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REVIEW

Lymphangiogenesis and Inflammation—Looking for the "Missing Pieces" of the Puzzle

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Abstract Several papers about lymphangiogenesis and inflammation focused on the detailed and complicated descriptions of the molecular pathways accompanying both non-tumor and tumor inflammatory-induced lymphatic vessel development. Many authors are tempted to present inflammatory-induced lymphangiogenesis in pathologic conditions neglecting the role of inflammatory cells during embryonic lymphatic vessel development. Some of the inflammatory cells are largely characterized in inflammatory-induced lymphangiogenesis, while others as mast cells, eosinophils, or plasma cells are less studied. No phenotypic characterization of inflammation-activated lymphatic endothelial cell is available in this moment. Another paradox is related to the existence of few papers regarding lymphangiogenesis inside lymphoid organs and for their related pathology. There are still several "missing pieces of such a big puzzle" of lymphangiogenesis and inflammation, with a direct impact on the ineffectiveness of the anti-inflammatory therapy as lymphangiogenesis inhibitors. The present paper will focus on the controversial issues of lymphangiogenesis and inflammation.

Keywords Lymphangiogenesis · Inflammation · Lymphatic vessels · Inflammatory cells · Endothelial cells

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Lymphangiogenesis: Step by Step from Friendship to Hostility

Embryonic lymphangiogenesis follows the development of the heart and blood vascular network (Al-Rawi et al. 2005; Kato et al. 2006). Endothelial cells forming cardinal vein endothelium have the ability to transform into lymphatic endothelial cells by the acquisition of lymphatic endothelial markers Prox 1, Sox18, or COUP-TFII. This process is called lymphatic endothelial cell specification and represents the most widely accepted theory regarding embryonic lymphatic vasculature development (Bernaudin et al. 2013; Kim et al. 2013; Kume 2010). Prox 1, Sox18 or COUP-TFII is overexpressed in the newly formed lymphatic vessel endothelium in the first stages of premalignant conditions (Cimpean et al. 2011, 2012; Palomba et al. 2010; Zhang et al. 2006) and also in the endothelial cells from the malignant tumor lymphatic vessels (Duong et al. 2012; Masuzawa et al. 2012; Ramani et al. 2012).

During embryonic and postnatal life, lymphatic vasculature network continues to remodel and to establish a specific phenotype of normal lymphatic vascular bed, characterized by the expression of LYVE-1, podoplanin (D2-40) and vascular endothelial growth factor receptor (VEGFR)-3 in quiescent lymphatic endothelial cells. Despite their relative reliability for lymphatic endothelium, none of these markers are exclusively expressed by the lymphatic endothelial cells. LYVE-1 is not restricted to the lymphatic endothelium, being present on the surface of yolk sac, placenta, and umbilical cord vessels and, also on intraembryonic blood vessels during prenatal development (El Filali et al. 2014; Lee et al. 2014). Apart from lymphatic endothelium, LYVE-1 is expressed in normal liver blood sinusoids, spleen endothelium, and activated tissue macrophages (Akishima et al. 2004; Mouta Carreira et al.

2001). VEGFR-3 tyrosine kinase activity is crucial for lymphatic vessel growth (Zarkada et al. 2015), but recently there were reported controversial data concerning its involvement in angiogenesis, mainly by controlling tip to stalk conversion at vessel fusion site (Nilsson et al. 2010; Tammela et al. 2011) and also by supporting sprouting, vessel branching, and endothelial cell proliferation (Cao et al. 1998; Tammela et al. 2008). In 2013, Gordon et al. (2013b) reported for the first time in humans that VEGFR-3 mutations or impairment of its phosphorylation during embryonic lymphangiogenesis correlated with mutation in VEGF-C (Gordon et al. 2013a) is causative for a defective lymphatic vessel development and primary lymphedema from Milroy disease. The VEGF-C/VEGFR-3 axis is expressed not only by lymphatic endothelial cells but also by a variety of human tumor cells. Activation of the VEGF-C/VEGFR-3 axis in lymphatic endothelial cells can facilitate metastasis by increasing the formation of lymphatic vessels (lymphangiogenesis) within and around tumors (Matsumoto et al. 2013).

Podoplanin seems to be the most reliable marker for lymphatic endothelial cells. Although, its expression is not restricted to endothelial cells from lymphatic vascular network, highlighting the basal cells of the normal stratified epithelia, Schwann cells, and myofibroblasts (Kanner et al. 2010) and also various tumor cells and cancer-associated fibroblasts (Neri et al. 2015; Raica et al. 2008), podoplanin remains a marker with high specificity for lymphatic endothelial cells.

CD34 sialomucin is a well-known marker for blood vessel endothelium. It is not expressed in lymphatic endothelial cells from normal tissues, but it is present in the lymphatic endothelial cells lining newly formed tumor lymphatic vessels (Fiedler et al. 2006). VEGFR-2 is highly expressed in lymphatic endothelial cells and promotes the expansion of lymphatic network during embryonic development (Dellinger et al. 2013; Nagy et al. 2002; Secker and Harvey 2015). Involvement of endothelial progenitor cells in lymphangiogenesis is not clearly defined. Previous papers reported the presence of a $CD34^+/CD133^+$ endothelial cells' subpopulation able to differentiate lymphatic endothelial cells (Salven et al. 2003). Recently, Tan et al. (2014) isolated a subpopulation of $CD34^+/VEGFR\text{-}3^+$ endothelial cells from human umbilical cord blood and demonstrated their ability to differentiate into lymphatic endothelial cells and subsequently organize into lymphatic capillary-like structures in three-dimensional matrix via VEGF-C/VEGFR-3 signaling pathway. Bone marrow-derived podoplanin-positive lymphatic progenitor cells play a crucial role in postnatal lymphangiogenesis (Lee et al. 2010), and its circulating levels are increased during pathologic condition as malignancies. High levels of circulating lymphatic progenitor endothelial cells were correlated with lymph node metastasis in ovarian cancer (Qiu et al. 2013), human small-cell lung carcinoma (Bogos et al. 2009), and breast cancer (Van't Hull et al. 2014).

Dynamic molecular profile of lymphangiogenesis suggests a heterogeneous population of lymphatic endothelial cells lining a vascular network highly specialized for interstitial fluid drainage and immune cell trafficking in normal conditions. These "friendly" routes become the most awful "enemies" in tumors being one of the dissemination routes for malignant cells and favoring nodal and distant metastasis. Lymphatic vascular network expansion is organ specific. Tissue-specific factors directly influence lymphatic vessel development, survival, and regeneration. A special attention has to be paid to bone marrow and lymphoid organs concerning lymphatic vessel types, presence, and distribution.

Bone marrow remains a mysterious organ regarding its involvement in the lymphatic system development. Bone marrow contains progenitor cells able to differentiate into lymphatic endothelial cells (Lee et al. 2010), but it has no lymphatic vessels. Also, inside the bone marrow, there are lymphocytes being produced from immature hematopoietic progenitor cells. During tumor dissemination, bone marrow represents one of the favorite sites where metastasis develops (Mareel et al. 2009), metastatic tumor cells arriving inside bone marrow through the blood vascular network not by lymphatic pathway. Spleen, thymus, and tonsils have efferent lymphatic vessels only compared with lymph nodes having both afferent and efferent lymphatic vessels.

It seems that lymphangiogenesis, presence, particular distribution, and morphology of lymphatic vessels inside bone marrow and lymphoid organs are directly influenced by lymphoid cell population which create a special microenvironment in close relationship with lymphatic endothelial cells, high endothelial venules (HEV), lymphocytes, and specific factors secreted by stromal cells.

Lymphotoxin α is produced by T cells in a small amount in normal state, and thus it acts in a paracrine manner to stimulate lymphangiogenesis in primary and secondary lymphoid organs. In contrast, lymphotoxin β inhibits lymphangiogenesis (Mounzer et al. 2010). Lymph node lymphangiogenesis is negatively regulated by T cells through interferon (IFN)-γ (Kataru et al. 2011). In vitro studies reported that proliferation and migration of lymphatic endothelial cells are inhibited by transforming growth factor β following lymphangiogenesis impairment. Crosstalk between lymph node lymphatics and HEV is mediated by lymphotoxin beta receptor (LTβR) expressed in both types of endothelial cells, and upregulation of LTβR by HEV produces an intense lymphangiogenic response (Oka et al. 2008).

Thymic epithelial cells seem to be key regulators of lymphatic vessel distribution and lymphangiogenesis inside the human thymus. Both cortical and medullary epithelial

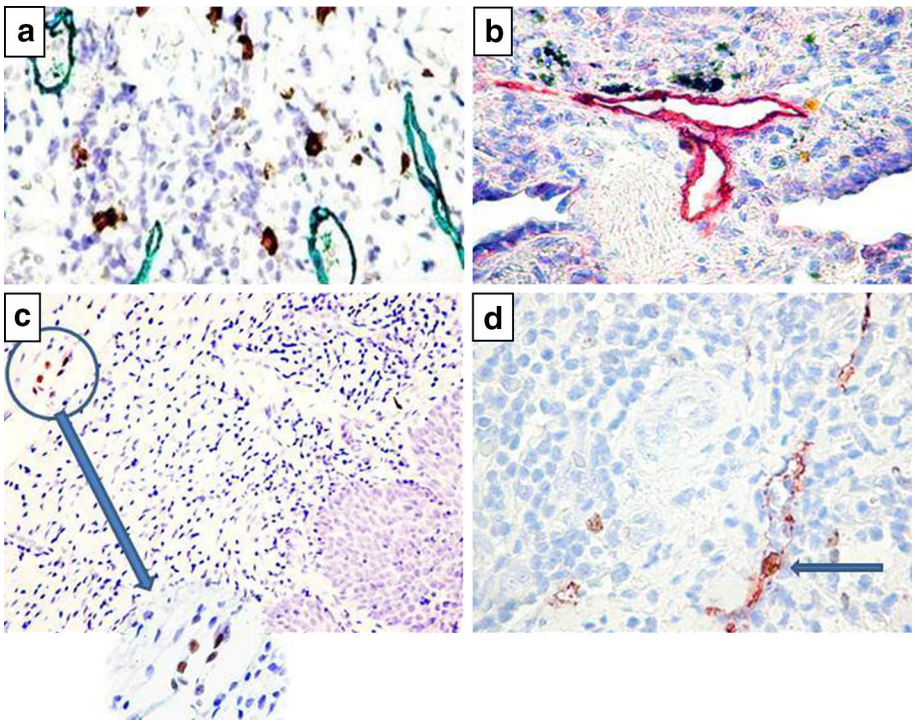

Fig. 1 Increased number of lymphatic vessels associated with inflammation from periodontal disease (**a**, highlighted with mouse anti-human monoclonal antibody against D2-40 antigen, appeared stained in *green* with Vina Green) and with a high number of mast cells (**a**, labeled with mouse anti-human monoclonal antibody against mast cell tryptase, clone AA1, stained in *brown*). Lung macrophages closely apposed to lymphatic vessels (**b**) in lung inflammatory state. Inflammation from early cervical lesions induces new lymphatic vessel development by promoting the differentiation of lymphatic endothelial cells expressing PROX 1 (**c**). Inflammatory tissue associated with breast cancer has abundant lymphatic vessels (also stained with D2-40) with discontinuous wall and Ki 67-positive proliferative endothelial cells (*arrow*, **d**)

cells of the normal human thymus express lymphatic markers as podoplanin (Raica et al. 2010), but this expression was reported to be involved in the thymoma pathogenesis and also it was not certified to have a role in thymus lymphangiogenesis. In normal human fetal and postnatal thymus, the presence of lymphatic vessels immunohistochemically positive for D2-40 was restricted to the interlobular connective tissue septae and thymus medulla. Lymphatic vessel density increases in thymomas, but the thymoma-related lymphangiogenesis pathways are not fully elucidated (Raica et al. 2010). Several experimental mouse models revealed that CCL21 and CCL19, two chemokines secreted by thymus medullary epithelial cells, are overexpressed by lymphatic vessels which increase in number during myasthenia gravis progression. Also, CCL21 and CCL19 exert a chemoattractant effect, by recruitment of peripheral B (especially naïve) and T cells responsible for the development of germinal centers specific for the thymus with myasthenia gravis.

According to Pearse (2006), there are no afferent lymphatic vessels in the normal thymus. In the inflammatory state of myasthenia gravis, during germinal center development, an afferent network of newly formed lymphatic vessels develops in the thymus, stimulated most probably by lymphangiogenic factors secreted by immune cells (Berrih-Aknin et al. 2009).

Lymphangiogenesis occurs in several pathologic circumstances varying from benign conditions as tissue repair (Shimoda and Kato 2006), arthritis (Schwarz et al. 2010; Zhang et al. 2007), and inflammatory bowel diseases (Algaba et al. 2013) or associated with various neoplasia (Cueni and Detmar 2008; Proulx et al. 2013; Schoppmann et al. 2013) (Fig. 1).

Inflammatory lymphangiogenesis was also reported during physiologic processes, and the best example can be considered immune cell-induced angiogenesis and lymphangiogenesis inside corpus luteum (Xu and Stouffer 2009). Gradual loss of progesterone production during ovarian cycle induces recruitment of inflammatory cells, mainly lymphocytes and macrophages which invade human corpus luteum (Best et al. 1996; Hameed et al. 1995; Takaya et al. 1997). First, lymphatic vessels were initially detected at the periphery of regressing corpus luteum (Xu and Stouffer 2009) and later inside it. This observations suggest lymphatic vessel recruitment from preexisting vessels surrounding corpus luteum, but this

hypothesis was not demonstrated yet. The most accepted lymphangiogenic mechanism in corpus luteum seems to be VEGF-C/VEGFR-3 axis stimulated by inflammatory cells (Nitta et al. 2011) together with a possible endothelin1 overexpression (Shirasuna et al. 2012) and particular hormonal milieu (Brown and Russell 2014).

These aspects described here are controversial and incompletely characterized. Inflammation is widely accepted as a promoter of lymphangiogenesis, but cellular and molecular pathways do not give a strong support for an efficient dual anti-inflammatory and anti-lymphangiogenic therapy which should be useful for several disease. The role of newly developed lymphatic network is not well understood for most of the pathologic conditions where an active inflammatory-induced lymphangiogenesis occurs. Except tumors where lymphatic vessels represent a potential route for dissemination and metastasis, the function of inflammatory-induced lymphatic vessels can be considered as a "missing piece" of several disease pathogenesis.

Controversial data regarding lymphatic vessels' role in induced inflammation have been published in the last years. In a study performed by Aebischer et al. (2014) about contact hypersensitivity-induced skin inflammation, the authors reported that lymphatic vessels have a time-dependent dual role. In the first stages (day 2), the uptake of the dye was reduced in lymphatic vessels of inflamed skin, while on day 9 the dye uptake was increased but the lymphatic vessels had a leaky appearance. The authors considered that these differences may be associated with time-dependent changes in tissue inflammation and in inflammation-induced lymphangiogenic remodeling, but they did not take into consideration and did not give any evidences about the development of new lymphatic vessels induced by inflammatory milieu. The leakage of lymphatic vessels on day 9 was explained in this study by a malfunction of preexistent lymphatic vessels which were unable to drain the huge amount of tissue edema induced by inflammation. The authors omitted to discuss about new lymphatic vessel formation induced by inflammation. The newly formed lymphatic vessels are also leaky because of their immaturity, and thus the assessment of lymphangiogenesis using PROX1, VEGFR-3, Sox18, and COUP-TFII (to identify the commitment of endothelial cells through a lymphatic lineage) would be necessary to complete their results. Other studies using two models of acute cutaneous inflammation (one of oxazolone-induced delayed-type hypersensitivity reactions and ultraviolet B irradiation) in VEGF-C and VEGF-D transgenic mice reported that the delivery of VEGF-C- and VEGFR-3-specific ligand significantly limited acute skin inflammation. They concluded that the increased network of lymphatic vessels in these mice significantly enhanced lymphatic drainage with no major change of the inflammatory cell infiltrate or the composition of the inflammatory cytokine milieu (Huggenberger et al. 2010, 2011; Kajiya et al. 2009).

Needs of tissue edema lymphatic drainage is the most common explanation for the inflammatory-induced lymphangiogenesis following allergic conditions (Huggenberger et al. 2011), immunization (Tan et al. 2013), rheumatoid arthritis (Bouta et al. 2014), diabetes (Klueh et al. 2014), or wound repair (Hall et al. 2013; Shimamura et al. 2009). For corneal repair, lymphangiogenesis inflammatory state is mandatory (Patel and Dana 2009), but the function of lymphatic vessels is completely unknown. Corneal lymphatic vessels regress before blood vessels after complete corneal healing (Ling et al. 2012). Human pterygium is a benign condition of the eye also showing an increased number of lymphatic vessels (Liu et al. 2012), following an active inflammatory-induced lymphangiogenesis through VEGF-C upregulation (Fukuhara et al. 2013) and PROX1 overexpression. For recurrent pterygium, the outgrowth of blood and lymphatic vessels provides evidences that immunological mechanism based on increased number of mast cells, lymphocytes, plasma cells, dendritic cells, and $CD4^+$ and $CD8^+$ T cells may play a role in recurrent pterygium lymphangiogenesis most probably due to lymphatic vessels' memory stimulated by recurrent inflammation (Kelley et al. 2013).

Cellular Master Players of Lymphangiogenesis and Inflammation: Relatives or Strangers?

Inflammatory-induced lymphangiogenesis is a result of simultaneous activation of antigen-presenting cells, tumor cells, endothelial cells, and immune cells. Lymphangiogenesis induction associated with an inflammatory milieu is not a limited process. Inflammatory cells do not exert just a local effect for lymphatic vessel development. Autocrine and paracrine action of cytokines and lymphangiogenic factors secreted by immune cells increases lymphangiogenesis by promoting new lymphatic vessel formation inside tissue with inflammation and by stimulating the development of bone marrow precursors of inflammatory cells.

Myeloid Cells

Myeloid cells are closely interconnected with the embryonic lymphatic vessel development (Böhmer et al. 2010) and continue to influence normal and pathologic lymphangiogenesis during postnatal life (Zumsteg and Christofori 2012). Myeloid cells were intensely studied as a potential source of cells with a progenitor phenotype able to differentiate into lymphatic endothelial cells. In an experimental study performed by Böhmer et al. (2010),

there was identified a subpopulation of Syk^+ myeloid cells comprising largely M2-polarized mononuclear cells, expressing VEGF-C/-D and chemokines. They stimulate lymphangiogenesis in vivo. Ablation of Syk gene produces the failure to separate blood and lymphatic vasculature (Abtahian et al. 2003). In pathologic conditions, especially in tumors, myeloid-derived suppressor cells (MDSC) are $CD11b^+$ cells known as incompletely differentiated progenitor cells (Peranzoni et al. 2010). A subset of MDSC with a particular $CD11b^+$ $M2^+$ phenotype is able to differentiate into lymphatic endothelial cell and to acquire lymphatic endothelial cell markers as VEGFR-3, podoplanin, and LYVE-1 (Maruyama et al. 2005; Shapira et al. 2011; Zumsteg et al. 2009). Involved in both tumor immunity and tumor lymphangiogenesis, MDSC increase in number in the blood, accumulate in the lymphoid organs of patients with malignancies (Schmid and Varner 2012), and promote tumor immune escape. Unfortunately, in this moment, myeloid cells are described as a heterogeneous population with a dual role in inflammation, tumor progression, and metastasis with an incompletely characterized phenotype for each subset and thus with no clear future statement as potential therapeutic targets.

Macrophages

Macrophages are not a new topic for inflammatory-induced lymphangiogenesis but less is known about their involvement in embryonic lymphangiogenesis. Recent studies performed by Gordon et al. (2010) on $PU.1^{-/-}$ and $Csf1r^{-/-}$ macrophage-deficient mouse embryos revealed that the absence of macrophages induced the development of hyperplastic dermal lymphatic vessels compared with those from wild-type mouse embryos. Also, the same team demonstrated that macrophages play a tissue-dependent role in lymphatic vascular development highlighting differences between lymphatic vessels' caliber and branching of $PU.1^{-/-}$ macrophage-deficient mice from dermis, lymphatic sacs, and pleural lymphatics. Together with enlarged lymphatic vessels described in dermis, there was reported small lymphatic sac and normal pleural lymphatic vessel development compared with wild-type mouse embryos (Harvey and Gordon 2012).

A controversial issue regarding macrophage involvement in lymphangiogenesis is its ability to act as progenitor cells able to transdifferentiate into lymphatic endothelial cells. Macrophages are often observed in histological sections, being interspersed among lymphatic endothelial cells, especially in tumor-associated lymphatic vessels. A subpopulation of LYVE-1-positive macrophages is reported to be closely related to blood (Schlereth et al. 2014) and lymphatic vessels (Gordon et al. 2010). All these data should support macrophages as a potential source of lymphatic progenitor cells, but other experimental data showed that LYVE-1-positive macrophages do not express Prox 1 and, thus, are unlikely to be underwent trans-differentiation into lymphatic endothelial cells (Gordon et al. 2010).

Inflammation-induced corneal lymphangiogenesis is drastically reduced after sunitinib treatment by reducing macrophage infiltration and subsequently VEGF-C levels (Detry et al. 2013).

Sunitinib (previously known as SU11248) is an oral, small-molecule, multi-targeted receptor tyrosine kinase inhibitor targeting all receptors for platelet-derived growth factor and VEGFRs, including VEGFR-3 (Gan et al. 2009). Sunitinib also inhibits KIT (CD117). It was the first drug which was simultaneously approved for two different indications by the FDA renal cell carcinoma and gastrointestinal stromal tumor (Quek and George 2010). Immune-mediated graft rejection is one of the most devastating and challenging events encountered after organ transplantation but also a good model of inflammation-induced lymphangiogenesis. An inflammatory infiltrate is present in the transplanted renal kidney, and renal grafts contained a high number of proliferating lymphatic vessels associated with an abundant content of inflammatory cells (Kerjaschki et al. 2004). Lymphangiogenesis occurs in islet allografts in response to inflammation and plays a key role in the islet inflammation in alloimmunity. Lymphatic vessel inhibition produced by sunitinib treatment of islet allografts significantly prolonged allograft survival by blocking lymphangiogenesis and a subsequently reduced infiltration of T cells into the grafts (Yin et al. 2011).

Macrophages can be regarded as the most intensely studied cellular inductors of lymphatic vessel development in pathologic conditions, and thus they will be briefly reviewed here. Macrophages accumulated on the sites of inflammation are important sources of lymphangiogenic growth factors as VEGF-C and VEGF-D (Loffredo et al. 2014). Also, macrophages have a high plasticity. At one moment in its development, any tumor has associated inflammatory cells. Macrophages are always present among them, and thus they already represent a therapeutic target in tumors (Fridlender and Albelda 2013). They are able to infiltrate tumor and inflammatory sites, to differentiate into antigen-presenting cells and to process the antigen through a direct influence of local stromal microenvironment (Ji 2012). But the therapy seems to be not so efficient because of macrophage heterogeneity and their ability of dynamical changes during inflammation and/or tumor progression. The presence of M1- and M2-type macrophages (with its subtypes M2a, M2b, and M2c) with different activatory and regulatory mechanisms during inflammation and tumor progression and their different involvement in lymphangiogenesis impair the success of

macrophage-targeted therapy (Gordon and Taylor 2005; Mantovani et al. 2004). The phenotypic switches between M1 and M2 macrophages during tumor progression or inflammation have not been described yet. This could be a weakness of therapeutic inefficiency. For this reason, recent experimental studies tried to "manipulate" tumor-associated macrophages to favor the efficacy of immunotherapy in cancer. Fridlender and Albelda (2013) and independently Long and Beatty (2013) demonstrated that the manipulation of tumor-associated macrophages can augment antitumor effects of cancer immunotherapy and is able to suppress angiogenesis, but no data are available about their effects on lymphangiogenesis suppression.

Eosinophils and Mast Cells

Eosinophils and mast cells are main cellular components of allergic reaction and asthma (Schuijs et al. 2013; Shukla et al. 2013), both conditions being characterized by lymphangiogenesis activation and increased number of lymphatic vessels due to the higher necessity of tissue fluid drainage. Indirect evidences of eosinophils involvement in lymphangiogenesis derived from few studies describing an increase of lymphatic vessel density in angiolymphoid hyperplasia with eosinophilia, a benign, uncommon idiopathic condition, characterized by cutaneous papules or nodules and an inflammatory infiltrate, consisting mainly of lymphocytes and eosinophils (Miteva et al. 2009). Eosinophils express CD30 and its expression is upregulated in certain conditions (Berro et al. 2004). CD30 is required for CCL21 expression (Bekiaris et al. 2009). CCL21 overexpression could induce a sustained inflammatory-mediated lymphangiogenesis in several pathologic conditions as myasthenia gravis (Berrih-Aknin et al. 2009) or thyroiditis (Furtado et al. 2007). Eosinophils also express CD44, a hyaluronan receptor with a 41 % homology with LYVE-1, able to bind to lymphatic endothelial cells and to facilitate leucocytes and tumor cell migration through lymphatic vessel wall. Tissue accumulation of eosinophils around lymphatic vessels should be explained by their affinity for lymphatic endothelium through a CD44/macrophage mannose receptor-mediated mechanism (Salmi et al. 2013), facilitating tissue lymphocyte infiltration and a subsequent inflammatory-induced lymphangiogenesis.

One of the most controversial cells involved in angiogenesis and lymphangiogenesis is mast cells. They are recognized as having a dual role in inflammatory process (Galli et al. 2008). Mature mast cells have a heterogeneous content of cytokines and proteolytic enzymes, but their phenotypic expression depends on other microenvironmental factors (Theoharides et al. 2012). Depending on the location and their maturation grade, mast cells secrete several vasoactive and proinflammatory mediators. In the presence of stem cell factor (SCF), mast cell production of proinflammatory cytokines is predominant, while a combination of SCF and interleukin (IL)-4 induces the production of Th2 cytokines, mostly (Bischoff et al. 2001). Also, mast cells promote tumor growth by their accumulation around tumors followed by induction of angiogenic switch (Crivellato et al. 2008). Knowing to be a rich source of proangiogenic factors as VEGF, IL-8, FGF2, MMP2, MMP9, Angpt1, and tumor necrosis factor (TNF)-α (Soucek et al. 2007, Genovese et al. 2012), mast cells produce lymphangiogenic growth factors as VEGF-C and VEGF-D also (Detoraki et al. 2009), but the role of mast cells in lymphangiogenesis is far to be elucidated.

Recent data highlighted a crosstalk between mast cells, different molecular subtypes of breast cancer, and tumor lymphatic vessels suggesting that mast cells in the tumor microenvironment are the key players involved in the development of tumor lymphatic vessels for some molecular subtypes of breast cancer (Raica et al. 2013). Other studies revealed that blocking mast cell migration and adhesion to squamous cell carcinomas led to decreased tumor-associated lymphangiogenesis and, as a result, decreased lymph node metastasis (Brideau et al. 2007). As a paradox, just scattered data can be found in the literature concerning lymphatic vessels of mast cell tumors (Shoyinka et al. 2005), and, moreover, no data about lymphangiogenesis in mastocytoma are available.

Blood vasculature and lymphatic vessels tend to follow a similar way inside the human body. Interaction between blood vessels and mast cells is highly characterized concerning intrinsic mechanisms and factors involved in this interaction. Little is known about mast cell effects on lymphatic vasculature. Compared with blood vessels characterized by a well-structured wall making them impermeable in normal conditions, initial lymphatic vessels have a thin wall and are highly permeable. During inflammation, mast cells act on lymphatic endothelial cells by upregulation of eNOS through histamine action and also through VEGF-C and VEGF-A secretion (Lahdenranta et al. 2009; Leak et al. 1995).

If the positive role of mast cells in tumor lymphangiogenesis seems to be accepted and supported by several studies, mast cells' involvement in embryonic lymphatic vessel development and inflammatory-induced lymphangiogenesis is still a field of debate. Mast cell-deficient c-kit mutant mice exhibit normal adult lymphatic vascular patterning in adult skin (Grimbaldeston et al. 2005). Also, no abnormalities in lymphatic vascular development and patterning have been observed for mouse mast cell protease 4-deficient mice. Most of the embryonic mast cells are immature, become mature during the embryonic development, and have an organ-specific evolution being more abundant in the dermis, muscle, or adrenal and less

abundant in the lung. Some questions should arise from these data: (1) If the mast cells are not required for the development of embryonic lymphatic vasculature, how they stimulate inflammatory- or tumor-induced lymphangiogenesis? (2) Are they able to promote activation of mature lymphatic endothelial cells? (3) Do mast cells from tumors have or have not a similar phenotype with those from inflammatory, benign, or premalignant lesions? (4) Which are the main mechanisms regarding mast cells' action on lymphatic endothelial cells? (5) Are mast cells able to induce lymphangiogenesis far from the site of inflammation or malignant process as it was suggested by two studies performed by Kunder et al. (2009, 2011)?

Plasma Cells

Indirect evidences suggested that plasma cells may have a role in lymphangiogenesis. Multiple myeloma can be considered the best model to study plasma cells' role in lymphangiogenesis. Zimmer et al. (2010) demonstrated that lymphangiogenesis is a prominent feature in multiple myeloma kidneys and that it is associated with a significant accumulation of macrophages, $CD20^+$ cells, and $CD27^+$ plasma cells.

Assessment of lymphangiogenic growth factors and their correspondent receptors in multiple myeloma is just beginning (Bao et al. 2009; Golenkov et al. 2013) and is done by using in vitro models. Lymphangiogenesis is completely unknown in such disease, despite the fact that myeloma cells have a high ability to spread through lymphatic route into lymph nodes mainly. Multiple myeloma cells secrete both angiogenic and lymphangiogenic growth factors as VEGF-A, VEGF-C, and VEGF-D (Kalitin et al. 2012; Vacca et al. 2003). Neither lymphatic vessel quantification nor lymphangiogenic growth factor receptor assessment has been reported for lymphatic endothelial cells from multiple myeloma.

B Cells, T Cells, and Dendritic Cells

B cells, T cells, and dendritic cells seem to have a close relationship during inflammatory-induced lymphangiogenesis. B and T cells are immune cells certified as having a lymphangiogenic role during tumor and no-tumor lymphangiogenic processes. B cells act on lymphatic endothelial cells through several mechanisms. They are found to drive lymph node lymphangiogenesis by VEGF-A/VEGFR-2 and VEGF-C/VEGFR-3 axis (Angeli et al. 2006) and also by dendritic cell trafficking modulation (Loffredo et al. 2014). While B cells and dendritic cells are accepted as positive regulators of lymphatic vessel development, several papers reported that T cells are negatively regulating the development of lymphatic vessels. In athymic nude mice, in which functional T cells are absent, a high density of $Lyve1^+$ lymphatic vessels can be observed in the lymph nodes (Kataru et al. 2011). It seems that IFN-γ is responsible for the crucial role of T cells ($CD4^+$ and $CD8^+$) in maintaining the homeostatic balance of lymphatic vessels.

Lymphatic Endothelial Cells

No clear phenotypic differences have been stated regarding lymphatic endothelial cells in tumor and non-tumor inflammatory conditions. Few papers reported changes in the lymphatic endothelial phenotype during dendritic cell transmigration through lymphatic vessel wall (Johnson and Jackson 2013; Russo et al. 2013). During lymphatic spread of tumor cells, lymphatic endothelial cells acquire a particular phenotype different from normal one, by expression of surface markers, some of them being common to those from inflammatory process (Farnsworth et al. 2006). Inflamed lymphatic endothelium exhibited increased Prox1 and VEGFR-3 expression preceding lymphangiogenesis in vivo. Increased expression of VEGFR-3 is mediated by Prox1 and NF-κB binding to its promoter (Flister et al. 2010). Lymphatic endothelial cells and the immune cells release growth factors and cytokines during inflammation. Also, lymphatic endothelial cell proliferation during acute inflammation is governed not only by VEGF-C and VEGF-D action through VEGFR-3 but also by other factors as FGF-2, PLGF-2, HGF, EGF, and KC/CXCL17 (Lachance et al. 2013). Recently, Teijeira et al. (2012) demonstrated that CD137 marker, originally described as a surface molecule present in activated T and NK cells, was upregulated in lymphatic endothelial cells on stimulation with TNF-α, lipopolysaccharide, and IL-1β. Tissue inflammation modulates gene expression of lymphatic endothelial cells and dendritic cell migration in a stimulus-dependent manner by Prox-1, VEGFR-3, and LYVE-1 significant downregulation following contact hypersensitivity (Vigl et al. 2011). In contrast, adhesion molecules ICAM-1 and VCAM-1 were upregulated in lymphatic endothelial cells in inflammatory conditions.

Therapeutic Impact of Association between Lymphangiogenesis and Inflammation

Targeted therapy against both lymphatic endothelial cells and inflammatory cells was extensively studied on corneal model of inflammatory-induced lymphangiogenesis. Treatment with corticosteroids resulted in a significant reduction of inflammatory corneal lymphangiogenesis by decreasing the lymphatic endothelial cell proliferation, macrophage recruitment, and proinflammatory cytokine

suppression (Hos et al. 2011). Sunitinib treatment drastically reduced pathologic corneal lymphangiogenesis by reducing F4/80$^+$ cell infiltration and by blocking VEGFR-2 phosphorylation induced by VEGF-A released by macrophages (Detry et al. 2013). Even ordinary aspirin has been reported to inhibit inflammatory-induced lymphangiogenesis with a potential decrease in metastatic spread, possibly through COX-II-dependent regulation of VEGF-C expression. COX-II independent mechanisms of inhibition of lymphangiogenesis by salicylates and other non-steroidal anti-inflammatory drugs have not been investigated (Yiannakopoulou 2012). Previous studies reported that D816V mast cells (imatinib resistant, having kinase domain mutations, the most frequent of which is the substitution in aminoacid 816, lead to direct activation of the catalytic domain), isolated from patients with systemic mastocytosis or from transgenic mice, are sensitive to rapamycin, whereas normal human or mouse mast cells are not (Gabillot-Carré et al. 2006). But, in a recent paper, Ji and Eshita (2014) highlighted that, despite the fact that mast cells play a certain role in complete Freund's adjuvant-induced intranodal lymphangiogenesis, and rapamycin inhibited lymphangiogenesis independent of mast cells. Effects of sodium cromoglycate on mast cells are well characterized, but no data about sodium cromoglycate therapy on lymphatic vessels and lymphangiogenesis are available till this moment. Brideau et al. (2007) suggested that endostatin can inhibit tumor lymphangiogenesis by decreasing the VEGF-C levels in the tumors, apparently via inhibition of mast cell migration and adhesion, and support the view that the biological effects of endostatin are not restricted to endothelial cells because endostatin also regulates tumor-associated inflammation and differentiation. All therapeutic options presented above are still in an experimental phase, and the beginning of clinical trials targeting lymphangiogenesis and inflammation is far to be initiated.

Heterogeneity of inflammatory cells hampers their individual characterization concerning involvement in the initiation and progression of lymphatic vessel development during inflammatory state from non-tumor and tumor conditions. Each of them has pros and cons as they may play a role in the activation and proliferation of lymphatic endothelial cells followed by an active lymphangiogenic process, but none of them are accepted today as a true therapeutic target. Limited information about inflammatory cell involvement in the embryonic development of lymphatic vessels represents one of the "missing piece" of inflammatory-induced lymphangiogenesis characterization. Also, some cells of the inflammatory process as macrophages or dendritic cells are extensively studied, while others as mast cells, plasma cells, or eosinophils are somehow neglected. Lack of well-certified cellular and molecular markers of interaction between lymphatic endothelial cells and inflammatory cells during lymphangiogenesis slows the development of targeted therapies with dual role in the inhibition of both inflammatory cells and activated lymphatic endothelial cells.

Inflammation is a common process associated with both benign and malignant conditions. Inflammatory microenvironment in non-tumor and tumor conditions was not clearly and differentially characterized. Various lymphangiogenic factors secreted by inflammatory cells are not released in the same time during inflammatory process, and thus, by acting in different moments of lymphangiogenic process, new lymphatic vessels seem to develop in a "wave like" fashion. This could be one reason of anti-inflammatory therapy failure regarding the complete and irreversible regression of lymphatic vessels. No phenotypic differences regarding the expression of specific endothelial markers able to differentiate between activated lymphatic endothelial cells from non-tumor and tumor conditions have been reported. In this moment, a combined anti-lymphangiogenic therapy against VEGFR-3 and non-specific anti-inflammatory drugs is experimentally evaluated, but preliminary clinical data do not strongly support its extensive use as common therapy especially in inflammation associated with malignant conditions. Specific targeted therapy directed to one type of inflammatory cells is far to be elucidated and applied. For example, several anti-mast cell drugs are known to reduce inflammation, but this action is not studied in correlation with a potential decrease of lymphatic vessel density. No data about therapies against plasma cells or eosinophils and their effects on lymphatic vessel development and distribution have been reported till now.

Five-Year View

Inflammation-induced lymphangiogenesis will remain an open field of debate for both researchers and clinicians. Extensive studies concerning inflammatory cells' influence on the embryonic development of lymphatic vessels would be useful for a better understanding of interaction between inflammatory cells and lymphatic vessels in the postnatal life. Identification of specific phenotypic changes of lymphatic endothelial cells in inflammatory-induced conditions must be of researcher's focus for the next years. Also, development of targeted therapies with a dual action on inflammatory and lymphatic endothelial cells should continue based on the preclinical data evaluation. For malignant disease, the triple interaction between inflammatory, malignant, and lymphatic endothelial cells is not fully characterized, nor the effects of tumor-associated inflammation on the initiation and progression of lymph

node lymphangiogenesis before or during metastatic process. Another unresolved problem with direct impact on the effectiveness of anti-lymphangiogenic and anti-inflammatory combined therapy in metastatic malignant disease is represented by the lack of evidences about a possible different phenotype of primary tumor lymphatic vessels developed in inflammatory conditions compared with newly formed lymphatic vessel endothelium from lymph nodes corresponding metastasis. The lymphoid tissue microenvironment involvement in the lymphatic vessels does not represent a research priority in this moment.

Lymphangiogenic growth factors were proved to be secreted by lymphoid tissue and bone marrow precursor cells. Knowing that bone marrow and thymus cortex (both locations rich in inflammatory cell precursors) have no lymphatics, a big question arises: would be possible here the presence of an endogenous inhibitor of lymphangiogenesis secreted in the thymus or bone marrow able to maintain these locations free of lymphatic vessels? If the answer will be "yes" in the next 5 years, the therapy of inflammatory-induced lymphangiogenesis would be definitely improved.

Conclusions

- Inflammation-induced lymphangiogenesis is a widely accepted concept, but intrinsic mechanisms regarding interaction between inflammatory cells and lymphatic endothelial cells are not fully characterized.
- Some of the inflammatory cells seem to interact with early stages of embryonic lymphatic vessel development, but the present studies were performed on animal models or in vitro studies only. No direct evidences of similar interactions were reported for the human embryo lymphangiogenesis yet.
- Macrophages, monocytes, and neutrophils are proved to be involved in inflammatory-induced lymphangiogenesis, but mast cells, plasma cells, and eosinophils still remain controversial.
- No endogenous inhibitor of lymphangiogenesis has been described in thymus cortex or bone marrow tissue. Its hypothetical (in this moment) existence, discovery, and characterization could improve the therapy against inflammatory-induced lymphatic vessel development.
- Phenotypic differences between lymphatic endothelial cells from tumor and non-tumor inflammatory-induced lymphatic vessels could be a clue for a differentiated anti-lymphangiogenic therapy.
- Inflammatory-induced lymphangiogenesis in primary tumor and its correspondent lymph node metastasis (a rich inflammatory microenvironment) might have different molecular mechanisms with a direct impact on a different lymphatic endothelial cell phenotype of lymphatic vessels from primary tumor and nodal metastasis.
- Induction of inflammatory lymphangiogenesis inside secondary metastatic sites through factors released by inflammatory cells associated with primary tumor before metastasis is microscopically or clinically evident suggested for mast cell granules able to traffic through preexistent lymphatic vessels, but these preliminary evidences need further studies to be accepted.
- There is no specific, targeted therapy for inflammatory-induced lymphangiogenesis in this moment. More specific therapeutic options would be developed and applied in preclinical and clinical trials after a deeper and more accurate evaluation of inflammation induced lymphangiogenesis in tumor and non-tumor conditions.
- Microenvironmental changes influenced by inflammatory cells alone or by cooperation between inflammatory and tumor cells are characterized.

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Compliance with ethical standards

Conflict of interest None declared.

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