheng Ji

Received: 11 May 2011 / Revised: 26 July 2011 / Accepted: 22 September 2011 / Published online: 8 October 2011

Abstract The lymphatic system provides important functions for tissue fluid homeostasis and immune response. Lymphangiogenesis, the formation of new lymphatics, comprises a series of complex cellular events in vitro or in vivo, e.g., proliferation, differentiation, and sprouting. Recent evidence has implied that macrophages act as a direct structural contributor to lymphatic endothelial walls or secret VEGF-C/-D and VEGF-A to initiate lymphangiogenesis in inflamed or tumor tissues. Bone marrow-derived macrophages are versatile cells that express different functional programs in response to exposure to microenvironmental signals, and can be identified by specific expression of a number of proteins, F4/80, CD11b, and CD68. Several causative factors, e.g., NF-κB, IL-1β, TNF-α, SDF-1, M-CSF, especially TonEBP/VEGF-C signaling, may be actively involved in macrophage-induced lymphangiogenesis. Alteration of macrophage phenotype and function has a profound effect on the development and progression of inflammation and malignancy, and macrophage depletion for controlling lymphangiogenesis may provide a novel approach for prevention and treatment of lymphatic-associated diseases.

Keywords Macrophages · Lymphangiogenesis · Lymphatic endothelial cells · Tumor · Inflammation · VEGF-A/-C/-D · VEGFR-2/-3

R.-C. Ji (✉)
Department of Human Anatomy,
Oita University Faculty of Medicine, Oita 879-5593, Japan
e-mail: JI@oita-u.ac.jp

Abbreviations

BMDCs	Bone marrow-derived cells
CEACAM-1	Carcinoembryonic antigen-related cell adhesion molecule-1
CLEVER-1	Common lymphatic endothelial and vascular endothelial receptor-1
CXCR-4	CXC chemokine receptor-4
ECM	Extracellular matrix
eNOS	Endothelial nitric oxide synthase
HSD	High-salt diet
HSV-1	Herpes simplex virus-1
IFP	Interstitial fluid pressure
IL-1β	Interleukin-1β
LECs	Lymphatic endothelial cells
LPS	Lipopolysaccharide
LYVE-1	Lymphatic vascular endothelial hyaluronan receptor-1
M-CSF	Macrophage colony-stimulating factor
MMP	Matrix metalloproteinase
MMR	Macrophage mannose receptor
MPS	Mononuclear phagocyte system
NF-κB	Nuclear factor-κB
Prox-1	Prospero-related homeobox-1
VEGF-A/-C/-D	Vascular endothelial growth factor-A/-C/-D
VEGFR-2/-3	Vascular endothelial growth factor receptor-2/-3
SDF-1	Stromal cell-derived factor-1
TAMs	Tumor-associated macrophages
TGF-β	Transforming growth factor-β
TLR-4	Toll-like receptor-4
TNF-α	Tumor necrosis factor-α
TonEBP	Tonicity-responsive enhancer binding protein

Introduction

The lymphatic system orchestrates the trafficking and recirculation of immune cells including lymphocytes and antigen-presenting cells like macrophages to the regional lymph node. Macrophages within different tissues as critical cells of the innate immune response, contribute to immediate and robust defense against microbial infections and to regulation of normal cell turnover and tissue remodeling [1, 2]. Macrophage-induced lymphangiogenesis may be explained in two ways. First, macrophages and/or other bone marrow-derived cells (BMDCs) are capable of transdifferentiating or incorporating into an endothelial phenotype, thereby making a direct structural contribution to the lymphatic wall [3, 4]. Second, activated macrophages produce lymphangiogenic factors, VEGF-C/-D and VEGF-A to stimulate division of preexisting lymphatic endothelial cells (LECs) or to further influence macrophage recruitment [5–8] (Fig. 1). In human diseases and animal models, tumor- or inflammation-induced lymphangiogenesis is greatly influenced by stromal cells, and mainly dependent on macrophage recruitment and activation [9–11]. LECs may, additionally, undergo similar cellular processes like proliferation, differentiation, and formation of new networks. In the past decade, lymphangiogenesis including its molecular and functional characteristics, and macrophages including their alternative activation in tumor and inflammation have been reviewed separately [9, 12–15]. The role of macrophages in the formation of new initial lymphatics remains to be elucidated.

Recently, much effort has been made to develop new agents targeting the activity of VEGF/VEGFR signaling pathways. Some studies have shown new evidence on macrophage-induced lymphangiogenesis, especially in the experiments of high-salt diet (HSD) and herpes simplex virus-1 (HSV-1) infection [16, 17]. However, a wide spectrum of factors may influence potential sites of anti-lymphangiogenic intervention. This review is to highlight the functional importance of macrophages as a source of VEGFs or as a direct lymphatic contributor in tumor- or inflammation-induced lymphangiogenesis. The analysis of the events leading to macrophage-induced lymphangiogenesis may provide new understanding of therapeutic options for lymphatic-associated diseases.

Biological features of macrophages in lymphangiogenesis

Activation and function of macrophages

Macrophages are released from the bone marrow as immature monocytes and circulate in the blood before extravasation across the endothelial barrier into the target tissue, where they differentiate into resident macrophages. Therefore, macrophages and monocytes belong to the myeloid lineage of leukocytes and, as such, originate from the same progenitor cells in the bone marrow as neutrophils, mast cells, and eosinophils [18]. Macrophages show high plasticity in cell lineage and acquire several functionally distinct phenotypes in response to factors present in the local tissue microenvironment [2, 19]. The plasticity of macrophages reflects their capability to switch from one lineage to another or to a mixed phenotype. Clearly, macrophages are multifunctional cells, capable of influencing a wide range of physiological and pathophysiological processes. In response to injury or inflammatory stimuli, monocytes are recruited from the bloodstream to the diseased tissues or malignant lesions, and can process antigen, migrate to lymphoid organs via lymphatics and differentiate into antigen presenting cells to participate in an effective immune response. The activation status of macrophages in tumor tissues may be modulated by local signals within the stromal microenvironment, like tumor necrosis factor (TNF)-α and hypoxia [20], but it is closely associated with tumor type and development stage. Thus, macrophages are found in every tissue of the body and, depending on the local microenvironment, acquire specialized functions including phagocytosis, antigen presentation, tissue remodeling, and secretion of a wide range of growth factors and cytokines [21].

On the basis of their secretory repertoire, receptor expression patterns and phenotypical criteria, macrophages are mainly divided into two subsets, classically activated and alternatively activated. Both activated macrophages can change their cellular morphology and secretory pattern as a result of appropriate stimulation. The subpopulations of activated macrophages possess different physiologies and perform distinct immunological functions [22]. Classically activated macrophages are monocytes stimulated with interferon (IFN)-γ, lipopolysaccharide (LPS), or in combination with interleukin (IL)-12 and IL-23, which differentiate into M1 macrophages. The subset of cells releases proteolytic enzymes including matrix metalloproteinase (MMP)-1, -2, -7, -9, and -12, which can degrade extracellular matrix (ECM) components like collagen, elastin and fibronectin [23, 24], and secrete nitric oxide and TNF-α [25]. M1 macrophages express high levels of pro-inflammatory cytokines and major histocompatibility complex molecules, capable of killing intracellular pathogens and tumor cells. They can induce the differentiation of T-helper (Th) 1 type responses or work as effector cells in Th1 cellular immune responses [15, 26]. Alternatively activated macrophages (M2 phenotype) are stimulated by Th2 cytokines, such as IL-4, IL-10, and IL-13. M2 macrophages actively contribute to the inflammatory response,

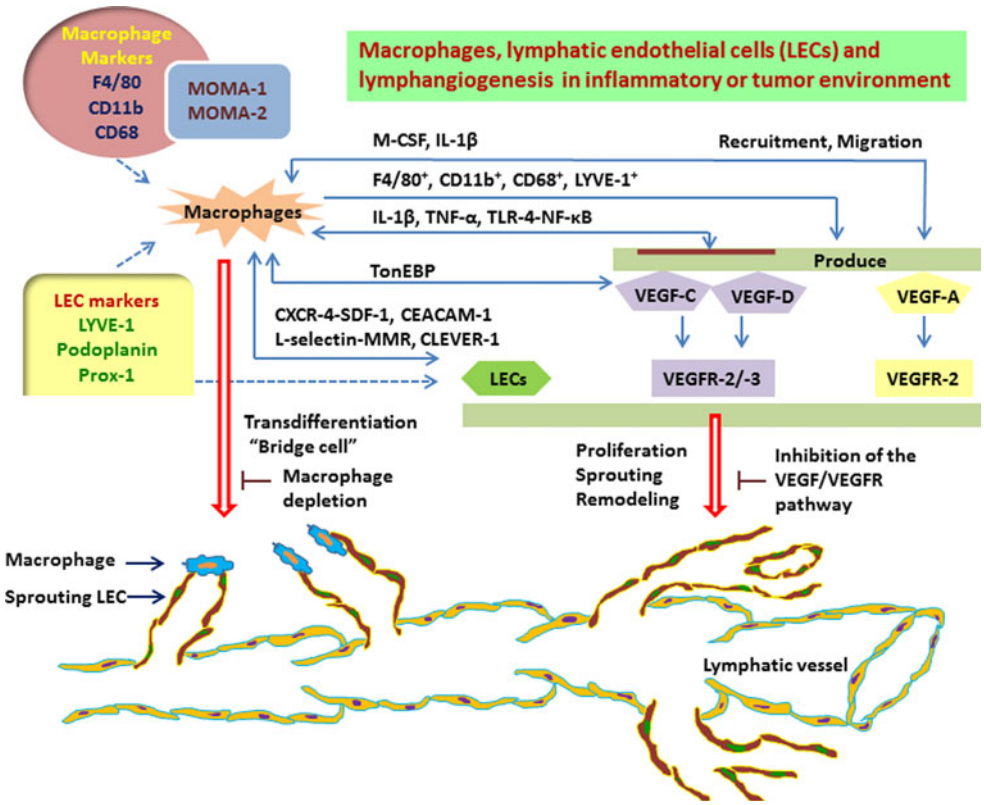

Fig. 1 The schema shows macrophage-induced lymphangiogenesis. Macrophages are capable of transdifferentiating into an endothelial phenotype, thereby making a direct structural contribution to the lymphatic wall. Moreover, macrophages can produce lymphangiogenic factors, VEGF-C/-D and VEGF-A to stimulate proliferation, sprouting and remodeling of preexisting lymphatics or to further influence macrophage recruitment

tumor growth, and stroma formation, and to the clearance of cell debris through producing several components involved in the synthesis of ECM [27, 28]. The molecules secreted by M2 macrophages have shown proliferative, angiogenic and antiinflammatory activities. The M2 macrophage phenotype can also produce factors that suppress T-cells proliferation and activity [29].

Both M1 and M2 phenotypes are important components of the innate and adaptive immune systems, the two subsets have their own biological features. The former macrophage is devoted to elicit chronic inflammation and tissue injury, whereas the latter tends to resolve inflammation and facilitate wound healing [26, 30]. The subdivision of M2 macrophages into M2a, M2b, and M2c subgroups according to their inducing stimuli may further explain the plasticity of macrophage phenotypes [28, 31]. Macrophages are known to express several VEGFRs, including constitutive expression of VEGFR-1/-3, and inducible expression of VEGFR-2. All of these VEGFRs are implicated in macrophage recruitment during inflammation [32, 33]. VEGFR-3 expressed by some BMDCs, e.g., macrophages and monocytes [34] has been shown to regulate adaptive immunity via mediating chemotaxis of antigen-presenting cells [32]. The macrophage function mediated by the factors like VEGF-A/-C/-D, interleukin-1β (IL-1β), and TNF-α, has indicated a possible link between LEC structures and macrophage differentiation. However, it remains to be validated whether the two macrophage subsets can cooperate with LECs in tumor- or inflammation-induced lymphangiogenesis.

It should be emphasized that the proliferative and patterning signals driving embryonic and pathological lymphangiogenesis are likely to be distinct. In $PU.1^{-/-}$ and $Csf1r^{-/-}$ mouse embryos, macrophages do not constitute the major source of prolymphangiogenic factors, including VEGF-C/-D for influencing dermal lymphatic sprouting, but instead regulate lymphatic caliber or patterning during embryonic development [35]. In addition, the cytoplasmic tyrosine kinase Syk-expressing myeloid population, which is largely comprised of M2-polarized monocytes, can stimulate lymphangiogenesis in vivo through secreting chemokines and growth factors. In $Syk^{-/-}$ embryos, the deregulated myeloid cells cause a lymphatic hyperplasia, leading to the formation of blood-lymphatic endothelial junctions or blood-lymphatic shunts [36]. Therefore, it is also interesting to know whether embryonic macrophages are tissue-specifically involved in morphogenetic or remodeling events, and what kind of physiological functions are being performed by these cells during embryonic lymphangiogenesis.

Macrophage mannose receptor (MMR) and LECs

MMR (also known as CD206), a 180-kDa transmembrane protein, is one of the best-characterized mannose-binding

lectins, participating in pathogen recognition, antigen presentation, and clearance of microorganisms and glycoproteins [37]. It is restricted to normal LECs including afferent and efferent lymphatics, and lymph node sinuses, but not to vascular endothelial cells [38, 39]. MMR is a ligand for L-selectin to mediate adhesion of lymphocytes to the lymphatics, suggesting that it may be involved in leukocyte trafficking. In human breast carcinomas, the intratumoral MMR expression is associated with the frequency of regional lymph node metastases via mediating entrance of tumor cells to lymphatics [40]. The uptake of phosphatidylserine-presenting liposomes by LPS-activated $F4/80^{+}$ macrophages upregulates the antiinflammatory phenotype and improves myocardial infarction repair, as reflected by enhanced expression of MMR and elevated secretion levels of transforming growth factor-β (TGF-β) and IL-10 [41]. In MMR-deficient mice, the adhesion of both normal lymphocytes and tumor cells to lymphatics is significantly decreased. The absence of MMR impairs trafficking of $CD4^{+}$, $CD8^{+}$ cells and B cells into the draining lymph nodes, but does not obviously affect lymphatic morphology or phenotype [37].

Similar to MMR expression, common lymphatic endothelial and vascular endothelial receptor 1 (CLEVER-1, also known as stabilin-1), an inducible vascular adhesion molecule, is present on lymphatic and sinusoidal endothelium as well as on a subset of M2 macrophages [42]. As a scavenger receptor, CLEVER-1 is implicated in binding of lymphocytes to LECs and high endothelial venules in the lymph nodes during inflammation and tumor metastasis [40, 43]. In this respect, both CLEVER-1 and MMR may be important mediators in cancer cell adhesion to LECs. The migration of lymphocytes and dendritic cells via the lymphatics is essential for controlling the nature and magnitude of the immune response [44]. However, the precise mechanisms of leukocyte migration from peripheral tissues into afferent lymphatics and from lymphoid organs into efferent lymphatics are not fully elucidated. Blockage of these molecules for selectively reducing leukocyte migration may provide a new approach to controlling tumor metastasis and inflammation by targeting LEC function.

The LECs, although initially envisioned as a passive, inert lymphatic lining, are now considered important in the regulation of cellular growth, differentiation, and inflammatory responses. Lymphangiogenesis is a complex, multistep process that involves expression of growth factors, ECM remodeling, endothelial cell migration, proliferation, and tube formation. Current knowledge shows that lymphangiogenesis is generally secondary to angiogenesis, but occurs independently of blood vessels, which may indicate their similarity and distinction in molecular and cellular biology. Definitely, the lymphatics have several features from blood vessels [45]. Thin-walled initial lymphatics are composed of a single layer of LECs, which are intercellularly connected by end-to-end, interdigitating, and overlapping junctions [46]. The blind-ended initial lymphatics express specific endothelial markers, responding to different growth factors, especially VEGF-C/-D, via activation of VEGFR-3. Unlike blood vessels, the lymphatics lack a basement membrane and are not surrounded by pericytes. The lymphatic endothelial layer is permeable and particularly suited for reabsorption of macromolecules. LECs with rich micropinocytotic vesicles are not only responsible for the recruitment of leucocytes into the lymphatics, but are also important in tumor cell metastasis. The pathomorphological changes in abnormal lymphatics mainly represent the size and density, and the number of sprouts, branches, and anastomoses. The dynamic alteration in dysfunctional lymphatics is usually shown in endothelial permeability, fluid absorption ability, valve movement, and lymph backflow. LEC alteration in intercellular junctions and molecular expressions, especially adhesion molecules, may provide potential morphological and functional basis for inflammatory process and tumor metastasis [47]. LECs in tumor and inflammatory tissues may express different surface markers than do "quiescent" steady state lymphatics [48]. LECs usually express three master control genes, Notch, COUP transcription factor 2, and prospero-related homeobox-1 (*Prox-1*), and their regulatory equilibrium in response to physiological and pathological stimuli may determine arteriovenous-lymphatic cell fate specification and LEC plasticity or reprogramming [49]. A process referred to as anastomosis depends on spatiotemporal relationship between macrophages and sprouting endothelial vessels. Macrophages serve both as 'bridge cells' and as guidance posts to mediate the specialized tip cells sent from endothelial cells at the sprouting forefront, to recognize and fuse with other tip cells [50, 51]. These tissue-resident macrophages located in the vicinity of vessel branches are polarized towards the M2 type [52]. They are proposed to release signals that alter the differentiation of endothelial cells and to rely on the ECM and cell-adhesion molecules on the cell surface including the fusing cells [51]. The preparation for fusion process is independent of VEGF that only induces tip-cell formation. Ideally, macrophages with their great mobility and flexibility, and affinity for tip-cell filopodia, appear suitable to help endothelial cells on different vessel segments to establish contact [50].

In this context, research efforts have been made to explore whether LECs are derived from circulating progenitors by transdifferentiation or from local preexisting lymphatics by cell division in adult lymphangiogenesis. Some researchers have indicated that macrophages contribute to lymphangiogenesis by processes other than the paracrine secretion of growth factors. LECs may be derived

through incorporation of circulating BMDCs and macrophage subsets [53–55]. To date, proliferation of preexisting lymphatics mainly accounts for postnatal lymphangiogenesis, there is still lacking direct experimental evidence demonstrating the adhesive interactions between the two kinds of cells, BMDCs or macrophages, and LECs. More investigation is necessary to elucidate the relative contribution of proliferation versus progenitor cell incorporation, and the subsequent morphological evolution of lymphatic formation and remodeling in different normal and pathological tissues. It would be interesting to know whether adhesion molecules mediate integration of activated macrophages into lymphatic endothelial walls, and whether the extremely low pressure in lymphatics favors the intercellular connection between macrophages and LECs. Also, the tumor itself may produce lymphangiogenic factors, which in turn modulate LEC dynamic structures and interactions with tumor cells.

Markers of prolymphangiogenic macrophages

For studying lymphangiogenesis, the specific markers for macrophage identification, F4/80, CD11b, and CD68 are frequently used to differentiate from LECs (Fig. 1). F4/80 is expressed in a wide range of mature tissue macrophages including Langerhans, Kupffer cells, microglia, macrophages located in the peritoneal cavity, lung, thymus and bone marrow stroma [12], suggesting that the expression is heterogeneous and varies during macrophage maturation and activation. F4/80-expressing macrophages with high phagocytic activity are transmigrated via local lymphatics into draining lymph nodes [56], to participate in destroying disease-producing microorganisms and removing dead tissue and other cellular debris [57]. CD11b is implicated in various adhesive interactions of myeloid cells (macrophages, monocytes, and granulocytes). CD11b monoclonal antibodies are known to modify many functions of myeloid cells. Inhibition of CD11b enhances tumor response to radiation by reducing myeloid cell recruitment [58]. CD68, the human homologue of macrosialin, is a member of the lysosomal-associated membrane protein family, and commonly regarded as a monocyte/macrophage marker [59]. Moreover, MOMA-1 (CD169) antibody against mouse metallophilic macrophage is immunohistochemically used to identify a clonogenic bone marrow progenitor specific for macrophages and dendritic cells [60]. MOMA-2 antibody is a marker for the broad detection of monocytes and macrophages in all mouse strains [61]. Other cell surface markers are for granulocytes (Gr-1), dendritic cells (CD11c) and B-lymphocytes (B220, CD45R).

Several endothelial markers are expressed by macrophages in tumor and inflammatory tissues. CD31 (platelet endothelial cell adhesion molecule-1, PECAM-1), a transmembrane glycoprotein is expressed by endothelial cells, and also expressed in certain tumors, including some vascular tumors [1, 62]. CD31 and macrophages play a key role in tissue regeneration, safely removing neutrophils, and tumor cell metastasis. The functionally important receptor in macrophage biology determines which cells will be phagocytosed through homophilic adhesion [63]. The lymphatic vascular endothelial hyaluronan receptor-1 (LYVE-1) with reproducible and highly sensitive staining characteristics, is widely used as a molecular marker for adult and embryonic LECs. LYVE-1 is also expressed in a considerable number of typical F4/80$^+$, CD11b$^+$ murine macrophages in tumor stroma and granulation tissue as well as in vitro [64, 65]. In the diabetic process, the occurrence of LYVE-1-expressing lymphatic compartments has been indicated to play a significant role in defective thymocyte differentiation and migration [62]. Identification of LYVE-1$^+$ macrophages has provided evidence supporting the possibility of macrophage coordination into LECs for lymphatic formation. The macrophage subpopulations expressed with LEC markers, *Prox-1,* LYVE-1 and podoplanin, are essential for lymphangiogenesis in different pathological processes, e.g., acute inflammation, wound healing and tumor metastasis.

The role of macrophages in tumor- and inflammation-induced lymphangiogenesis (Tables 1, 2)

VEGF-C/-D/VEGFR-3 signaling pathway is a crucial regulator of macrophage-induced lymphangiogenesis

Inflammatory and immune cells constitute the major cellular compartment of inflammation stroma, where intercellular communication is mediated through secretion of growth factors, chemokines, proteases, and other ECM components. Moreover, inflammatory molecules and cells are recognized as important contributors to lymphangiogenesis [13, 66]. Macrophages are a key component of inflammatory infiltrates of primary and secondary tumor tissues. The mechanisms underlying the recruitment and function of the tumor-associated macrophages (TAMs) have been an area of intense research over the past decade. Accumulating evidence in different types of human cancers and animal tumor models has indicated that TAMs receive signals from diverse cells within the tumor microenvironment in inducing angiogenesis, lymphangiogenesis, suppressing antitumor immunity, and in facilitating early events of the metastatic cascade [47, 67–71]. Pathological lymphangiogenesis is mainly triggered by growth factors such as VEGF-A and VEGF-C/-D, the members of VEGF family, through binding

to their receptors VEGFR-2 and VEGFR-3 [8, 72, 73] (Fig. 1). VEGF-C and -D have a similar domain structure, both undergo proteolytic processing in a similar manner and, in humans, both share the same receptor-binding specificity [74]. These secreted glycoproteins mainly signal via the tyrosine kinase receptor VEGFR-3 on the LEC surface, and result in lymphatic growth in vitro and in vivo [52, 75]. Macrophages are also a rich source of VEGF-A. Despite its initial recognition as a potent factor inducing and amplifying angiogenic response, VEGF-A has been shown to promote lymphangiogenesis, acting both indirectly via the recruitment of VEGF-C/-D-producing inflammatory cells and directly via VEGFR-2 [76, 77]. TAMs are mainly concentrated in the interphase between the tumor and normal tissue, and thus tumor-induced lymphangiogenesis is closely related with peritumoral inflammation. LYVE-1^+ macrophages and LECs are adjacent at the invasive edge of a metastatic melanoma with severe inflammation, and the peritumoral mononuclear inflammatory cells also express VEGF-C [5]. This suggests that the inflammation reaction and VEGF-C expressing TAMs are involved in the onset and maintenance of tumor lymphangiogenesis, phagocytosis of tumor cell debris, and immune complexes containing tumor-associated antigens [78]. Furthermore, some cytokines and chemokines are clearly implicated in the recruitment of TAMs, including macrophage colony-stimulating factor-1 (M-CSF, also known as CSF-1). Circulating monocytes are shown to be recruited by tumor-derived chemotactic factors into the tumor tissues, and differentiated into macrophages, where they exhibit a distinct phenotype similar to alternatively activated macrophages, and switch on *de novo* synthesis of VEGF-C/-D [69, 79].

Macrophages play a critical role in the emergence and resolution of inflammation and in the maintenance of tissue homeostasis through remodeling and repair. These cells display a high degree of heterogeneity and can adapt or alter their phenotype to suit the microenvironment in which they reside [18]. In human diffuse alveolar damage and onchocerca nodule, $CD68^+$/VEGF-C^+ or $CD68^+$/LYVE-1^+ macrophages are aggregated around newly formed lymphatics or even colocalized with LECs in the proliferative stage [80, 81]. In murine chronic respiratory tract and peritoneal infection models, abundant macrophage infiltration is closely associated with upregulated expression of VEGF-C/-D and increased vessel density [10, 82]. Under the circumstances, lymphangiogenesis may provide effective conduits for removal of excess interstitial fluid and leaked proteins derived from blood vessels. Impaired lymphangiogenesis combined with inflammatory fibrosis may interfere with interstitial clearance and lead to sluggish lymph flow, and even lymphedema [14, 83]. However, it still remains unsolved whether the lymphangiogenesis is the sole determinant to compensate for increased leakage.

The interplay between macrophages and lymphangiogenic regulators reflects complex changes in inflammation and tumor microenvironment

Proinflammatory cytokine-induced activation of macrophages reciprocally interacts with VEGF-C/-D/VEGFR-3 or VEGF-A/VEGFR-2 signaling. The activity of IL-1β is mediated by upregulation of VEGF-C/-D and VEGF-A, together with macrophage recruitment in response to inflammatory stimuli [84]. IL-1β and TNF-α can activate the transcription factor nuclear factor-κB (NF-κB) by inducing phosphorylation, ubiquitination, and subsequent degradation of IκB by the proteasome pathway [85]. NF-κB is a master mediator of many cellular processes to regulate various pathways that impact on the function of TAMs. NF-κB-induced expression of inflammatory cytokines, e.g., IL-6, by macrophages may contribute to tumor cell survival and proliferation in inflammation-linked tumor onset, but modulation of NF-κB activation in TAMs in established tumors maintains their immunosuppressive and tumor-promoting phenotype [31]. Local tissue inflammation may cause lymph nodes to undergo a transient but profound remodeling, with volume expansion, lymphoid hyperplasia, and markedly increased lymph node lymphangiogenesis [86]. Lymph node lymphangiogenesis is induced by VEGF-A secreted from inflamed tissues rather than regional draining lymph nodes [87]. B lymphocyte accumulation is also suggested to be a major reason for lymph node lymphangiogenesis and increased lymph flow [44, 88]. However, extensive infiltration of TAMs may potentially contribute to signaling of lymphangiogenesis within the draining lymph nodes.

Contribution of ECM to lymphangiogenesis and tumor metastasis has been supported by comprehensive clinical data and experimental tumor models [11]. The cytokines produced by macrophages interact with appropriate target cells for lymphatic proliferation in a favorable environment, in which ECM remodeling is regulated by macrophage secretion of proteinases, e.g., MMP-2 and MMP-9 [11]. Interactions between stromal components and tumor or inflamed cells may serve as a crucial mediator for initiating lymphangiogenesis. Tumor-associated stromal cells are different from their counterparts in normal tissues. The fibroblasts can produce growth factors and ECM proteins for proliferation and survival of tumor cells [89]. Fibroblast elimination in vivo has profound effects on immune polarization that is associated with decreased tumor lymphangiogenesis and suppression of spontaneous breast cancer metastasis [90]. Hyaluronic acid constitutes a major part of ECM and provides a favorable microenvironment for cell proliferation and migration in tissue injury and repair [11]. A hyaluronan-rich tumor stroma has greatly

Table 1 Tumor-associated macrophages (TAMs) and tumor-induced lymphangiogenesis

Animal models or human diseases	Macrophages ($F4/80^+$, $CD11b^+$, $CD68^+$, LYVE-1^+) and other biological features (cytokine/chemokine/growth factor)	References
VEGF-C-overexpressing human melanomas /female Swiss/c (nu/nu) nude mice	VEGF-C induces macrophage chemotaxis and recruitment, revealing a potential function of VEGF-C as an immunomodulator	[7]
	$CD11b^+$, $F4/80^+$ macrophages and all LYVE-1^+ lymphatics express VEGFR-3	
	Increased densities of peritumoral macrophages in the skin surrounding VEGF-C-transfected melanomas are correlated with the extent of tumor growth suppression	
Squamous carcinoma/human uterine cervix	VEGF-C and VEGF-D are produced by activated macrophages with expression of a panel of specific markers including CD68	[68]
	VEGF-C-expressing macrophages produce VEGFR-3 and induce peritumoral lymphangiogenesis	
Primary cutaneous malignant melanoma/human	LYVE-1^+ macrophages express VEGF-C in metastatic tumors	[5]
	The low levels of VEGF-C expression by tumor cells are complimented by stromal sources like peritumoral macrophages	
Intradermal tumor models/C57BL/6 mice Malignant melanoma/human	LYVE-1 expression occurs in a subset of $CD11b^+$, $F4/80^+$ tissue macrophages that preferentially co-express stabilin-1 and contribute to lymphatic vessel count	[64]
	Double labeling with macrophage and LEC markers can clearly differentiate LYVE-1^+ lymphatics from LYVE-1^+ tumor-infiltrating macrophages	
Ovarian cancer /CbyJ.Cg-Foxn1nu/J mice	VEGF-C/-D and VEGF-A from $CD11b^+$/LYVE-1^+ macrophages induce dysfunctional lymphangiogenesis	[145]
	Infiltrating macrophages are not involved in LEC incorporation	
Osteosarcoma/osteopetrotic (op/op) mice	M-CSF contributes to the appearance of LYVE-1^+ macrophages and postnatal lymphangiogenesis	[92]
	M-CSF deficiency reduces the abundance of LYVE-1^+ and LYVE1^- macrophages, resulting in defects in vascular and lymphatic development	
	M-CSF inhibition effectively suppresses tumor angiogenesis and lymphangiogenesis	
	Retinal macrophages express high levels of MMP-2 and MMP-9 but not VEGF	
Insulinoma / Rip1Tag2 transgenic mice	About 80% GFP^+ BMDC within the tumors are $F4/80^+$ macrophages	[55]
TRAMP-C1 prostate cancer transplantation/C57Bl/6 mice	$F4/80^+$/LYVE-1^+ macrophages exist in the tumor periphery	
	BMDC integrate into tumor-associated lymphatic vessels in vivo	
	Macrophages contribute to tumor lymphangiogenesis by processes rather than the secretion of lymphangiogenic factors	
	Macrophages can convert into LECs and integrate into cord-like structures formed by LECs in vitro	

promoted intratumoral lymphangiogenesis [91]. LYVE-1^+ macrophages preferentially coexpressing the hyaluronan receptor-like molecule CLEVER-1, have been found to mimic sprouting and collapsed lymphatics in murine tumor models and excisional wound healing [64]. M-CSF contributes to the appearance of LYVE-1^+ macrophages in the osteosarcoma and its deficiency will result in defects in lymphatic development [92]. Clearly, differentiation of LYVE-1^+ lymphatics from LYVE-1^+ tumor-infiltrating macrophages should be undertaken by double immunohistochemical staining in addition with individual-specific markers (Fig. 1).

Macrophages and VEGF ligands, however, may not be the sole lymphangiogenic modulators. The process of lymphatic formation may be independent of the VEGFR-3 ligands VEGF-C and -D or of macrophage infiltration. In HSV-1-induced lymphangiogenesis, macrophage recruitment and increased TGF-β_1 have shown no effect on VEGF-C or VEGF-D expression in LECs, and macrophages are not a detectable source of VEGF-A [17]. TGF-β signaling negatively regulates lymphangiogenesis in inflammatory and certain tumor tissues. A high level of TGF-β1 expression is associated with abnormal lymphatic architecture and dilated lymphatics, and its inhibition significantly accelerates lymphangiogenesis during wound repair [93]. TGF-β blockade may thus prevent abnormality of diaphragmatic lymphatics and improve ascites drainage in orthotopic human ovarian carcinoma model [94]. Also, inhibition of endogenous TGF-β signaling by TβR-I inhibitor induces early lymphatic development in mouse

Table 2 Inflammatory macrophages and inflammation-induced lymphangiogenesis

Animal models or human diseases	Macrophages ($F4/80^+$, $CD11b^+$, $CD68^+$, LYVE-1^+) and other biological features (cytokine/chemokine/growth factor)	References
Suture-induced inflammatory corneal model/transgenic mice for VEGF-$A^{164/164}$ or VEGF-$A^{188/188}$	$CD11b^+$ $F4/80^+$ macrophages in inflamed corneas release lymphangiogenic factors VEGF-C/-D	[76]
	VEGF-A-recruited macrophages upregulate VEGF-C/-D to induce inflammatory lymphangiogenesis	
	Macrophage recruitment is an essential mediator of the (indirect) lymphangiogenic effect of VEGF-A	
Corneal transplantation/BALB/c and CB17 SCID mice	Bone marrow-derived $CD11b^+$ macrophages with expression of LYVE-1 and *Prox-1* in inflamed corneal stromata physically contribute to pathological lymphangiogenesis	[4]
	Macrophages alone can form LYVE-1/podoplanin-positive tube-like structures and transdifferentiate into LECs	
Chronic respiratory tract infection with *Mycoplasma pulmonis*/C3H or C57BL/6 mice	Airway macrophages express VEGFR-3 ligands VEGF-C or VEGF-D	[10]
	VEGFR-3 signaling is the principal driving force for lymphatic growth	
Renal transplants/human	Macrophages may serve as lymphatic endothelial progenitors or as a major source of VEGF-C and other lymphangiogenic factors	[53]
	Once integrated into the lymphatic endothelial layer, the donor-derived endothelial cells do not retain any macrophage markers, such as CD11b or CD68	
	VEGFR-3^+ macrophages with high developmental plasticity may transdifferentiate into LECs	
Impaired diabetic wound healing/db/db mice	$F4/80^+$ macrophages promote lymphatic formation during the early stages of wound healing	[147]
	The mRNA levels of VEGFR-3 and its ligands VEGF-C and VEGF-A are reduced in db/db-derived macrophages	
	IL-1β stimulation can rescue diabetic macrophage function that induces lymphatic formation and accelerates wound healing via increased VEGFR-3 and VEGF-C expression	
Inflamed cornea (corneal micropocket assay)/C57BL/6 mice	IL-1β-induced lymphangiogenesis is mediated by upregulation of VEGF-C/-D and VEGF-A, together with recruitment and activation of $F4/80^+$ macrophages, and can be suppressed by NF-κB inhibition with suppression of VEGF-C/-D and VEGF-A expression	[84]
Excisional wound healing/C57B6 mice	Increased infiltration of $F4/80^+$ macrophages is associated with decreased scarring	[93]
	Increased TGF-β_1 is associated with abnormal lymphatic architecture and dilated lymphatics	
	TGF-β_1 inhibition directly promotes lymphangiogenesis and macrophage infiltration, independent of VEGF-C/-D	
LPS-induced peritoneal inflammation/C3H/HeN (HeN, normal TLR4 signaling), C3H/HeJ (HeJ, TLR4 gene missense mutation)	$CD11b^+$/$F4/80^+$ macrophages that infiltrate into diaphragmatic lymphatics can induce lymphangiogenesis by secreting VEGF-C/-D	[137]
	TLR-4 signaling in LECs is a critical initiator of LPS-induced inflammatory lymphangiogenesis by chemotactic recruitment of activated macrophages	
Bacteria-induced acute ear skin inflammation/GFP^+ (C57BL/6J genetic background) and K14-VEGF-C transgenic mice	Lymphangiogenesis is related with recruitment of $CD11b^+$/Gr-1^+ macrophages in the inflamed skin and draining lymph nodes	[138]
	VEGF-C/-D and VEGF-A are derived from the $CD11b^+$/Gr-1^+ macrophages	
	A subset of $CD11b^+$/Gr-1^+/$F4/80^+$ macrophages, and VEGF-C/-D and VEGF-A are major mediators for pathogen clearance and inflammation resolution through lymphatic expansion and enhanced lymph flow	

Table 2 continued

Animal models or human diseases	Macrophages ($F4/80^+$, $CD11b^+$, $CD68^+$, LYVE-1^+) and other biological features (cytokine/chemokine/growth factor)	References
Idiopathic pulmonary fibrosis/human	Activated $CD11b^+$ alveolar macrophages can transdifferentiate into LECs to form podoplanin/LYVE-1-positive lymphatic-like vessels in vitro	[54]
	VEGF-C and -D are not the driving molecules of lymphangiogenesis in idiopathic pulmonary fibrosis	
Onchocerciasis (Onchocerca nodule)/human	$CD68^+$/LYVE-1^+ macrophages are recruited to lymphangiogenic area and integrated into endothelial lining of the nodule lymphatics	[80]
HSD/male Sprague–Dawley rats and Swiss-129 Sv mice	HSD increases TonEBP, VEGF-C mRNA and protein expression in macrophages	[16]
	TonEBP binds promoter of the gene encoding VEGF-C and causes VEGF-C secretion by macrophages	
	TonEBP-VEGF-C signaling in MPS cells is a major determinant of lymphangiogenesis and extracellular volume	
LPS-induced peritonitis/FVB/N and GFP^+ mice (C57BL/6J genetic background)	$CD11b^+$ macrophage-derived VEGF-C/D play a key role in LPS-induced aberrant lymphangiogenesis, and lymphatic remodeling in a paracrine manner	[82]
Corneal infection with HSV-1/C57BL/6 mice (GFP expression under chicken β-actin promoter)	HSV-1 directly induces lymphangiogenesis through VEGF-A/VEGFR-2 signaling	[17]
	VEGF-A is derived from HSV-1-infected epithelial cells rather than $F4/80^+$ macrophages	
	BMDCs do not transdifferentiate into LECs	

embryonic stem cells, and promotes lymphangiogenesis in mouse models of chronic peritonitis and pancreatic cancer [95]. T cell-secreted interferon-γ reduces the number of *Prox-1*$^+$ LECs and directly inhibits lymph node lymphangiogenesis without prolymphangiogenic stimulation from macrophages [96]. Obviously, the main factors and mediators governing inflammation-induced lymphangiogenesis are not well defined. In recent few years, animal cornea has been widely used for studying inflammatory lymphangiogenesis, because it is an avascular, alymphatic, and immune privileged tissue. The optimum experimental conditions in the transparent cornea can be easily determined, and the results can be analyzed under stereo and dynamic observation. In a mouse corneal transplant model, both BMDCs and a subpopulation of $CD11b^+$ macrophages have been shown to contribute to the induction of lymphangiogenesis. Bone marrow-derived $CD11b^+$ macrophages, which coexpress LEC markers like LYVE-1 and *Prox-1*, can coalesce with LECs in inflamed corneal stromata, and have the ability to assemble into lymphatic-like structures in vitro [4], although LECs may show a down-regulation of LYVE-1 expression during inflammation [97]. Dynamic regulation of inflammatory cascade will require intervention at multiple levels simultaneously targeting a variety of cytokines, chemokines, and growth factors, which influence lymphangiogenesis and macrophage recruitment to the inflamed tissues. The development of animal models concerning LEC and macrophage plasticity will lead us to understand the mechanisms that are involved in lymphangiogenesis regulation.

A novel idea for lymphangiogenesis: macrophages act as a direct structural contributor to lymphatics

Noteworthily, some studies have indicated that coexpression of macrophage and lymphatic markers and, incorporation of $CD11b^+$ macrophages into newly formed lymphatics occur during the early stage of lymphatic formation [4, 64, 76]. The cell incorporation into LECs most likely occurs in the diseased condition rather than in the normal tissues. Bone marrow derived endothelial precursor cells with VEGFR-2 or VEGFR-3 expression are extravagated from blood vessels and subsequently incorporate into newly formed lymphatics, and irradiation of bone marrow can remarkably suppress lymphatic growth in a mouse corneal model [98]. Myeloid-derived suppressor cells originate in the bone marrow from common myeloid progenitor and often differentiate into $CD11b^+$ $Gr1^{med}$ $F4/80^{low/-}$ IL-$4R\alpha^+$ cells [31]. With tumor growth, these cells increase in the blood and some of them are recruited to the tumor site, where they often express the macrophage marker F4/80 and differentiate into TAMs [99, 100]. When cocultured in vitro with LECs, bone marrow-derived macrophages incorporate predominantly at the tips and branch points of growing cord-like structures, suggesting they may instigate lymphatic sprouts after being recruited to LECs [55]. In the bronchoalveolar lavage fluid

from subjects with idiopathic pulmonary fibrosis, short-fragment hyaluronic acid enhances LEC migration and proliferation, and $CD11b^+$ alveolar macrophages can transdifferentiate into LECs and form lymphatic-like vessels in vitro [54]. In human renal transplants, recipient-derived lymphatic progenitor cells directly transdifferentiate into LECs, contributing to *de novo* lymphangiogenesis. The potential candidates for lymphatic progenitors are considered to be VEGFR-3^+ tissue macrophages with developmental plasticity [53].

In the few years, progress has been made in understanding the link between precursor cells or macrophages and LECs, but there is still controversy concerning cell incorporation in lymphangiogenesis, even for the same tumor cell lines. Actually, some issues still remain to be solved, (1) Does the LEC incorporation from migrated macrophages occur in either initial or collecting lymphatics?; (2) How do the incorporating cells participate in functional intercellular junctions, valve formation, and arrangement of perilymphatic elements in the surrounding tissue?; (3) When do the anchoring filaments, a characteristic feature of initial lymphatics appear to attach LECs to interstitial collagen fibrils? Macrophages belong to the myeloid cell lineage and derive from myeloid progenitor cells. If macrophages physically contribute to LECs or differentiate into lymphatic prototype in tumor and inflammatory tissues, they are assumed to eventually lose their phenotype upon integration.

New insights into macrophage-induced lymphangiogenesis

Recently, some studies have revealed an unexpected evolutionary link between macrophages and lymphangiogenesis, which may help to mediate and coordinate the functions of many other signaling factors and pathways.

HSD and lymphangiogenesis

Lymphangiogenesis is physiologically required for the maintenance of interstitial fluid balance and diffusion of protein molecules. Interstitial fluid flow and migration of LECs with VEGF-C expression are supposed to modulate or even direct lymphangiogenesis [101]. It is known that extracellular fluids in the intravascular and interstitial compartments are normally in equilibrium. Sodium-potassium-ATPase activity in the cell membrane can be rapidly adjusted in response to environmental changes like sodium intake in maintaining the balance. Mononuclear phagocyte system (MPS) including macrophages may also regulate various homeostatic processes. Extracellular accumulation of Na^+ may inevitably lead to retention of interstitial fluid.

VEGF-C as an osmosensitive, hypertonicity-driven gene is intimately involved in extracellular volume and blood pressure homeostasis. Tonicity-responsive enhancers play a crucial role in hypertonicity-induced transcriptional stimulation of the sodium/myo-inositol cotransporter, sodium/chloride/betaine cotransporter, and aldose reductase. The tonicity-responsive enhancer binding protein (TonEBP) is a transcription factor that stimulates transcription through its binding to sequences of the tonicity-responsive enhancers via a Rel-like DNA binding domain [102]. A recent study reported that HSD leads to interstitial hypertonic Na^+ accumulation and volume retention in the rat skin, where TonEBP/VEGF-C signaling in infiltrating MPS cells provide a buffering mechanism for modulating interstitial Na^+ clearance and increasing endothelial nitric oxide synthase (eNOS) protein expression [16]. TonEBP binds the promoter of the gene encoding VEGF-C and causes VEGF-C secretion by macrophages [16], indicating a Na^+-induced hyperplasia of the lymphatic network [9]. Thus, VEGF-C secretion exposed to osmotic stress does not stem from an inflammatory response. MPS/TonEBP/VEGF-C activity shows a "M2 feature" of MPS cell function. The dermal interstitium in which MPS cells reside, represents a separate tissue-specific, extracellular microenvironment that is not necessarily reflected by changes in serum electrolyte concentrations [103]. MPS-driven and VEGF-C-mediated lymphatic network modification has an important role in the extrarenal regulation of interstitial electrolyte and volume balance.

The lymphatic system collects excess tissue fluid, macromolecules, and cells from the ECM and transports them back into the blood circulation. The connection between stromal matrix and slender lymphatics is directly influenced by interstitial fluid pressure (IFP). Several factors contribute to an increased IFP in tumor tissues, e.g., vascular abnormalities, fibrosis, and contraction of the interstitial matrix. Increased IFP in many solid tumors forms an obstacle in tumor treatment as it results in inefficient uptake of therapeutic agents [104]. Lymphatic permeability is considered to be a crucial factor for modulating IFP and interstitial environment. Increased lymphatic vessel density and area fraction, and high IFP within tumor tissues may facilitate access of tumor cells into the peripheral lymphatics [46]. Netrin-4, a laminin-related secreted protein for lymphangiogenesis, can stimulate lymphatic permeability in vitro and in vivo by activating small GTPases and Src family kinases/FAK and down-regulating tight junction proteins, thus promoting to tumor dissemination [105]. Indeed, high IFP prevailing in the tumor tissues may affect lymphangiogenesis via integrin-mediated signal transduction [106]. After an excessive salt intake, local hypertonicity is sensed by macrophages that stimulate lymphatic growth via inducing VEGF-C

secretion, creating a third fluid compartment that buffers the increased body Na^+ and volume [107]. It should be noted that macrophages are exposed to high Na^+ concentrations, where the uniquely stored sodium is not free but supposedly bound to proteoglycans. Therefore, several issues need to be addressed in the future, (1) How can osmotically inactive Na^+ induce a hypertonic state that is sensed by macrophages [107]?; (2) Do macrophages have a limited capacity to produce VEGF-C in interstitial hypertonic Na^+ environment?; (3) Does the TonEBP/VEGF-C regulatory axis exist in the kidney?

HSV-1 and inflammatory lymphangiogenesis

HSV-1, differing from other viruses, does not encode molecular mimics of any known angiogenic factors. Herpes simplex keratitis results from an infection with HSV-1, depending on aberrant host responses to antigen within the immune-privileged cornea. HSV-1 infection induces the production of many angiogenic factors, e.g., VEGF, IL-6, MMP-9, and platelet-derived growth factor [108–110], suggesting that normal equilibrium may be disrupted between proangiogenic and antiangiogenic stimuli to induce an angiogenic switch [111].

In contrast to established inflammatory models, however, HSV-1-induced lymphangiogenesis occurs independently of macrophage recruitment and VEGF-C/-D expression, but dependently on VEGF-A/VEGFR-2 signaling, indicating that BMDCs do not transdifferentiate to LECs after corneal HSV-1 infection [17]. Furthermore, continuous cytokine exposure is not required for the maintenance of mature lymphatics, whereas withdrawal of lymphangiogenic stimuli may elicit destruction of immature lymphatics [74, 112]. In comparison with VEGF-C/-D-induced lymphangiogenesis, the newly formed lymphatics induced by VEGF-A/VEGFR-2 have some structural and functional characteristics, showing a relatively dilated, leaky, and poorly functional phenotype [77, 113, 114]. However, HSV-1-induced lymphatics remain intact and functional well beyond resolution of the infection. The molecular mechanism that explains the phenotype of LECs is still unknown.

M-CSF and CEACAM-1

Numerous tumor-derived chemoattractants interacting with tyrosine kinase receptors, such as M-CSF and VEGFs, are thought to ensure the recruitment of macrophages into tumor tissues [12]. M-CSF signaling through its receptor CSF1R (CD115, c-fms) is a critical regulator of the differentiation, proliferation and survival of macrophages and monocytes, and the expression of specific cell surface markers [115, 116]. M-CSF receptor signaling contributes to myeloid cell-mediated angiogenesis through regulating the recruitment of $CD11b^+F4/80^+$ TAMs [117]. M-CSF-induced VEGF secretion has suggested its direct role in monocyte/macrophage-mediated angiogenesis. M-CSF functions as an angiogenic switch for promoting the formation of high-density vessel networks in the primary breast tumor [115]. Recombinant M-CSF can induce tube and network formation in human umbilical vein endothelial cells, through transcriptional regulation [118]. M-CSF and VEGF have close overlapping functions in the support of osteoclastic bone resorption. In the op/op mouse (ablated for M-CSF), the osteopetrotic phenotype is rescued by a single injection of human recombinant VEGF-A, showing that VEGF-A can substitute for M-CSF in vivo [119]. Moreover, TNF has been found to induce M-CSF-dependent cells, including osteoclast precursors and monocytes, to produce VEGF-C through NF-κB, leading to significantly increased lymphangiogenesis [120]. M-CSF inhibition effectively suppresses tumor lymphangiogenesis, but the continuous inhibition does not affect the healthy lymphatics outside tumors [92]. Modulation of the signaling molecules in the recruitment and function of distinct subsets of tumor-infiltrating myeloid cells will improve the cancer treatment.

The carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM-1), a member of the immunoglobulin superfamily, has been implicated in various intercellular adhesion and intracellular signaling, for controlling growth and differentiation of normal and cancer cells, especially for regulating both lymphoid and myeloid cell types [121]. CEACAM-1 acts as a potent angiogenic factor and as a major morphogenic effector for VEGF [122]. Recently, CEACAM-1 has been reported to trigger reprogramming of vascular endothelial cells to lymphangiogenesis. The switch factor is colocalized with podoplanin in LECs of tumor tissues, and its expression appears earlier in the lymphatics than in tumor-associated blood vessels and before tumor invasion [123]. CEACAM-1 overexpression in human umbilical vein endothelial cells leads to an upregulation of VEGF-C/-D and VEGFR-3, and interestingly, the endothelial cells transfected with CEACAM-1 show enhanced expression of lymphatic markers *Prox-1*, podoplanin, and LYVE-1 [123]. In experimental cutaneous leishmaniasis, B6.Ceacam-$1^{-/-}$ mice show impairment of lymphangiogenesis, indicating that lymphatic formation is affected by the loss of CEACAM-1^+/$CD11b^{high}$ cells. The myeloid cells do not constitute definite LEC precursors, and thereby can neither fuse nor transdifferentiate into lymphatics [48]. Therefore, the issue has been debated as to how macrophages contribute physically to the developing lymphatic wall, resulting from lack of dynamic data to support the cellular integration mechanism.

SDF-1-CXCR-4 axis

The chemokine, stromal cell-derived factor-1 (SDF-1, also called CXCL-12) is critical to bone marrow stem cell development, and its receptor CXCR-4 is moderately expressed in some subsets of leukocytes including monocytes/macrophages [124]. Murine embryos lacking SDF-1 or CXCR-4 show multiple lethal defects, including impaired bone marrow lymphoid and myeloid hematopoiesis [125, 126]. The SDF-1-CXCR-4 axis regulates the adhesion of hematopoietic cells to fibronectin and other ligands, and the interaction of hematopoietic cells with endothelial and stromal cells, through stimulating VEGF secretion and activating integrins in the bone marrow microenvironment [127]. SDF-1 acts as a chemoattractant for human $CD34^+$ progenitor cells [128], which may explain the mobilization of hematopoietic progenitors to peripheral blood. CD31-expressing endothelial cells have been suggested to be a source of SDF-1. SDF-1 gene expression is regulated by the transcription factor hypoxia-inducible factor-1 in endothelial cells, resulting in selective in vivo expression of SDF-1 in ischemic tissue in the direct proportion to reduced oxygen tension [129].

The tumor cells expressing CXCR-4 for specific LEC-secreted SDF-1 may approach and invade preexisting or newly formed lymphatics [130]. In extramammary Paget's disease, LECs of subcapsular sinuses and lymph node-resident macrophages have an ability to produce SDF-1. The epithelial-mesenchymal transition-related features may promote lymphatic metastasis by activating SDF-1-CXCR4 axis [131]. In human breast carcinoma, stromal fibroblasts promote tumor growth and angiogenesis through elevated SDF-1 secretion [132]. Blocking the SDF-1-CXCR4 interactions with neutralizing anti-CXCR4 or anti-SDF-1 antibodies can significantly impair these migratory responses, indicating that SDF-1 is one of the main chemotactic factors for cancer cells in the ECM [133].

Recently, SDF-1 has been described to induce recruitment, infiltration and ongoing retention of bone marrow-derived LYVE-1^+ macrophages in regulating formation of vascular networks [21], suggesting that the reciprocal relationship between CXCR4-expressing macrophages and SDF-1-expressing vascular-stromal cells is involved in angiogenesis. Moreover, proteolytic enzymes such as MMP-9 play a role in the process by degrading SDF-1 in bone marrow, increasing expression of CXCR-4 and mobilizing maturing leukocytes, progenitors, and stem cells [134]. Macrophage function, which is potentially coordinated by SDF-1-CXCR-4 signaling, may contribute to enhanced regional lymphangiogenesis.

LPS-Toll-like receptor-4 (TLR-4)-NF-κB signaling pathway

Macrophages and endothelial cells are pivotal components in the development of innate immune responses against invading microorganisms. LPS or endotoxin is a potent activator of cells including macrophages, monocytes, and endothelial cells in the inflammatory process [135]. Bacterial LPS induces actin reorganization, increased paracellular permeability, and disruption of endothelial monolayer integrity and survival signaling events in vitro through caspase cleavage of adherens junction proteins [136]. In LPS-induced peritonitis of mice, $CD11b^+$ macrophage-derived VEGF-C and -D induce abnormal and dysfunctional lymphangiogenesis, exhibiting an impaired peritoneal fluid drainage [82]. The substantial increase in lymphatic vessel density is resulted from preexisting LECs and reversible remodeling [137], but not from transdifferentiation of BMDCs. LPS-induced recruitment of $CD11b^+$/Gr-1^+ macrophages that are demonstrated to be $F4/80^+$ cells, is also involved in inflammation resolution [138].

Endothelial cells of lymphatics and blood vessels express TLR-4 and respond to LPS. Bacterial LPS activates NF-κB through TLR-4 in the cultured human dermal microvessel endothelial cells [139]. TLR-4 expression in LEC intracellular region can enhance recruitment and infiltration of $CD11b^+$ macrophages around lymphatics. However, LPS-TLR4 signaling in LECs does not directly promote cell proliferation, migration, and tube formation, and expression of lymphangiogenic molecules [137]. NF-κB-dependent mediator, LPS increases VEGFR-3 expression and responsiveness of LECs to VEGFR-3 ligands. Inflammation-induced NF-κB signaling precedes lymphatic-specific upregulation of VEGFR-3 in vivo, and NF-κB activates VEGFR-3 transcription in cultured LECs [140]. This suggests that LEC stimulation by NF-κB-dependent cytokines has the potential to amplify lymphangiogenic signaling by increasing VEGFR-3 expression. Thus, LPS-TLR4-NF-κB signaling in LECs may be a crucial pathway that conveys LPS-induced inflammation and lymphangiogenesis, but the mechanistic basis of its regulation of lymphatic-specific genes has not been well defined.

In addition, eNOS and IL-6 have been indicated to mediate macrophage-induced lymphangiogenesis. eNOS expressed in LECs regulates the initial lymph flow by acting on collecting lymphatics in vivo [141], and mediates VEGF-C-induced lymphangiogenesis, peritumor lymphatic hyperplasia and tumor metastasis, through VEGFR-2/-3 signaling dependent on PI3 K-mediated Akt activation [142]. The virus-induced expression of cytokines and chemokines is primarily due to stimulation of one or more of the depicted signal transduction cascades [143]. In

human cytomegalovirus-infected LEC cultures, many of these factors are involved in the physiological regulation of endothelial cell properties. The lymphangiogenesis is supposed to be through an indirect mechanism that relies on the stimulation of IL-6 and secretion of granulocyte-macrophage colony-stimulating factors from infected cells, but is independent of VEGF [144].

Macrophage depletion and its prospective use

Either systemic depletion of all BMDCs by irradiation or local depletion of macrophages with administration of clodronate liposomes prior to tissue injury can significantly inhibit lymphangiogenesis. In combination with blockage of VEGF/VEGFR signaling pathways, macrophage depletion may provide a potent target for upcoming gene or protein therapy in controlling tumor- or inflammation-induced lymphangiogenesis to prevent tumor metastasis and attenuate inflammatory responses.

In an ovarian cancer model, selective depletion of macrophages with additional inhibition of VEGF-C/-D and VEGF-A signaling with soluble VEGFR-3 and VEGF-Trap has indicated that VEGF-A/-C/-D from $CD11b^{+}$/$LYVE\text{-}1^{+}$ macrophages are responsible for mediating aberrant lymphangiogenesis [145]. During acute skin inflammation, depletion of macrophages including $CD11b^{+}/Gr\text{-}1^{+}$ macrophages and, blockade of VEGF-C/-D or VEGF-A result in delayed antigen clearance and inflammatory cell migration, and decreased LPS-induced lymphangiogenesis and regional lymph flow [138]. In organ and tissue transplantation, lymphatics are thought to be important mediators of immune processes, acting as a conduit for foreign antigens and antigen-presenting cells, such as macrophages. Preexisting blood vessels and lymphatics are an established risk factor for the immune rejection. Absence of lymphatics in the recipient bed prior to transplantation is suggested to promote subsequent graft survival. The local depletion of VEGF-C/-D expressing macrophages leads to substantial inhibition of corneal lymphangiogenesis [76]. Moreover, integrin $\alpha 5\beta 1$ plays an important role in VEGFR-3-mediated lymphangiogenesis in vivo, and its functional inhibition by small molecule antagonists has significantly blocked the outgrowth of new lymphatics into the cornea in a dose-dependent manner [146]. In diabetic macrophages, on the other hand, the mRNA levels of VEGFR-3 and its ligands VEGF-C/-A are notably decreased, resulting in delayed lymphangiogenesis. Application of IL-1β-treated db/db-derived macrophages to diabetic wounds induces new lymphatic formation and accelerates the healing process [147]. During the past decade, increasing evidence has indicated that macrophages surrounding tumor or inflamed tissues are actively involved in pathological lymphangiogenesis, and thus alteration of macrophage phenotype and function has a significant impact on LEC proliferation and sprouting. This may reveal the potential of specific anti-lymphangiogenic therapy in the treatment of lymphatic-associated diseases.

Conclusions

Recent advances have been made in understanding the mechanisms of macrophages as a direct result of secreted growth factors, VEGF-C/-D and VEGF-A, or even as integrated cells to fuse with LECs in lymphangiogenesis (Fig. 1). However, multiple macrophage phenotypes have differential functions and exert divergent effects on surrounding cells. Further study of the factors that stimulate or inhibit macrophages and that accelerate or delay regression of newly formed lymphatics may offer novel therapeutic approaches for lymphedema formation, inflammatory process, and tumor metastasis.

References

1. Muller WA, Randolph GJ (1999) Migration of leukocytes across endothelium and beyond: molecules involved in the transmigration and fate of monocytes. J Leukoc Biol 66:698–704
2. Martinez FO, Helming L, Gordon S (2009) Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol 27:451–483
3. Kerjaschki D (2005) The crucial role of macrophages in lymphangiogenesis. J Clin Invest 115:2316–2319
4. Maruyama K, Ii M, Cursiefen C, Jackson DG, Keino H, Tomita M, Van Rooijen N, Takenaka H, D'Amore PA, Stein-Streilein J, Losordo DW, Streilein JW (2005) Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. J Clin Invest 115:2363–2372
5. Dadras SS, Paul T, Bertoncini J, Brown LF, Muzikansky A, Jackson DG, Ellwanger U, Garbe C, Mihm MC, Detmar M (2003) Tumor lymphangiogenesis: a novel prognostic indicator for cutaneous melanoma metastasis and survival. Am J Pathol 162:1951–1960
6. Mandriota SJ, Jussila L, Jeltsch M, Compagni A, Baetens D, Prevo R, Banerji S, Huarte J, Montesano R, Jackson DG, Orci L, Alitalo K, Christofori G, Pepper MS (2001) Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. EMBO J 20:672–682
7. Skobe M, Hamberg LM, Hawighorst T, Schirner M, Wolf GL, Alitalo K, Detmar M (2001) Concurrent induction of lymphangiogenesis, angiogenesis, and macrophage recruitment by vascular endothelial growth factor-C in melanoma. Am J Pathol 159:893–903
8. Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, Prevo R, Jackson DG, Nishikawa S, Kubo H, Achen MG (2001) VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. Nat Med 7:186–191
9. Alitalo K, Tammela T, Petrova TV (2005) Lymphangiogenesis in development and human disease. Nature 438:946–953

10. Baluk P, Tammela T, Ator E, Lyubynska N, Achen MG, Hicklin DJ, Jeltsch M, Petrova TV, Pytowski B, Stacker SA, Ylä-Herttuala S, Jackson DG, Alitalo K, McDonald DM (2005) Pathogenesis of persistent lymphatic vessel hyperplasia in chronic airway inflammation. J Clin Invest 115:247–257
11. Ji RC (2006) Lymphatic endothelial cells, lymphangiogenesis, and extracellular matrix. Lymphat Res Biol 4:83–100
12. Lewis CE, Pollard JW (2006) Distinct role of macrophages in different tumor microenvironments. Cancer Res 66:605–612
13. Ji RC (2007) Lymphatic endothelial cells, inflammatory lymphangiogenesis, and prospective players. Curr Med Chem 14:2359–2368
14. Ji RC (2008) Lymphatic endothelial cells, lymphedematous lymphangiogenesis, and molecular control of edema formation. Lymphat Res Biol 6:123–137
15. Tabas I (2010) Macrophage death and defective inflammation resolution in atherosclerosis. Nat Rev Immunol 10:36–46
16. Machnik A, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, Machura K, Park JK, Beck FX, Müller DN, Derer W, Goss J, Ziomber A, Dietsch P, Wagner H, van Rooijen N, Kurtz A, Hilgers KF, Alitalo K, Eckardt KU, Luft FC, Kerjaschki D, Titze J (2009) Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. Nat Med 15:545–552
17. Wuest TR, Carr DJ (2010) VEGF-A expression by HSV-1-infected cells drives corneal lymphangiogenesis. J Exp Med 207:101–115, S1-2
18. Coffelt SB, Hughes R, Lewis CE (2009) Tumor-associated macrophages: effectors of angiogenesis and tumor progression. Biochim Biophys Acta 1796:11–18
19. Mancino A, Lawrence T (2010) Nuclear factor-kappaB and tumor-associated macrophages. Clin Cancer Res 16:784–789
20. Stout RD, Suttles J (2004) Functional plasticity of macrophages: reversible adaptation to changing microenvironments. J Leukoc Biol 76:509–513
21. Cho CH, Koh YJ, Han J, Sung HK, Jong Lee H, Morisada T, Schwendener RA, Brekken RA, Kang G, Oike Y, Choi TS, Suda T, Yoo OJ, Koh GY (2007) Angiogenic role of LYVE-1-positive macrophages in adipose tissue. Circ Res 100:e47–e57
22. Edwards JP, Zhang X, Frauwirth KA, Mosser DM (2006) Biochemical and functional characterization of three activated macrophage populations. J Leukoc Biol 80:1298–1307
23. Gibbs DF, Shanley TP, Warner RL, Murphy HS, Varani J, Johnson KJ (1999) Role of matrix metalloproteinases in models of macrophage-dependent acute lung injury. Evidence for alveolar macrophage as source of proteinases. Am J Respir Cell Mol Biol 20:1145–1154
24. Chizzolini C, Rezzonico R, De Luca C, Burger D, Dayer JM (2000) Th2 cell membrane factors in association with IL-4 enhance matrix metalloproteinase-1 (MMP-1) while decreasing MMP-9 production by granulocyte-macrophage colony-stimulating factor-differentiated human monocytes. J Immunol 164:5952–5960
25. Sorimachi K, Akimoto K, Ikehara Y, Inafuku K, Okubo A, Yamazaki S (2001) Secretion of TNF-alpha, IL-8 and nitric oxide by macrophages activated with *Agaricus blazei* Murill fractions in vitro. Cell Struct Funct 26:103–108
26. Gordon S (2003) Alternative activation of macrophages. Nat Rev Immunol 3:23–35
27. Gratchev A, Guillot P, Hakiy N, Politz O, Orfanos CE, Schledzewski K, Goerdt S (2001) Alternatively activated macrophages differentially express fibronectin and its splice variants and the extracellular matrix protein betaIG-H3. Scand J Immunol 53:386–392
28. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M (2004) The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 25:677–686
29. Gordon S, Taylor PR (2005) Monocyte and macrophage heterogeneity. Nat Rev Immunol 5:953–964
30. Mosser DM (2003) The many faces of macrophage activation. J Leukoc Biol 73:209–212
31. Hagemann T, Biswas SK, Lawrence T, Sica A, Lewis CE (2009) Regulation of macrophage function in tumors: the multifaceted role of NF-kappaB. Blood 113:3139–3146
32. Chen L, Hamrah P, Cursiefen C, Zhang Q, Pytowski B, Streilein JW, Dana MR (2004) Vascular endothelial growth factor receptor-3 mediates induction of corneal alloimmunity. Nat Med 10:813–815
33. Chung ES, Chauhan SK, Jin Y, Nakao S, Hafezi-Moghadam A, van Rooijen N, Zhang Q, Chen L, Dana R (2009) Contribution of macrophages to angiogenesis induced by vascular endothelial growth factor receptor-3-specific ligands. Am J Pathol 175:1984–1992
34. Hamrah P, Chen L, Cursiefen C, Zhang Q, Joyce NC, Dana MR (2004) Expression of vascular endothelial growth factor receptor-3 (VEGFR-3) on monocytic bone marrow-derived cells in the conjunctiva. Exp Eye Res 79:553–561
35. Gordon EJ, Rao S, Pollard JW, Nutt SL, Lang RA, Harvey NL (2010) Macrophages define dermal lymphatic vessel calibre during development by regulating lymphatic endothelial cell proliferation. Development 137:3899–3910
36. Böhmer R, Neuhaus B, Bühren S, Zhang D, Stehling M, Böck B, Kiefer F (2010) Regulation of developmental lymphangiogenesis by Syk(+) leukocytes. Dev Cell 18:437–449
37. Marttila-Ichihara F, Turja R, Miiluniemi M, Karikoski M, Maksimow M, Niemelä J, Martinez-Pomares L, Salmi M, Jalkanen S (2008) Macrophage mannose receptor on lymphatics controls cell trafficking. Blood 112:64–72
38. Linehan SA, Martínez-Pomares L, Stahl PD, Gordon S (1999) Mannose receptor and its putative ligands in normal murine lymphoid and nonlymphoid organs: in situ expression of mannose receptor by selected macrophages, endothelial cells, perivascular microglia, and mesangial cells, but not dendritic cells. J Exp Med 189:1961–1972
39. Martens JH, Kzhyshkowska J, Falkowski-Hansen M, Schledzewski K, Gratchev A, Mansmann U, Schmuttermaier C, Dippel E, Koenen W, Riedel F, Sankala M, Tryggvason K, Kobzik L, Moldenhauer G, Arnold B, Goerdt S (2006) Differential expression of a gene signature for scavenger/lectin receptors by endothelial cells and macrophages in human lymph node sinuses, the primary sites of regional metastasis. J Pathol 208:574–589
40. Irjala H, Alanen K, Grénman R, Heikkilä P, Joensuu H, Jalkanen S (2003) Mannose receptor (MR) and common lymphatic endothelial and vascular endothelial receptor (CLEVER)-1 direct the binding of cancer cells to the lymph vessel endothelium. Cancer Res 63:4671–4676
41. Harel-Adar T, Mordechai TB, Amsalem Y, Feinberg MS, Leor J, Cohen S (2011) Modulation of cardiac macrophages by phosphatidylserine-presenting liposomes improves infarct repair. Proc Natl Acad Sci USA 108:1827–1832
42. Karikoski M, Irjala H, Maksimow M, Miiluniemi M, Granfors K, Hernesniemi S, Elima K, Moldenhauer G, Schledzewski K, Kzhyshkowska J, Goerdt S, Salmi M, Jalkanen S (2009) Clever-1/Stabilin-1 regulates lymphocyte migration within lymphatics and leukocyte entrance to sites of inflammation. Eur J Immunol 39:3477–3487
43. Salmi M, Koskinen K, Henttinen T, Elima K, Jalkanen S (2004) CLEVER-1 mediates lymphocyte transmigration through vascular and lymphatic endothelium. Blood 104:3849–3857

44. Angeli V, Ginhoux F, Llodrà J, Quemeneur L, Frenette PS, Skobe M, Jessberger R, Merad M, Randolph GJ (2006) B cell-driven lymphangiogenesis in inflamed lymph nodes enhances dendritic cell mobilization. Immunity 24:203–215
45. Ji RC, Eshita Y, Xing L, Miura M (2010) Multiple expressions of lymphatic markers and morphological evolution of newly formed lymphatics in lymphangioma and lymph node lymphangiogenesis. Microvasc Res 80:195–201
46. Ji RC (2006) Lymphatic endothelial cells, tumor lymphangiogenesis and metastasis: new insights into intratumoral and peritumoral lymphatics. Cancer Metastasis Rev 25:677–694
47. Ji RC (2009) Lymph node lymphangiogenesis: a new concept for modulating tumor metastasis and inflammatory process. Histol Histopathol 24:377–384
48. Horst AK, Bickert T, Brewig N, Ludewig P, van Rooijen N, Schumacher U, Beauchemin N, Ito WD, Fleischer B, Wagener C, Ritter U (2009) CEACAM1^{+} myeloid cells control angiogenesis in inflammation. Blood 113:6726–6736
49. Kang J, Yoo J, Lee S, Tang W, Aguilar B, Ramu S, Choi I, Otu HH, Shin JW, Dotto GP, Koh CJ, Detmar M, Hong YK (2010) An exquisite cross-control mechanism among endothelial cell fate regulators directs the plasticity and heterogeneity of lymphatic endothelial cells. Blood 116:140–150
50. Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, Peri F, Wilson SW, Ruhrberg C (2010) Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. Blood 116:829–840
51. Schmidt T, Carmeliet P (2010) Blood-vessel formation: Bridges that guide and unite. Nature 465:697–699
52. Tammela T, Zarkada G, Wallgard E, Murtomäki A, Suchting S, Wirzenius M, Waltari M, Hellström M, Schomber T, Peltonen R, Freitas C, Duarte A, Isoniemi H, Laakkonen P, Christofori G, Ylä-Herttuala S, Shibuya M, Pytowski B, Eichmann A, Betsholtz C, Alitalo K (2008) Blocking VEGFR-3 suppresses angiogenic sprouting and vascular network formation. Nature 454:656–660
53. Kerjaschki D, Huttary N, Raab I, Regele H, Bojarski-Nagy K, Bartel G, Kröber SM, Greinix H, Rosenmaier A, Karlhofer F, Wick N, Mazal PR (2006) Lymphatic endothelial progenitor cells contribute to de novo lymphangiogenesis in human renal transplants. Nat Med 12:230–234
54. El-Chemaly S, Malide D, Zudaire E, Ikeda Y, Weinberg BA, Pacheco-Rodriguez G, Rosas IO, Aparicio M, Ren P, MacDonald SD, Wu HP, Nathan SD, Cuttitta F, McCoy JP, Gochuico BR, Moss J (2009) Abnormal lymphangiogenesis in idiopathic pulmonary fibrosis with insights into cellular and molecular mechanisms. Proc Natl Acad Sci USA 106:3958–3963
55. Zumsteg A, Baeriswyl V, Imaizumi N, Schwendener R, Rüegg C, Christofori G (2009) Myeloid cells contribute to tumor lymphangiogenesis. PLoS One 4:e7067
56. Bellingan GJ, Caldwell H, Howie SE, Dransfield I, Haslett C (1996) In vivo fate of the inflammatory macrophage during the resolution of inflammation: inflammatory macrophages do not die locally, but emigrate to the draining lymph nodes. J Immunol 157:2577–2585
57. Zeisberger SM, Odermatt B, Marty C, Zehnder-Fjällman AH, Ballmer-Hofer K, Schwendener RA (2006) Clodronate-liposome-mediated depletion of tumour-associated macrophages: a new and highly effective antiangiogenic therapy approach. Br J Cancer 95:272–281
58. Ahn GO, Tseng D, Liao CH, Dorie MJ, Czechowicz A, Brown JM (2010) Inhibition of Mac-1 (CD11b/CD18) enhances tumor response to radiation by reducing myeloid cell recruitment. Proc Natl Acad Sci USA 107:8363–8368
59. Holness CL, Simmons DL (1993) Molecular cloning of CD68, a human macrophage marker related to lysosomal glycoproteins. Blood 81:1607–1613
60. Fogg DK, Sibon C, Miled C, Jung S, Aucouturier P, Littman DR, Cumano A, Geissmann F (2006) A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. Science 311:83–87
61. Kermani P, Rafii D, Jin DK, Whitlock P, Schaffer W, Chiang A, Vincent L, Friedrich M, Shido K, Hackett NR, Crystal RG, Rafii S, Hempstead BL (2005) Neurotrophins promote revascularization by local recruitment of TrkB^{+} endothelial cells and systemic mobilization of hematopoietic progenitors. J Clin Invest 115:653–663
62. Ji RC, Kurihara K, Kato S (2006) Lymphatic vascular endothelial hyaluronan receptor (LYVE)-1- and CCL21-positive lymphatic compartments in the diabetic thymus. Anat Sci Int 81:201–209
63. Brown S, Heinisch I, Ross E, Shaw K, Buckley CD, Savill J (2002) Apoptosis disables CD31-mediated cell detachment from phagocytes promoting binding and engulfment. Nature 418:200–203
64. Schledzewski K, Falkowski M, Moldenhauer G, Metharom P, Kzhyshkowska J, Ganss R, Demory A, Falkowska-Hansen B, Kurzen H, Ugurel S, Geginat G, Arnold B, Goerdt S (2006) Lymphatic endothelium-specific hyaluronan receptor LYVE-1 is expressed by stabilin-1+, F4/80+, CD11b+ macrophages in malignant tumours and wound healing tissue in vivo and in bone marrow cultures in vitro: implications for the assessment of lymphangiogenesis. J Pathol 209:67–77
65. Ji RC, Eshita Y, Kato S (2007) Investigation of intratumoural and peritumoural lymphatics expressed by podoplanin and LYVE-1 in the hybridoma-induced tumours. Int J Exp Pathol 88:257–270
66. Guo R, Zhou Q, Proulx ST, Wood R, Ji RC, Ritchlin CT, Pytowski B, Zhu Z, Wang YJ, Schwarz EM, Xing L (2009) Inhibition of lymphangiogenesis and lymphatic drainage via vascular endothelial growth factor receptor 3 blockade increases the severity of inflammation in a mouse model of chronic inflammatory arthritis. Arthritis Rheum 60:2666–2676
67. Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420:860–867
68. Schoppmann SF, Birner P, Stöckl J, Kalt R, Ullrich R, Caucig C, Kriehuber E, Nagy K, Alitalo K, Kerjaschki D (2002) Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. Am J Pathol 161:947–956
69. Pollard JW (2004) Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 4:71–78
70. Mantovani A, Allavena P, Sica A, Balkwill F (2008) Cancer-related inflammation. Nature 454:436–444
71. Moussai D, Mitsui H, Pettersen JS, Pierson KC, Shah KR, Suárez-Fariñas M, Cardinale IR, Bluth MJ, Krueger JG, Carucci JA (2011) The human cutaneous squamous cell carcinoma microenvironment is characterized by increased lymphatic density and enhanced expression of macrophage-derived VEGF-C. J Invest Dermatol 131:229–236
72. Karkkainen MJ, Haiko P, Sainio K, Partanen J, Taipale J, Petrova TV, Jeltsch M, Jackson DG, Talikka M, Rauvala H, Betsholtz C, Alitalo K (2004) Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. Nat Immunol 5:74–80
73. Björndahl MA, Cao R, Burton JB, Brakenhielm E, Religa P, Galter D, Wu L, Cao Y (2005) Vascular endothelial growth factor-a promotes peritumoral lymphangiogenesis and lymphatic metastasis. Cancer Res 65:9261–9268

74. Karpanen T, Alitalo K (2008) Molecular biology and pathology of lymphangiogenesis. Annu Rev Pathol 3:367–397
75. Achen MG, Jeltsch M, Kukk E, Mäkinen T, Vitali A, Wilks AF, Alitalo K, Stacker SA (1998) Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). Proc Natl Acad Sci USA 95:548–553
76. Cursiefen C, Chen L, Borges LP, Jackson D, Cao J, Radziejewski C, D'Amore PA, Dana MR, Wiegand SJ, Streilein JW (2004) VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. J Clin Invest 113:1040–1050
77. Hong YK, Lange-Asschenfeldt B, Velasco P, Hirakawa S, Kunstfeld R, Brown LF, Bohlen P, Senger DR, Detmar M (2004) VEGF-A promotes tissue repair-associated lymphatic vessel formation via VEGFR-2 and the alpha1beta1 and alpha2beta1 integrins. FASEB J 18:1111–1113
78. Barbera-Guillem E, Nyhus JK, Wolford CC, Friece CR, Sampsel JW (2002) Vascular endothelial growth factor secretion by tumor-infiltrating macrophages essentially supports tumor angiogenesis, and IgG immune complexes potentiate the process. Cancer Res 62:7042–7049
79. Sica A, Rubino L, Mancino A, Larghi P, Porta C, Rimoldi M, Solinas G, Locati M, Allavena P, Mantovani A (2007) Targeting tumour-associated macrophages. Expert Opin Ther Targets 11:1219–1229
80. Attout T, Hoerauf A, Dénécé G, Debrah AY, Marfo-Debrekyei Y, Boussinesq M, Wanji S, Martinez V, Mand S, Adjei O, Bain O, Specht S, Martin C (2009) Lymphatic vascularisation and involvement of Lyve-1^+ macrophages in the human onchocerca nodule. PLoS One 4:e8234
81. Yamashita M, Iwama N, Date F, Shibata N, Miki H, Yamauchi K, Sawai T, Sato S, Takahashi T, Ono M (2009) Macrophages participate in lymphangiogenesis in idiopathic diffuse alveolar damage through CCL19-CCR7 signal. Hum Pathol 40:1553–1563
82. Kim KE, Koh YJ, Jeon BH, Jang C, Han J, Kataru RP, Schwendener RA, Kim JM, Koh GY (2009) Role of $CD11b^+$ macrophages in intraperitoneal lipopolysaccharide-induced aberrant lymphangiogenesis and lymphatic function in the diaphragm. Am J Pathol 175:1733–1745
83. Xing L, Ji RC (2008) Lymphangiogenesis, myeloid cells and inflammation. Expert Rev Clin Immunol 4:599–613
84. Watari K, Nakao S, Fotovati A, Basaki Y, Hosoi F, Bereczky B, Higuchi R, Miyamoto T, Kuwano M, Ono M (2008) Role of macrophages in inflammatory lymphangiogenesis: enhanced production of vascular endothelial growth factor C and D through NF-kappaB activation. Biochem Biophys Res Commun 377:826–831
85. Handa O, Naito Y, Takagi T, Shimozawa M, Kokura S, Yoshida N, Matsui H, Cepinskas G, Kvietys PR, Yoshikawa T (2004) Tumor necrosis factor-alpha-induced cytokine-induced neutrophil chemoattractant-1 (CINC-1) production by rat gastric epithelial cells: role of reactive oxygen species and nuclear factor-kappaB. J Pharmacol Exp Ther 309:670–676
86. Drayton DL, Liao S, Mounzer RH, Ruddle NH (2006) Lymphoid organ development: from ontogeny to neogenesis. Nat Immunol 7:344–353
87. Halin C, Tobler NE, Vigl B, Brown LF, Detmar M (2007) VEGF-A produced by chronically inflamed tissue induces lymphangiogenesis in draining lymph nodes. Blood 110:3158–3167
88. Harrell MI, Iritani BM, Ruddell A (2007) Tumor-induced sentinel lymph node lymphangiogenesis and increased lymph flow precede melanoma metastasis. Am J Pathol 170:774–786
89. Kalluri R, Zeisberg M (2006) Fibroblasts in cancer. Nat Rev Cancer 6:392–401
90. Liao D, Luo Y, Markowitz D, Xiang R, Reisfeld RA (2009) Cancer associated fibroblasts promote tumor growth and metastasis by modulating the tumor immune microenvironment in a 4T1 murine breast cancer model. PLoS One 4:e7965
91. Koyama H, Kobayashi N, Harada M, Takeoka M, Kawai Y, Sano K, Fujimori M, Amano J, Ohhashi T, Kannagi R, Kimata K, Taniguchi S, Itano N (2008) Significance of tumor-associated stroma in promotion of intratumoral lymphangiogenesis: pivotal role of a hyaluronan-rich tumor microenvironment. Am J Pathol 172:179–193
92. Kubota Y, Takubo K, Shimizu T, Ohno H, Kishi K, Shibuya M, Saya H, Suda T (2009) M-CSF inhibition selectively targets pathological angiogenesis and lymphangiogenesis. J Exp Med 206:1089–1102
93. Clavin NW, Avraham T, Fernandez J, Daluvoy SV, Soares MA, Chaudhry A, Mehrara BJ (2008) TGF-beta1 is a negative regulator of lymphatic regeneration during wound repair. Am J Physiol Heart Circ Physiol 295:H2113–H2127
94. Liao S, Liu J, Lin P, Shi T, Jain RK, Xu L (2011) TGF-{beta} blockade controls ascites by preventing abnormalization of lymphatic vessels in orthotopic human ovarian carcinoma models. Clin Cancer Res 17:1415–1424
95. Oka M, Iwata C, Suzuki HI, Kiyono K, Morishita Y, Watabe T, Komuro A, Kano MR, Miyazono K (2008) Inhibition of endogenous TGF-beta signaling enhances lymphangiogenesis. Blood 111:4571–4579
96. Kataru RP, Kim H, Jang C, Choi DK, Koh BI, Kim M, Gollamudi S, Kim YK, Lee SH, Koh GY (2011) T lymphocytes negatively regulate lymph node lymphatic vessel formation. Immunity 34:96–107
97. Johnson LA, Prevo R, Clasper S, Jackson DG (2007) Inflammation-induced uptake and degradation of the lymphatic endothelial hyaluronan receptor LYVE-1. J Biol Chem 282:33671–33680
98. Religa P, Cao R, Bjorndahl M, Zhou Z, Zhu Z, Cao Y (2005) Presence of bone marrow-derived circulating progenitor endothelial cells in the newly formed lymphatic vessels. Blood 106:4184–4190
99. Kusmartsev S, Gabrilovich DI (2005) STAT1 signaling regulates tumor-associated macrophage-mediated T cell deletion. J Immunol 174:4880–4891
100. Nagaraj S, Gabrilovich DI (2008) Tumor escape mechanism governed by myeloid-derived suppressor cells. Cancer Res 68:2561–2563
101. Boardman KC, Swartz MA (2003) Interstitial flow as a guide for lymphangiogenesis. Circ Res 92:801–808
102. Miyakawa H, Woo SK, Dahl SC, Handler JS, Kwon HM (1999) Tonicity-responsive enhancer binding protein, a rel-like protein that stimulates transcription in response to hypertonicity. Proc Natl Acad Sci USA 96:2538–2542
103. Machnik A, Dahlmann A, Kopp C, Goss J, Wagner H, van Rooijen N, Eckardt KU, Müller DN, Park JK, Luft FC, Kerjaschki D, Titze J (2010) Mononuclear phagocyte system depletion blocks interstitial tonicity-responsive enhancer binding protein/vascular endothelial growth factor C expression and induces salt-sensitive hypertension in rats. Hypertension 55:755–761
104. Heldin CH, Rubin K, Pietras K, Ostman A (2004) High interstitial fluid pressure—an obstacle in cancer therapy. Nat Rev Cancer 4:806–813
105. Larrieu-Lahargue F, Welm AL, Thomas KR, Li DY (2010) Netrin-4 induces lymphangiogenesis in vivo. Blood 115:5418–5426
106. Wiig H, Keskin D, Kalluri R (2010) Interaction between the extracellular matrix and lymphatics: consequences for lymphangiogenesis and lymphatic function. Matrix Biol 29:645–656

107. Marvar PJ, Gordon FJ, Harrison DG (2009) Blood pressure control: salt gets under your skin. Nat Med 15:487–488
108. Lee S, Zheng M, Kim B, Rouse BT (2002) Role of matrix metalloproteinase-9 in angiogenesis caused by ocular infection with herpes simplex virus. J Clin Invest 110:1105–1111
109. Hayashi K, Hooper LC, Chin MS, Nagineni CN, Detrick B, Hooks JJ (2006) Herpes simplex virus 1 (HSV-1) DNA and immune complex (HSV-1-human IgG) elicit vigorous interleukin 6 release from infected corneal cells via Toll-like receptors. J Gen Virol 87:2161–2169
110. Kaye S, Choudhary A (2006) Herpes simplex keratitis. Prog Retin Eye Res 25:355–380
111. Sottile J (2004) Regulation of angiogenesis by extracellular matrix. Biochim Biophys Acta 1654:13–22
112. Jain RK (2003) Molecular regulation of vessel maturation. Nat Med 9:685–693
113. Nagy JA, Vasile E, Feng D, Sundberg C, Brown LF, Detmar MJ, Lawitts JA, Benjamin L, Tan X, Manseau EJ, Dvorak AM, Dvorak HF (2002) Vascular permeability factor/vascular endothelial growth factor induces lymphangiogenesis as well as angiogenesis. J Exp Med 196:1497–1506
114. Kajiya K, Hirakawa S, Detmar M (2006) Vascular endothelial growth factor-A mediates ultraviolet B-induced impairment of lymphatic vessel function. Am J Pathol 169:1496–1503
115. Lin EY, Nguyen AV, Russell RG, Pollard JW (2001) Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med 193:727–740
116. Hamilton JA (2008) Colony-stimulating factors in inflammation and autoimmunity. Nat Rev Immunol 8:533–544
117. Priceman SJ, Sung JL, Shaposhnik Z, Burton JB, Torres-Collado AX, Moughon DL, Johnson M, Lusis AJ, Cohen DA, Iruela-Arispe ML, Wu L (2010) Targeting distinct tumor-infiltrating myeloid cells by inhibiting CSF-1 receptor: combating tumor evasion of antiangiogenic therapy. Blood 115:1461–1471
118. Eubank TD, Galloway M, Montague CM, Waldman WJ, Marsh CB (2003) M-CSF induces vascular endothelial growth factor production and angiogenic activity from human monocytes. J Immunol 171:2637–2643
119. Niida S, Kaku M, Amano H, Yoshida H, Kataoka H, Nishikawa S, Tanne K, Maeda N, Nishikawa S, Kodama H (1999) Vascular endothelial growth factor can substitute for macrophage colony-stimulating factor in the support of osteoclastic bone resorption. J Exp Med 190:293–298
120. Zhang Q, Lu Y, Proulx ST, Guo R, Yao Z, Schwarz EM, Boyce BF, Xing L (2007) Increased lymphangiogenesis in joints of mice with inflammatory arthritis. Arthritis Res Ther 9:R118
121. Gray-Owen SD, Blumberg RS (2006) CEACAM1: contact-dependent control of immunity. Nat Rev Immunol 6:433–446
122. Ergün S, Kilik N, Ziegeler G, Hansen A, Nollau P, Götze J, Wurmbach JH, Horst A, Weil J, Fernando M, Wagener C (2000) CEA-related cell adhesion molecule 1: a potent angiogenic factor and a major effector of vascular endothelial growth factor. Mol Cell 5:311–320
123. Kilic N, Oliveira-Ferrer L, Neshat-Vahid S, Irmak S, Obst-Pernberg K, Wurmbach JH, Loges S, Kilic E, Weil J, Lauke H, Tilki D, Singer BB, Ergün S (2007) Lymphatic reprogramming of microvascular endothelial cells by CEA-related cell adhesion molecule-1 via interaction with VEGFR-3 and Prox1. Blood 110:4223–4233
124. Murdoch C (2000) CXCR4: chemokine receptor extraordinaire. Immunol Rev 177:175–184
125. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR (1998) Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 393:595–599
126. Lapidot T, Petit I (2002) Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. Exp Hematol 30:973–981
127. Kijowski J, Baj-Krzyworzeka M, Majka M, Reca R, Marquez LA, Christofidou-Solomidou M, Janowska-Wieczorek A, Ratajczak MZ (2001) The SDF-1-CXCR4 axis stimulates VEGF secretion and activates integrins but does not affect proliferation and survival in lymphohematopoietic cells. Stem Cells 19:453–466
128. Aiuti A, Webb IJ, Bleul C, Springer T, Gutierrez-Ramos JC (1997) The chemokine SDF-1 is a chemoattractant for human $CD34^+$ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of $CD34^+$ progenitors to peripheral blood. J Exp Med 185:111–120
129. Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, Gurtner GC (2004) Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 10:858–864
130. Royston D, Jackson DG (2009) Mechanisms of lymphatic metastasis in human colorectal adenocarcinoma. J Pathol 217:608–619
131. Hirakawa S, Detmar M, Kerjaschki D, Nagamatsu S, Matsuo K, Tanemura A, Kamata N, Higashikawa K, Okazaki H, Kameda K, Nishida-Fukuda H, Mori H, Hanakawa Y, Sayama K, Shirakata Y, Tohyama M, Tokumaru S, Katayama I, Hashimoto K (2009) Nodal lymphangiogenesis and metastasis: role of tumor-induced lymphatic vessel activation in extramammary Paget's disease. Am J Pathol 175:2235–2248
132. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL, Weinberg RA (2005) Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 121:335–348
133. Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verástegui E, Zlotnik A (2001) Involvement of chemokine receptors in breast cancer metastasis. Nature 410:50–56
134. Kollet O, Dar A, Shivtiel S, Kalinkovich A, Lapid K, Sztainberg Y, Tesio M, Samstein RM, Goichberg P, Spiegel A, Elson A, Lapidot T (2006) Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. Nat Med 12:657–664
135. Opal SM, Cohen J (1999) Clinical gram-positive sepsis: does it fundamentally differ from gram-negative bacterial sepsis? Crit Care Med 27:1608–1616
136. Bannerman DD, Sathyamoorthy M, Goldblum SE (1998) Bacterial lipopolysaccharide disrupts endothelial monolayer integrity and survival signaling events through caspase cleavage of adherens junction proteins. J Biol Chem 273:35371–35380
137. Kang S, Lee SP, Kim KE, Kim HZ, Mémet S, Koh GY (2009) Toll-like receptor 4 in lymphatic endothelial cells contributes to LPS-induced lymphangiogenesis by chemotactic recruitment of macrophages. Blood 113:2605–2613
138. Kataru RP, Jung K, Jang C, Yang H, Schwendener RA, Baik JE, Han SH, Alitalo K, Koh GY (2009) Critical role of $CD11b^+$ macrophages and VEGF in inflammatory lymphangiogenesis, antigen clearance, and inflammation resolution. Blood 113:5650–5659
139. Faure E, Equils O, Sieling PA, Thomas L, Zhang FX, Kirschning CJ, Polentarutti N, Muzio M, Arditi M (2000) Bacterial lipopolysaccharide activates NF-kappaB through toll-like receptor 4 (TLR-4) in cultured human dermal endothelial cells. Differential expression of TLR-4 and TLR-2 in endothelial cells. J Biol Chem 275:11058–11063
140. Flister MJ, Wilber A, Hall KL, Iwata C, Miyazono K, Nisato RE, Pepper MS, Zawieja DC, Ran S (2010) Inflammation

induces lymphangiogenesis through up-regulation of VEGFR-3 mediated by NF-kappaB and Prox1. Blood 115:418–429
141. Hagendoorn J, Padera TP, Kashiwagi S, Isaka N, Noda F, Lin MI, Huang PL, Sessa WC, Fukumura D, Jain RK (2004) Endothelial nitric oxide synthase regulates microlymphatic flow via collecting lymphatics. Circ Res 95:204–209
142. Lahdenranta J, Hagendoorn J, Padera TP, Hoshida T, Nelson G, Kashiwagi S, Jain RK, Fukumura D (2009) Endothelial nitric oxide synthase mediates lymphangiogenesis and lymphatic metastasis. Cancer Res 69:2801–2808
143. Mogensen TH, Paludan SR (2001) Molecular pathways in virus-induced cytokine production. Microbiol Mol Biol Rev 65:131–150
144. Fiorentini S, Luganini A, Dell'oste V, Lorusso B, Cervi E, Caccuri F, Bonardelli S, Landolfo S, Caruso A, Gribaudo G (2011) Human cytomegalovirus productively infects lymphatic endothelial cells and induces a secretome that promotes angiogenesis and lymphangiogenesis through interleukin-6 and granulocyte-macrophage colony-stimulating factor. J Gen Virol 92:650–660
145. Jeon BH, Jang C, Han J, Kataru RP, Piao L, Jung K, Cha HJ, Schwendener RA, Jang KY, Kim KS, Alitalo K, Koh GY (2008) Profound but dysfunctional lymphangiogenesis via vascular endothelial growth factor ligands from $CD11b^{+}$ macrophages in advanced ovarian cancer. Cancer Res 68:1100–1109
146. Dietrich T, Bock F, Yuen D, Hos D, Bachmann BO, Zahn G, Wiegand S, Chen L, Cursiefen C (2010) Cutting edge: lymphatic vessels, not blood vessels, primarily mediate immune rejections after transplantation. J Immunol 184:535–539
147. Maruyama K, Asai J, Ii M, Thorne T, Losordo DW, D'Amore PA (2007) Decreased macrophage number and activation lead to reduced lymphatic vessel formation and contribute to impaired diabetic wound healing. Am J Pathol 170:1178–1191