Lymphatic Vessels: Structure and Function

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Lymphatic vessels are part of the lymphatic system. The vessels evolved phylogenetically only after it became necessary for multicellular organisms to remove fluids and proteins from tissue and return them to the bloodstream [1]. In humans, the lymphatic system begins to develop between the sixth and seventh week of embryonic development, at a time when the cardiovascular system is already functioning [2].

In an article on the drainage function of lymphatic vessels Phylida Brown states that Hippocrates (approximately 400 BC) described vessels bearing "white blood." In 1622, the Italian physician Gasparo Asellius discovered lymphatic vessels in the mesenterium of a fed dog, and he described them as "milk veins." However, the findings of three British anatomists, William Hunter, William Hewson and William Cruikshank, published during the period 1740 to 1787 were to prove hugely influential in lymphatic vessel anatomy. They dubbed lymphatic vessels (lymphatics) "absorbents" (vasa absorbantia), because their function is to absorb liquid waste. Moreover, Hewson already acknowledged the fact that lymphatic vessels evolved to produce a substance called lymph, which includes small particles (now known to be lymphocytes) essential to body growth and health. In 1995, Terence Ryan pointed out that the findings of the above mentioned three anatomists were cited in an edition of *Encyclopaedia Britannica* as early as 1806, and its readers were introduced to the function of lymphatic vessels.

Lymphatic vessels are part of the lymphatic and immune system in the body. Their draining function is very important especially during an inflammation

The 20th century brought significant advances in lymphatic system research, as new findings were published on recirculation of lymphocytes and proteins, on the ultrastructure of lymphatic capillaries, on the spontaneous contractility of lymphatic vessels, and on the transport of microorganisms by the lymph. These discoveries enabled lymphatic vessels to be visualized in vivo using vital stains, contrast substances, and radionuclide lymphangiograms [3].

The anatomic area of the lymphatic system is extensive. Olszewski [1] identified the following to be a part of the lymphatic system: interstitium, lymphatic vessels, lymphatic organs, and their mobile messengers – migrating cells. The lymphatic system functions as one whole, despite being made up of numerous differently arranged lymphatic organs across the entire body, as well as billions of individual, free-moving lymphocytes that circulate in the bloodstream, lymph and interstitial fluids. Lymphatic organs are connected by two vessel systems – the lymphatic vessel system and the blood vessel system [4]. The lymphatic tissue of a young person weighing 70 kg contains approximately 10^{12} lymphocytes, i.e., 1 kg [5]. It must be noted that the lymphatic system is part of the immune system.

Lymphatic vessels form a sort of "second circulatory system" in the body – lymphatic circulation. However, our knowledge is rudimentary when compared to our knowledge of blood vessels. In recent years, scientists in several laboratories began to study lymphatic vessels intensively and found evidence to support the fact that the "second circulatory system" is crucial for the normal functioning of the immune system and that it plays an important role in the pathogenesis of numerous diseases; for example, cancer, lymphedema, asthma, and various inflammatory diseases [6].

Lymphatic vessels form a drainage system in the body, running parallel with veins and collecting lymph from the whole body. The system of lymphatic vessels includes lymphatic capillaries, prenodal lymphatic vessels and postnodal lymphatic vessels, which converge into larger lymphatic vessels to bring lymph into the ductus thoracicus and ductus lymphaticus dexter that lead into the confluences of large veins. In the histological picture, valves similar to those in veins are found in the lumina of lymphatic collector vessels. Their function is to prevent the backwards flow of lymph.

It is hard to differentiate lymphatic vessels – especially lymphatic capillaries – from small blood capillaries in a histological examination of bioptic and especially necrotic human material. This fact has helped lymphatic vessels to escape detection by pathologists, and certainly contributed to the fact that lymphatic vessel research was always second to that of blood vessels over the years. A significant turn came in 1990 with the discovery of molecules that specifically control the development and growth of lymphatic vessels

(lymphangiogenesis), and with the identification of molecules specific for the endothelium of lymphatic capillaries [7].

Of the above mentioned molecules, one of the first was the vascular endothelial growth factor receptor-3 with its ligand VEGF-C [8]. In recent years, scientists explored the possibility of using VEGF-C and VEGF-D growth factors, which are known to cause lymphangiogenesis, in the treatment of tissue edema in various diseases and in diabetic wound healing [9]. Baluk et al. [10] described the effect of growth factors on lymphatic vessels in mice with experimentally induced chronic respiratory tract infection. The inhibition of VEGFR-3 completely prevented the growth of lymphatic vessels but not blood vessels. Insufficient lymphatic vessel growth increased the edema of the mucosa and decreased the hypertrophy of regional lymph nodes. Application of VEGF-C or VEGF-D evoked lymphangiogenesis but did not cause angiogenesis of blood vessels.

Several years ago, Banjeri et al. [11] identified a specific protein on the surface of lymphatic endothelial cells and macrophages and named it LYVE-1. They found it to be a receptor for hyaluronan. This receptor is located in the cell wall of lymphatic vessels, yet it was not found on blood vessels. Using LYVE-1 antibodies, they managed to visualize the endothelium of lymphatic vessels in tissue sections from several organs, including the skin [12]. Another molecule that can be used to identify lymphatic vessels in tissue sections, podoplanine, is a membranous glycoprotein found in endothelial cells of lymphatic capillaries. It has not been found in blood capillaries.

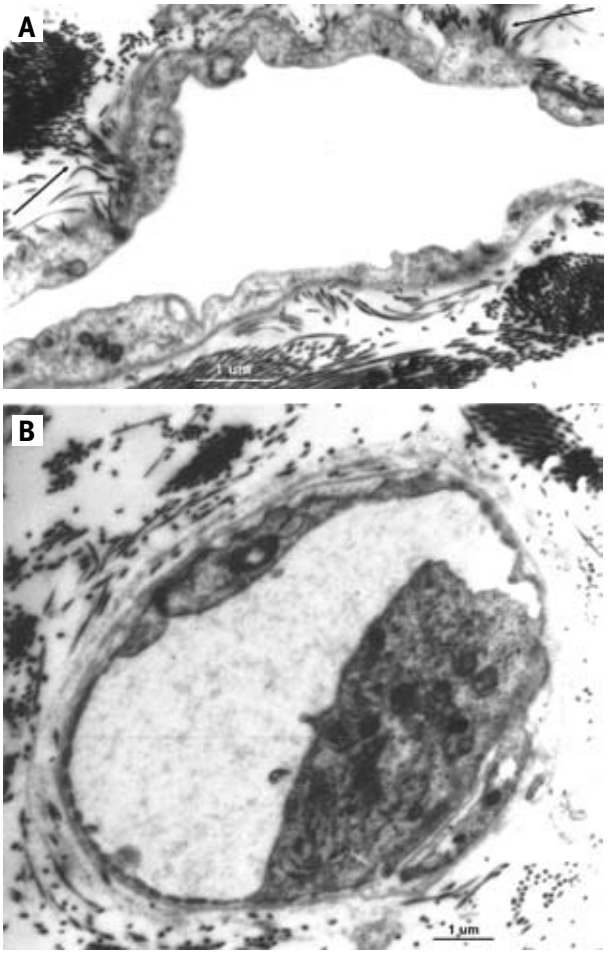
Lymphatic capillaries, often called initial lymphatics, are the thinnest lymphatic vessel. Similar to blood capillaries, lymphatic capillaries are an integral part of connective tissue, especially loose connective tissue [Figure 1].

Both blood capillaries and lymphatic capillaries are crucial to microcirculation in the loose connective tissue. Microcirculation also includes tissue channels that are the morphological substrate of extravascular microcirculation in the loose connective tissue [13]. By means of microcirculation, loose connective tissue facilitates cell nutrition and drainage of metabolism products. It is also the site of inflammatory processes.

The morphological background of microcirculation is formed by blood capillaries, the interstitium of connective tissue, and lymphatic capillaries. Tissue channels are situated in the interstitium [13]. The interstitium consists of the extravascular space between capillary walls and tissue cells. Components of the interstitium include intercellular substances (matrix), tissue fluid and controlling immune cells. The intercellular matrix is made up of fibers (collagen, elastic and reticular fibers) and amorphous substance. The amorphous substance comprises glycosaminoglycans, proteoglycans and glycoproteins. The major part of the amorphous substance is formed by glycosaminoglycan, i.e., hyaluronan. Hyaluronan forms a supporting construction for the migration and adherence of immune cells in connective tissue. Natural hyaluronan is a polymer with high molecular weight of usually over 10^6 D. In the case of an inflammation, the intercellular matrix accumulates hyaluronan fragments with low molecular weight. Hyaluronan is not only a static structural element of the interstitium but is also subject to constant metabolic turnover. During this process, hyaluronan enters lymphatic capillaries and is subsequently transported within the prenodal lymph into regional lymph nodes, where approximately 90% of it

Specialized interendothelial junctions of lymphatic capillaries enable drainage of tissue fluid, immune cells and debris from the interstitium of connective tissue to the lymph nodes

Figure 1. Electron microscopic microphotographs depicting the ultrastructure of the lymphatic capillary **[A]** and blood capillary **[B]**. There are anchoring filaments (arrows) from the adjacent connective tissue attached to the cell membrane of lymphatic capillary endothelium. The blood capillary endothelium is fenestrated and surrounded by a basement membrane.



VEGF = vascular endothelial growth factor receptor

undergoes degradation and the remaining 10% is transported by efferent lymph into the blood circulation to be metabolized later in the liver [14].

It is well known that hyaluronan may also act as a signaling molecule for cells. Interactions between the intercellular matrix and molecules on the cell surface play an important role in cell migration. Cells interact with each other through ubiquitous recognizing molecules called adhesive molecules [15]. Cell migration is crucial for morphogenesis during embryonic development. It plays an important role later in tissue repair and immunological control.

In contrast to erythrocytes, leukocytes act mostly outside the blood flow. Leukocytes exit blood circulation and enter the surrounding interstitial connective tissue where they perform immunological control [Figure 2].

Important functions of leukocytes include the identification of antigens, the destruction of invasive bacteria, and the removal of debris. The migration of leukocytes in the interstitium involves receptors acting as "legs" that help moving cells to adhere to the intercellular matrix or other cells.

Lymphatic vessels play an important role in the homeostasis of extracellular fluid. The average human body weighing 65 kg contains 3 L of blood plasma and 12 L of interstitial fluid. Up to 8–12 L of afferent lymph are produced each day, of which 4–8 L of ultrafiltrate are reabsorbed into the bloodstream in the lymphatic nodes. Lymphatic vessels transport 4 L of efferent lymph into the bloodstream daily. The concentration of proteins in plasma, interstitial fluid, afferent lymph, and efferent lymph is 70 g/L, 20–30 g/L, 20–30 g/L, and 60 g/L, respectively. The fluid turnover (including the volume of fluid reabsorbed in the lymph nodes) reaches up to two-thirds of the total volume of interstitial fluid every 24 hours.

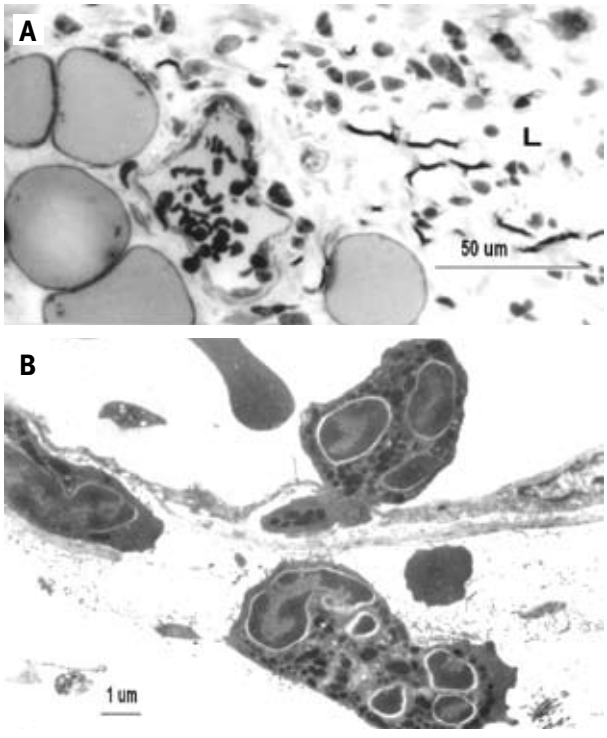
The accumulation of tissue fluid in the interstitium can cause edema in the afflicted area. Edema may occur if microvascular filtration (in the blood capillaries and venules) exceeds the lymphatic drainage for a sufficiently long period. This may be caused by a high rate of filtration or a weak flow of lymph, or a combination of both [16].

In steady state, the extravasation of fluids and proteins from blood vessels is balanced by lymphatic drainage and a return into the bloodstream. The lymphatic system is far more important in achieving homeostasis in tissues than was previously thought. Recently, it was even shown that the skin on the lower extremities contains a denser and more extensive network of lymphatic capillaries than the skin of the upper extremities [17]. Due to orthostasis, the lower extremities have a higher filtration pressure and a higher influx of fluids. Those authors [17] state that the capacity for lymph transport in the lower extremities is greater in order to compensate the higher influx of interstitial fluid caused by the effects of orthostasis and gravity.

As early as the turn of the 20th century, Starling [18] successfully demonstrated that lymphatic vessels play an important role in the regulation of hydrostatic and oncocytic pressure in the interstitium [18]. Plasmatic proteins that had entered the extravascular area (the interstitium) may return to the blood in two ways: a fraction will return through the cell wall of blood vessels, while the greater part will reach the lymphatic capillaries and will be led by the system of lymphatic vessels around and back into the bloodstream. A dynamic equilibrium is normally reached between filtration, reabsorbtion into blood capillaries, and extra tissue fluid drainage (clearance) into lymphatic capillaries, which is why no edema occurs

Edematous fluid containing more than 10 g/L of proteins is considered to be high protein fluid and signals the inflammatory origin of such edema. Plasma proteins are taken up from the interstitial tissue by lymphatic capillaries as well as by proteolysis – mainly in cases of high protein edema [19]. Proteolysis is facilitated by multiple types of cells, of which macrophages are the most important. While macrophages remain important for inflammation, we should not forget that they are present in healthy tissue as well. It has been found that 1 cm^3 of loose connective tissue contains approximately 10^7 macrophages. Their amount increases during the inflammatory process (almost tenfold). Földi and Casley-Smith [19]

Figure 2. Microphotograph **[A]** and electron microscopic microphotograph **[B]** showing the diapedesis of leukocytes – neutrophilic granulocytes – through the wall of postcapillary venule. The leukocytes are visualized passing from the venular lumen into the interstitium. The microphotograph also visualized the lymphatic capillary (L) near the venule, and a lymphocyte in its lumen.



highlighted the role of macrophages in the development and retention of chronic inflammation. Activated macrophages produce chemokines. Macrophages also play an active role in phagocytosis and cleave proteins. Lymphologists presume that tissue proteolysis is a physiological process that provides building material for tissue cells. They believe that proteolysis in tissues plays an important role in the inflammatory process.

However, lymphatic vessels are not simply passive drainage tubes draining interstitial tissue fluid. Spontaneous contractility of lymph vessels is utilized in lymph transport. Active contractions of lymph vessels were described by Hewson as early as 1774, but their important role was recognized only recently. Regular contractions of lymph vessels at a frequency of 2–4 per minute were observed in vitro in lymphatic vessels isolated from cattle mesenterium. Lymphatic vessels were even found to be able to pump fluid against the hydrostatic gradient. Spontaneous contractility of prenodal lymphatic vessels has been observed in humans, and these contractions were demonstrated to be driving the lymph [20]. Therefore, the contractility of lymphatic vessels is seen as an important driving force of lymph propulsion. The lymph flow is controlled by neuroendocrine mechanisms. Catecholamines have been proved to promote the contractility of lymphatic vessels and foster lymph flow both in vitro and in vivo. Both adrenergic and cholinergic nerves were detected in lymphatic vessel walls.

Another physiological function of the lymph vessel system includes the transport of blood cells. The peripheral (afferent, prenodal) lymph contains rare erythrocytes from the interstitium. Tissue fluid that had not been absorbed into blood vessels is drained away through lymphatic vessels, together with proteins, macromolecules and cells that become part of the lymph upon their entry into lymphatic capillaries. Also, small lymphocytes, plasma cells, macrophages, monocytes, neutrophilic and eosinophilic granulocytes, and large basophilic cells were all found in the prenodal lymph cannulated from lymphatic vessels from limbs and some organs of sheep [21]. The afferent lymph is populated mainly by monocytes, because they continuously exit the bloodstream, migrate, undergo differentiation in the interstitium and perform their function, and enter the lymphatic capillaries [21]. Monocytes, macrophages and dendritic cells are usually not found in the efferent lymph [22].

The existence of lymphatic capillaries influences lymphocyte recirculation and immune cell movement through the interstitium of connective tissue in peripheral organs (e.g., intestine, skin, kidney and others)

The central (efferent, postnodal) lymph contains noticeably more blood cells, because lymph nodes are where flowing lymph receives lymphocytes from postcapillary venules. This process was described by Gowans and Knight in 1964 [23]. Through the postnodal lymph, lymphocytes return to the bloodstream; this is called lymphocyte physiological recirculation. Those researchers [23] described a modified endothelium in the postcapillary venules of the lymph nodes which gives these cells a specific appearance under the microscope, and accordingly named them high endothelial venules. The specialized endothelium of postcapillary venules in the lymph nodes enables subpopulations of lymphocytes to move from the bloodstream into the lymph more readily than would be the case for any other tissue [4]. The extravasation of lymphocytes begins with the interaction between lymphocytes and high endothelial venules, which is in turn made possible by the specific interaction between the receptor and the ligand. Morgan and Holt [24] found that only lively, completely functional lymphocytes enter the lymph nodes from the bloodstream, having a normal cell surface capable of interaction with the endothelium of high endothelial venules.

It was later found that lymphocytes also continuously migrate (recirculate) from the bloodstream into the lymph in the intestine, through lymphatic tissue called gut-associated lymphoid tissue [25]. By cannulating the lymph from afferent lymphatic vessels in sheep, it was found that lymphocytes also recirculate in the skin, kidney and other organs. Recirculating lymphocytes carry out immune control in almost all tissues, and they are responsible for spreading immune responses and distributing immune memory in the entire organism [26]. In regulating immune responses the important role of vitamin D was stressed by Toubi and Shoenfeld [27].

The majority of mature lymphocytes continuously recirculate from the blood into tissues, and back again into the bloodstream through lymph at a rate of once or twice a day. The 12–24 hour cycle of recirculation is repeated again until the cell finds its antigene, or dies [28].

Postcapillary venules that resemble high endothelial venules in the lymph node paracortex are also found in tissue afflicted by chronic inflammatory processes. These vessels are often surrounded by a large number of lymphocytes [29]. It is known that a large number of adhesive molecules, cytokines and chemokines take part in the migration of immune cells from the bloodstream into tissues [28]. However, only a few experiments aimed at studying the mechanisms of immune system cell entry from interstitial connective tissue into afferent lymphatic vessels have been carried out [22]. Irjala and co-authors [30] identified a molecule which they named the "common lymphatic endothelial and vascular endothelial receptor-1" (CLEVER-1) that mediates the bonds of lymphocytes to both high endothelial venules and lymphatic vessels. The authors suggest that CLEVER-1 regulates the recirculation of lymphocytes and is active in the migration of leukocytes to the sites of inflammation. It was recently found that the exit of T lymphocytes from

CLEVER-1 = common lymphatic endothelial and vascular endothelial receptor-1

peripheral tissues into lymphatic vessels is dependent on the chemokine receptor CCR7 [31].

The migration of subpopulations of small lymphocytes is tissue-specific. Naïve lymphocytes are programmed to recirculate through the lymph nodes. In contrast to naïve lymphocytes, memory and effector lymphocytes exit the blood and migrate through loose connective tissue situated in the peripheral organs, e.g., in the intestinal mucosa, lung interstitium, skin, or joints [28].

In studying the lymphatic system during the ontogenetic development of sheep, Cahill and team [5] found extensive recirculation of T lymphocytes and dendritic cells through peripheral tissues. The authors concluded that the considerable recirculation of T lymphocytes is the same feature of the fetal immune system as liveborn animals.

By cannulating lymphatic vessels, lymphologists found in as early as 1980 that lymphocyte migration differs considerably from the migration of other cells from the bloodstream. Lymphocytes migrate from the bloodstream into lymph even if an exogenous antigene is absent. Lymphatic capillaries are entered by antigens from interstitial connective tissue. It may be said that almost all natural immune system stimulation is caused by the entry of an antigene (e.g., viruses, bacteria, allergens) through intact or damaged skin or mucosa. As soon as antigens enter the interstitium in connective tissue, they quickly enter the lymph through specialized interendothelial junctions in the walls of lymphatic capillaries. Afferent lymphatic vessels then deliver the antigens into the regional lymph node in which the immune response is induced. This response is then "sent" into the entire body through lymphatic and blood circulation [4]. The study of the contents of efferent lymph taken from cannulated efferent lymphatic vessels of experimental animals has shown that up to 5 ml of lymph per hour and 30–50 million lymphocytes per hour can be taken from one lymph node at ease, weighing approximately 1 g. Approximately 90% of small lymphocytes in lymph flowing from the node had entered it from the blood through high endothelial venules, 2–3% were proliferated in the lymph node, and 5–10% arose from the peripheral (afferent) lymph. The afferent lymph contains 10–20% macrophages and some small lymphocytes, while the efferent lymph contains almost no macrophages but 20–30% of small lymphocytes. The fate of macrophages that enter the lymph node from the afferent lymph is not clear, but about 10^7–10^8 macrophages will vanish completely every day in a lymph node weighing 1 g [4]. A lymph node in which an immune response is taking place shows changes in cell migration. The migration of lymphocytes from the bloodstream to the lymph increases.

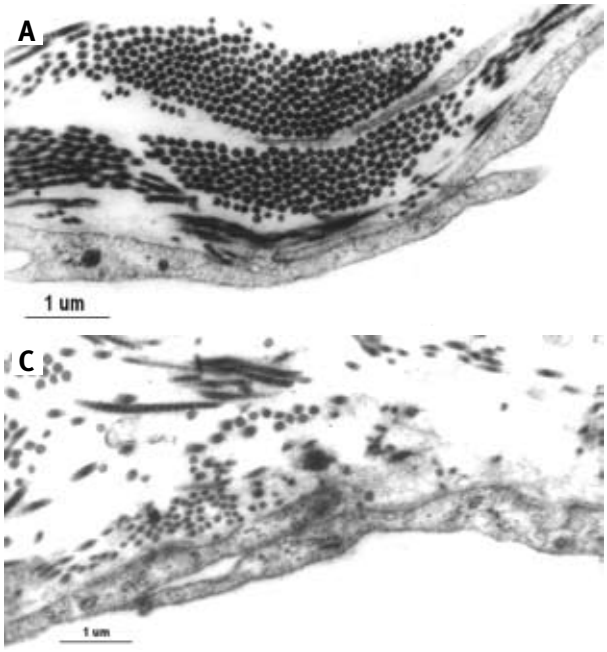
Afferent lymphatic vessels enable the transport of other cells of the immune system, such as dendritic cells, from peripheral organs (skin, synovial membrane, and others) to the regional lymph nodes. Dendritic (antigen-presenting) cells meet foreign antigens in the skin. However, lymph nodes are the optimal place for presenting antigens to T lymphocytes; therefore, it is necessary for dendritic cells to enter the lymphatic capillaries and travel to the lymph nodes in the prenodal lymph. At present, we know that dendritic cells are attracted to the lymphatic capillaries (initial lymphatic vessels) by the CCL21 and CCR7 chemokines [32].

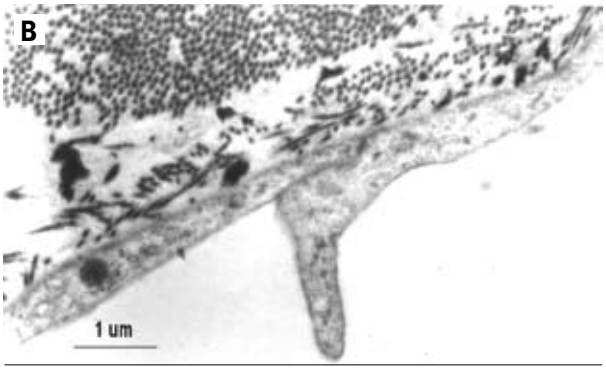
The drainage function of lymphatic vessels is crucial for inflammatory reactions in the interstitium. During an inflammation, lymphatic vessels even proliferate, and lymphangiogenesis occurs. This process was described in 1937 by Pullinger and Florey [33], who stressed that debris is removed from the place affected by inflammation by the lymphatic vessels, either directly or through phagocytic cells. Olszewski [34] reported having found cell debris in the afferent lymph obtained by cannulating superficial lymphatic vessels in lower extremities of humans. The afferent lymph contained apoptotic cells. He described apoptosis in 20% of lymphocytes in the afferent lymph. In addition, he observed fragments of membranes, nuclei, mitochondria and fibrinogen in human afferent lymph by electron microscopy. Macrophages and cell debris were also reported to have been found in the lymphatic capillaries of the synovial membrane obtained from operation material from synovectomies in patients with rheumatoid arthritis and juvenile idiopathic arthritis [35,36]. The observation of lymphocytes, monocytes, macrophages and cell debris in the lumina of some lymphatic capillaries confirmed the drainage function of lymphatic vessels in the synovial membrane.

The system of lymphatic vessels forms a functional entity with the pre-lymphatic tissue channels situated in the interstitium of connective tissue. Using electron microscopy, these tissue channels were described by Casley-Smith [37]. The results of microscopic observations have suggested that the most peripheral part of the lymphatic system is a completely open system of tissue channels. The traditional concept of the blind ending (or beginning) of lymphatic vessels is seen by lymphologists to be a result of the retrograde filling methods used in morphology.

In conclusion, we would like to describe the histological structure of the most delicate lymphatic vessels – lymphatic capillaries, and answer the following question: How do the immune cells that perform immunological control in connective tissue enter the lymphatic capillaries from the interstitium? The walls of lymphatic capillaries are composed of endothelial cells. Lymphatic capillaries are not lined by a basement membrane. The surrounding connective tissue fibers are directly anchored to the endothelial cells of lymphatic capillaries [Figure 1]. This fact was recognized by Pullinger and Florey in 1935 using a light microscope to study the skin of experimental animals with edema [38]. Endothelial cells of lymphatic capillaries are interlinked with intercellular junctions, the details of which were revealed by the method of

Figure 3. Electron microscopic microphotographs **[A,B,C]** depicting the ultrastructure of specialized intercellular junctions between the endothelial cells of lymphatic capillaries. Specialized interendothelial junctions consist of overlapping extensions of adjacent endothelial cells. Bundles of collagen fibers and elastic fibers are depicted in the surrounding connective tissue.

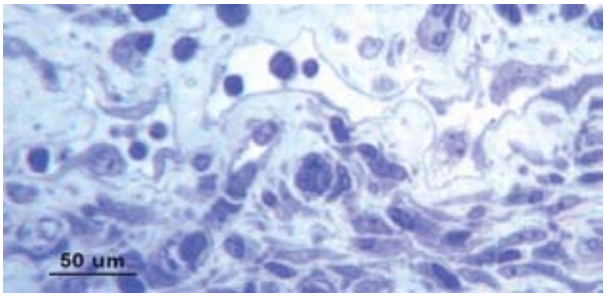




transmission electron microscopy. As described by Leak and Burke in 1996 [39], specialized inter-endothelial junctions play a substantial role in the draining function of lymphatic capillaries. These specialized junctions consist of mutually overlapping endothelial extensions. Cell membranes of overlapping endothelial extensions are not connected by intercellular adhesive junctions. The adjacent connective tissue fibers (anchoring filaments) are anchored only to the external extension. While the external extension of the endothelial cell is firmly attached to the adjacent connective tissue by anchoring filaments, the internal extension (flap), which is unattached, may act as a single valve. When the interstitial pressure rises, the internal extension bends into the lumen of the lymphatic capillary, creating a direct communication between the interstitium space and the lymphatic capillary. As soon as the pressure in the lymphatic capillary lumen exceeds the pressure in the adjacent tissue, the internal extension will cover up the external extension [Figure 3]. This mechanism guarantees a one-way flow to transport interstitial fluid, large molecules and cells from the interstitium into the lumen of the lymphatic capillary. Specialized inter-endothelial junctions may open up as much as several micrometers [40]. As the specialized inter-endothelial junctions are similar to valves both in their morphology and function, they were later named endothelial microvalves or primary valves.

Lymphatic capillaries are part of the microcirculation in the connective tissue. Immune cells that perform immunological control in peripheral organs enter the lymphatic capillaries from the interstitium [Figure 4].

Figure 4. Microphotograph depicting lymphatic capillaries. Their lumina contain several lymphocytes and macrophages (semithin resin section stained with toluidine blue).



These cells are then further transported in lymph by prenodal lymphatic vessels into lymph nodes, while some of them are subject to recirculation into the blood circulation.

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