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Review Article

Lymphatics and its Role in Inflammation, Lymphoedema, Cancer and Immunity

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Abstract

Advances in our understanding of the structure and function of the lymphatic system have made it possible to identify its role in a variety of disease processes. Because it is involved not only in fluid homeostasis but also in immune cell trafficking, the lymphatic system can mediate and ultimately alter immune responses. Our rapidly increasing knowledge of the molecular control of the lymphatic system will inevitably lead to new and effective therapies for patients with lymphatic dysfunction. In this review, we discuss the anatomy and physiological function of lymphatic vessel function and explore how the lymphatic system contributes too many disease processes, including cancer and lymphedema. This review highlights the most recent developments in lymphatic biology and how the lymphatic system contributes to the pathogenesis of various diseases involving immune and inflammatory responses and its role in disseminating tumor cells.

Keywords: Lymphatics; Cancer; Immunity; Lymphoedema

Introduction

Lymphatics are network of blind ended capillaries which are found in all tissues and organs except central nervous system and bone marrow [1]. These are large flattened tube lined by attenuated endothelium. After empty of fluid and macro-molecules, it gets collapsed; hence, it is not easily seen in histopathology of various organs [2]. Lymphatics are described in literature since long time. Hippocrates in 400BC described that there are vessels which contain white cells. Later on, in 1627 BC, Aselli et al. [3] re-identified the lymphatic vessels. They found milky veins in the gut of well-fed dog. Actual study of lymphatics was done between 1902 and 1909 by Sabin et al. [4,5].

Lymphatics collect extracellular fluid present in interstitium and drain into veins. Lymphatic capillaries consist of Endothelial Cells (LEC) supported by thin discontinuous basement membrane with sparse pericytes. LEC are linked to each other with button like intercellular junction which opens in response to increased interstitial fluid pressure [6]. Button like discontinuous intercellular junction allows the collection of interstitial fluid and its content. Wide gap also allows entry of Dendritic Cells (DC).

Lymphatics drain into collecting lymphatic vessel and ultimately into the thoracic duct. Fluid is returned to the blood vessels through lymphatic venous connection at the junction of jugular and subclavian veins [7]. LEC have oakleaf shaped appearance which overlaps each other and valve like structure which opens when tissue fluid pressure is greater [8]. Contrary, if fluid pressure is higher inside lymphatic channels then valve close and trap newly formed lymphatics inside.

DC and antigen presenting cells enter the lymphatics due to chemokines CCL21 and gradient produced by LEC and interstitial flow [9-12].

Lymphatic capillaries merge into Collecting Lymphatic Vessels (CLV). LEC of collecting duct are tightly connected to each other by zipper like junctional pattern. CLV are invested by smooth muscle cells and have intraluminal valve. The vessel segments between two valves are called as lymphangion. Their valve causes unidirectional lymph flow. Smooth muscle cells are scattered around the valve but more organized along the lymphangion.

The smooth muscle cells act as active muscular pumping unit [1] and help in contraction of lymph vessels to drive lymph through lymph nodes towards blood circulation [13,14]. Usually active pumping by lymphatic smooth muscles governs lymph flow but passive forces such as pulsatile blood flow, smooth muscle contraction, fluid pressure gradient and gravity drive lymph flow [15].

Endothelial cells derived nitric oxide and neurotransmitters regulate contraction of lymphatics. Nitric Oxide (NO) is produced by Nitric Oxide Synthase (NOS) which is of three types- endothelial NOS (eNOS), inducer NOS (iNOS) and neuronal NOS (nNOS). Out of these, eNOS is expressed by LEC [16,17] calcium signalling also help in lymphatic contraction. Rise of calcium in the cytoplasm activate myosin light chain kinases which generate contractile force through cross bridging of actin and myosin light chain [18-20]. Some large lymphatics have nerve in their wall that alters contraction.

Some neurotransmitters eg noradrenalin, isoproterenol [21], substance P [21], histamine [22] and endothelin 1 also affect contraction by altering the calcium influx (Table 1) [23].

Function of lymphatics

1. Maintains tissue fluid balance.

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Table 1: Differences between lymphatics and blood capillaries.

Blood Capillaries	Lymphatic Capillaries
1. Blood vessel endothelial cells have fenestra and Weibel-Palade bodies.	1. No fenestra, no Weibel Palade bodies in the endothelium.
2. Factor VIII related antigens are present in endothelial cells of blood vessels.	2. Factor VIII related antigens are not present in lymphatic endothelial cells.
3. Basement membrane is continuous in capillaries	3. Basement membrane is discontinuous in lymphatic capillaries
4. Pericytes are present at the periphery of the capillaries	4. Either no pericytes or sparse pericytes in some area
5. No elastic fibers in blood capillaries	5. Lymphatic capillaries are supported by elastic fibers which helps in identification of lymphatics in skin
6. Prox1, VESFR3, LYVE1, Podoplanin are absent in capillary endothelial cells [2,7].	6. LEC expresses Prox1, VEGFR3, LYVE1, Podoplanin which helps in identification [24,25].

2. Lymphatics control microcirculation in the tissues because it drains away macromolecules that leaks from the blood vessels and also removes debris of daily wear and tear. Lymphatics removes 10% of interstitial fluid [24,2].

3. It transports lipids absorbed in the gut to the blood circulation [2]. These are specialized types of lymphatics of intestine which are called as lacteals which takes dietary fat- and fat-soluble vitamins and transport them to the venous channel [2,7].

4. It is involved in metastasis of cancer cells and also involved in the progression of cancer cells [2].

5. Lymphatics also takes away bacteria from the intestine hence blockade of lymphatics leads to accumulation of debris, lipid and protein in the interstitium which promotes inflammatory state rich in white blood cells and macrophages. Later cells help in deposition of collagen which causes distortion of epidermis, dermis and adipose tissue [24].

6. It transports antigen presenting cells and lymphocytes to lymph nodes to initiate adaptive immune response.

Role of lymphatics in cancer progression

Lymph node is the common site for solid tumor metastasis. Tumour cells secrete Vascular Endothelial Growth Factors (VEGF)-VEGF-C [26-28] VEGF-D [29] and VEGF-A [30] which promotes spread of tumour to sentinel lymph node. Macrophage also secrete VEGF-C and causes lymphatic network expansion even before arrival of metastatic cancer cells which has been found in metastasis of squamous cell carcinoma to the lung and lymph node [26,31].

Tumour derived VEGF-C and D increases contraction of proximal collecting lymphatic vessels and increases lymph flow which helps in tumour cell dissemination. Lymphangiogenesis is increased in premetastatic tumour draining lymph node even prior to metastasis [32]. There is also remodelling of high endothelial venules. [33,34].

Recruitment of myeloid cells, reduction of effector lymphocytes number and function which together increases metastasis [35].

Besides this, other changes have been found in stromal cells, cytokine signalling molecules and chemokines receptors, elevated prostaglandins E2 in subcapsular sinus. Elevated IL-10, TGFβ, GMCSF in tumour draining lymph nodes help in tumour metastasis [36-38].

Myeloid cells promote immunosuppressive environment and facilitate tumour growth [39]. Breast cancer cells have increased expression of chemokine receptors, CXCR4 and CCR7 which correlates with lymph node metastasis.

Blocking of CXCR4 signalling and VEGFR3 reduces lymphangiogenesis and lymph nodes metastasis in carcinoma lung [40-42].

Tumour draining lymph nodes have environment which suppresses immune response which is as follows:

- Metastatic and premetastatic lymph nodes have fewer effector T cells [39].
- Myeloid Derived Suppressor Cells (MDSC) helps in recruitment of T regulatory cells (Treg). These cells suppress proliferation of effector T cells and its function [43,44].
- LEC can present self and tumour antigen through MHC class I, molecule but due to lack of costimulatory molecules (B7.1, B7.2) causes deletion of self-reactive naïve CD8 T cells.
- LEC also expresses inhibitory ligand PDL1 and causes deletion of auto reactive T cells.
- Cancer cells produces remodelling of HEV which impairs immune cells trafficking to the lymph node. [33].
- Tumour specific T cells become tolerant by expressing CTLA-4 and PD-1.
- B cells present tumour antigen to CD4 and CD8 lymphocytes in draining lymph nodes and gets activated and secretes antibody which does not check growth of tumour cells [2].

Lymphedema

This is a progressive pathological condition where lymphatic vessels fail to drain fluid from interstitial space and return to the blood. This results in accumulation of protein rich fluid in interstitial space which produces inflammation, adipose tissue hypertrophy and progressive fibrosis ultimately which causes functional impairment and deformity [45,2].

Based on etiology of lymphedema, it is classified into primary and secondary lymphedema.

Primary lymphedema

It is rare. Its incidence is about 1.2/100000 population [46]. Usually patients are young and less than 20 years of age. It is due to dysplasia of lymph vessels and is associated with congenital syndrome [47]. Lymphatics may be hypoplastic or hyperplastic due to functional defects in lymphatics [7]. Mostly extremities are affected due to insufficient drainage but drainage in viscera is also affected [47].

Milroy's disease

It is a congenital lymphedema with autosomal dominant inheritance [48,49]. It is due to mutation in VEGF-C/VEGFR3 axis or developmental pathology. Another type of genetic lymphedema found at puberty is called as distichiasis syndrome. Patient presents with lymphedema with double rows of eye lashes (distichiasis). It is due to mutation in FOXC2 gene [50]. Which encodes fork head related transcriptional factors which are required for formation of lymphatic valves and recruits pericytes to lymphatics.

Hypotrichosis-lymphedema-telangiectasia syndrome which is

due to SOX-18 mutation. SOX-18 helps in development of blood vessels and also lymphatic vessel [51].

Secondary lymphedema

It is multifactorial caused by trauma, infection, radiation, surgery, obesity and malignancy [46,52]. It develops at later stage and progresses to chronic condition. In developed countries, it occurs after surgery or radiotherapy and affects upper extremities. Treatment of gynaecological and urological malignancy, carcinoma prostate and melanoma usually produce lymphedema of lower extremities [45]. Incidence of cancer related lymphedema after lymph node dissection or biopsy forms maximum cases (63.4%). Besides surgery and radiation, chemotherapy, hormonal therapy or targeted therapy can also produce lymphedema [53,54].

In developing countries, most common cause is filariasis caused by Wuchereria bancrofti (90%) [55,56]. It usually produces lymphedema of lower legs, chyluria and elephantiasis [57].

Pathogenesis of secondary lymphedema

Lymphatic stasis induces CD4 positive TH2 helper cell which secretes cytokines that produces fibrosis, adipose tissue deposits and lymphatic dysfunction [58]. TGFβ, secreted by TH2 cells produces fibrosis. There is correlation between disease severities with fibrosis [59]. In addition to fibrosis, TGFβ inhibits lymphatic vessel formation after radiation therapy [60,61]. Fibrosis also inhibits lymphatic regeneration [62]. Blockade of TGFβ1 promotes lymphatic regeneration, improves fibrosis and decreases TH2 cells [63].

Treatment of patients of lymphedema with VEGFC recombinant protein stimulates growth of cutaneous lymph vessel and regeneration [49,64].

Role of lymphatic system in disease process

In acute inflammation whether sterile or after infection, there is increased lymphangiogenesis [65]. This causes adequate fluid and antigen drainage to lymph node to enable antigen processing and adequate immune response. It also maintains tissue fluid homeostasis. (Liao and von der Weid 2015) [66]. Macrophages, vascular endothelial growth factor C and B cells increases lymphangiogenesis but T cells inhibit it [67-70].

Role of lymphatic in chronic inflammation

Prolonged exposure to injurious agent causes accumulation of lymphocytes which forms Tertiary Lymphoid Organs (TLO). This is because high endothelial venules which stimulate lymphocyte homing and lymphocyte collection at affected site. TLO tries to stop inflammation due to injurious agent. [71,72].

Hyperplasia of lymphatics and increased lymphangiogenesis have been reported in skin of psoriatic patients [72], in intestinal mucosa of ulcerative colitis [73], joints of experimental inflammatory arthritis [74], in kidney of transplant rejection [75]. VEGF-A also causes delayed type of hypersensitivity reaction. Antibody to VEGF R1 & R2 and VEGF-A inhibits chronic inflammatory response.

Role of lymphatics in transplant rejection

Ingrowth of lymphatics in corneal transplant causes transport of effector lymphocytes and antigen presenting cells into graft which accelerates rejection of graft [2]. In kidney transplant LEC secretes CCL21 which attracts CCR7 positive cells and transport it to lymph node and generate adaptive immune response [75]. Lymphatic vessels and lymphangiogenesis in transplant determine the outcome of graft. In early stage, it is beneficial because it reduces oedema and inflammation. In later stages, it is detrimental to graft. Blocking of lymphangiogenesis improves graft survival [76].

Role of lymphatics in arthritis

Lymphangiogenesis is increased in arthritis. Number of lymphatic vessels shows positive correlation with severity of synovial inflammation. VEGF-C and receptors VEGFR2 and R3 are expressed in increased density in inflamed synovium [77,78].

Role of lymphatics in lipid homeostasis

Lymphatic vessels mediate uptake of lipids from intestine. Lymph also promotes differentiation of preadipocytes to mature adipocytes. [79,80]. Lymphatic dysfunction causes adult onset obesity due to leakage of lymph from mesenteric lymphatic vessels which promotes lipid accumulation in adipocytes [80,7].

Role of lymphatics in hypertension

Lymphatics have role in maintaining blood pressure. In experimental animals, in salt induced hypertension, sodium accumulates in skin and produces increased density of interstitial lymphatic network and hyperplasia of lymphatics. It is due to increased VEGF-C secretion by macrophages [81]. In human beings also, increased secretion of VEGF-C has been found in patients where blood pressure changes adjusting their salt content [82].

Role of lymphatic in immune response

Secondary lymphoid organs, eg, lymph node, spleen, Peyer's patches, collect foreign antigen and antigen presenting cells to activate antigen specific lymphocytes. In peripheral tissue, special type of lymphatic capillaries are found which are called as initial lymphatics. These allow soluble material and cells to enter the lymphatics. The fluid and cells are called as lymph which is transported to regional lymph nodes. Lymph node helps in concentration and filtration of lymph fluid. Non stimulated (naive) and activated lymphocytes exit lymph node via efferent lymphatic vessels [83].

Cells which enter through afferent lymphatics are mostly memory T cells and dendritic cells whereas efferent lymphatics contain cells, which are mostly B cells and T cells which have surveyed antigens.

In the paracortex of lymph nodes, blood vessels develop into specialized structure called as high endothelial venules which expresses peripheral node address in (PNAd) and attaches with L-selectin expressing naïve lymphocytes. Binding leads to chemokine activation which produces tight binding of T lymphocytes with integrins. This results in transendothelial migration of lymphocytes [84-86]. Lymph node contains various types of reticular cells called as fibroblastic, follicular and marginal stromal cell which express various types of chemokine receptors that attracts various types of immune cells. Folicular stromal cells express CCL19, CCL21 which attracts CCR7 positive T cells and dendritic cells and its migration in paracortical area.

Follicular stromal cells, DC and marginal reticular cells express CXCL13 that helps in movement of CXCR5 expressing B cells into cortex of lymph nodes.

LEC contains lipid signalling molecules called as sphingosine-1-phosphate which facilitate egress of lymphocytes from lymph node into efferent lymphatics [87,88]. Entry of lymph node with DC, T cells and macrophages helps in generating the immune response to foreign antigens. It also prevents autoimmune response to self-antigen

because DC in lymphoid tissue are immature and expresses low level of co-stimulatory molecules hence, self-reactive T cells are deleted by anergy or inhibited by Treg cells [89,90].

Conclusion

Lymphatics are specialized vessels which collect extracellular fluid from interstitium and transport it to veins. They do not have continuous basement membrane but instead has elastic fibers to support its structure. It helps in maintaining tissue pressure, controls microcirculation, transports lipid absorbed from gut to blood circulation and helps in clearing bacteria. Lymphatics along with lymph nodes initiate adaptive immune response and prevents autoimmunity by anergy of self-reactive T cells or B cells. Lymphatics help in tumour metastasis. Blockade of lymphatics after cancer, surgery, radiation, chemotherapy, trauma or parasitic infection produces lymphedema.

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