Gut Lymphatic Vessels

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Lymphatic vessels play a distinctive role in draining fluid, molecules and even cells from interstitial and serosal spaces back to the blood circulation. Lymph vessels of the gut, and especially those located in the villi (called lacteals), not only serve this primary function, but are also responsible for the transport of lipid moieties absorbed by the intestinal mucosa and serve as a second line of defence against possible bacterial infections.

Keywords: lymphatic vessel ; lacteal ; microbiota

1. Foreword

The lymphatic system is a fascinating and still partially undiscovered fluid transport system that lies in parallel with the blood circulation and complements it by returning the liquid filtered from the blood capillaries towards the interstitial spaces back to the blood stream. Its role is fundamental in maintaining a functional fluid volume and composition in various areas of the body, preventing organ failure. In this review, we will briefly discuss the general mechanisms of lymph drainage and propulsion, and then focus on the most recent findings that pertain to the exquisite, peculiar environment of the initial lymphatic vessels of the gut, the lacteals. They have recently been the site of extensive research because of the pivotal role that the close association between lacteals and microbiota exerts on the whole-body homeostasis.

2. General Overview of the Lymphatic System

The lymphatic network is widely distributed throughout the body, arising as lymphatic *capillaries*, thin-walled vessels devoid of lymphatic muscle (LM), connected to the extracellular matrix by anchoring filaments, forming primary valves[1]. Lymphatic capillaries then drain into progressively larger and converging *collecting lymphatics*, which are equipped with a LM layer owning unique features (as it displays skeletal, cardiac, and smooth muscle contractile elements[2]), and possess intraluminal valves[3], separating adjacent vessel segments named "*lymphangions*", the functional contracting pump units of the lymphatic system. The proper function of the lymphatic system is critically related to the development of pressure gradients between the vessel's segments and/or surrounding tissue. According to Starling's Law[4], lymph formation depends upon the transmural pressure gradient (ΔP_{TM}) between intraluminal (P_{Lymph}) and interstitial (P_{int}) hydraulic pressures ($\Delta P_{TM} = P_{Lymph} - P_{int}$). Lymph propulsion is due to the intraluminal hydraulic pressure gradient (ΔP_{Lymph}) across adjacent *lymphangions* ($\Delta P_{Lymph} = P_{L,1} - P_{L,2}$), acting against an overall opposite pressure gradient[5]. In most tissues' lymphatic capillaries, P_{Lymph} is almost slightly subatmospheric[4], whereas in the venous system, the intraluminal pressure is ~10 cmH_2O. However, exceeding the transvalve ΔP_{Lymph} (1–1.5 cmH_2O) is enough to guarantee the proper lymph propulsion to the downstream lymphangion, against an adverse hydraulic pressure gradient and the force of gravity[5].

ΔP_{TM} and ΔP_{Lymph} are deeply affected by different mechanisms, either involving the spontaneous contraction of the vessel itself ("*intrinsic*" mechanism) or mechanical stresses originating in the surrounding tissues ("*extrinsic*" mechanisms). The intrinsic mechanism is predominant in vessels located in soft tissues and body areas experiencing no significant tissue displacement, such as mesenteric lymphatics. It relies on spontaneous contractions of the vessel triggered by pacemaker cells in the LM layer[6][7] and then transmitted to electrically coupled LM cells in the vessel's wall[8]. Different pacemaking mechanisms have been proposed, such as Spontaneous Transient Depolarisations (STDs)[9][10] induced by calcium-dependent chloride currents or I_f-like currents, due to hyperpolarisation-activated cyclic nucleotide (HCN) channels, similarly to what occurs in the heart sinoatrial node[11][12]. Hence, in analogy to the cardiac cycle, LM intrinsic activity generates phasic contractions, displaying an active systolic phase, which forces lymph *propulsion* to the adjacent vessel segment, and a passive diastolic phase, due to LM relaxation, which favours lymphangion fluid *refilling*. The whole mechanism can be described in terms of contraction frequency and ejection fraction or stroke volume[13][14].

Lymph flow dynamics and the surrounding microenvironment can deeply affect lymphatic spontaneous contractions. Changes in transmural and/or intraluminal pressures, lymph flow-induced wall shear stress, nitric oxide, histamine, fluid osmolarity, local tissue temperature and neuronal modulation by the autonomous nervous system can significantly alter contraction frequency (i.e., chronotropic effect) and/or contraction amplitude (i.e., inotropic effect), continuously modulating and adapting lymph drainage and transport to current needs[15][16][17][18][19][20][21][22][23][24][25][26]. Impaired intrinsic contractility, as well as lymphatic vessels obstruction, may lead to oedema development as a result of tissue fluid imbalance[4]. The extrinsic mechanism, on the other hand, is related to mechanical stresses arising in surrounding tissues then transmitted to the lymphatic vessels by means of fibrous elements of the extracellular matrix[1]. It typically involves vessels located in areas of the body which experience cyclical movements such as the heart or skeletal muscle, lymphatics undergoing cardiogenic activity or respiratory movements, intestinal motility, external compression and arteriolar vasomotion[17][27][28][29][30][31][32]. These mechanisms rhythmically exert external forces compressing and expanding lymphatic vessels, thus dramatically affecting primary and intraluminal valves dynamics and both ΔP_{TM} and ΔP_{Lymph} .

Intrinsic and extrinsic mechanisms may coexist according to area on the body: their relevance depends upon the sources of extrinsic forces ranging from blood vessels' vasomotion caused by the pulsatile blood flow, to skeletal muscle fibres' contraction. Indeed, in the rat diaphragmatic lymphatic network, both intrinsic and extrinsic mechanisms cooperate as the contraction of the skeletal muscle fibres is adequate to sustain lymph flow in vessels of the tendinous and medial muscle regions, but it is not sufficient in the muscular periphery adjacent to the chest wall, where intrinsic contractions are required to prevent fluid accumulation[33][34][35]. However, if extrinsically related mechanisms are sufficient to generate lymph flow-supportive pressure gradients for proper lymph propulsion, when flow rates are elevated, lymphatic vessels generally display their own flow-induced inhibition of the spontaneous contractions, and lymphatics behave like conduits[36].

3. The Lymphatic System of the Intestine and Mesentery

The organisation of the lymphatic network greatly varies among different body areas. In the intestine, a three-level distribution of lymphatic vessels can be identified: (a) in the small intestinal villi, (b) in the submucosa and (c) in the smooth muscle layer surrounding the mucosa[37]. The blind-ended lymphatic capillaries, also known as intestinal *lacteals* (**Figure 1**), are exclusively located in the centre of villi, normally reaching 60–70% of the villus length[38], which is, however, variable among different intestine tracts. Indeed, villi length decreases from the duodenum to the jejunum and distal ileum. As a result, according to the absorptive properties of the intestinal epithelium, lacteals are longest in the duodenum, where most nutrient uptake occurs. Lacteals merge at the villi basis, forming the submucosal network. Intestinal villi contain a blood vascular capillary network and 1–10 central lacteals, providing a route for absorbed nutrient distribution[39][40]. Water-soluble molecules enter blood vessels and are transported to the portal vein; conversely, lipids and other lipophilic molecules of large size such as chylomicrons enter lymphatic vessels, which then reach the blood circulatory system without passing through the liver. Such a privileged delivery route can also be used to enhance the bioavailability of oral lipophilic drugs, thus improving the efficacy of therapeutical strategies[41][42].

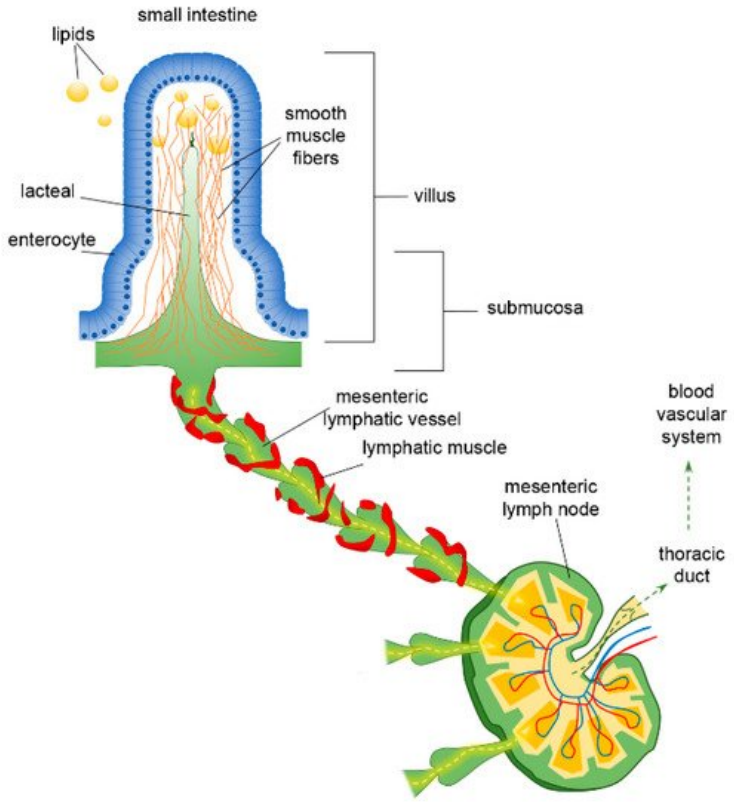

Figure 1. Functional organisation of lymphatic capillaries (*lacteals*), submucosal and mesenteric collecting vessels in the intestine. Dietary lipids are absorbed at the epithelial surface of the intestine, entering lacteals by paracellular and/or transcellular mechanisms. Lacteals are located at the centre of the intestinal villus, surrounded by villus smooth muscle fibres. They merge at the villus basis forming the submucosal network and then *lymph* is propelled along mesenteric collecting vessels endowed with a lymphatic muscle mesh. Lymph passes through mesenteric lymph nodes, ultimately reaching the venous circulatory system via the thoracic duct.

As other lymphatic capillaries, lacteals are non-contracting vessels, having no LM elements in their vessels' walls nor intraluminal valves. Therefore, lymph drainage by intestinal lymphatics is deeply affected by extrinsic forces related to vasomotion and intestinal motility[43][44]. Indeed, the pulsatile activity of neighbouring arteries as well as villous motility may easily mechanically deform lymphatics. Lacteals are surrounded by villus smooth muscle fibres, organised in a tree-like structure (**Figure 1**), whose contractile activity exerts extrinsic forces contributing to enhance intestinal lymph and blood flow, propelling lymph at velocities up to 150 µm/s[45], with a positive effect on lipid absorption[44]. Lacteals' periodic squeezing due to the contraction of those longitudinally oriented smooth muscle fibres is critically modulated by neurohormonal factors released by the autonomic nervous system. Thus, in the intestinal lymphatic network, neuromodulation may exert a mixed modulatory role by acting on both intrinsic and extrinsic mechanisms of drainage and propulsion[45]. Moreover, the contraction of smooth muscle layers in the intestinal wall gives rise to a compressive stress on lacteals and gut lymphatics, favouring vessel squeezing and lymph propulsion. On the contrary, when smooth muscle relaxes, lymphatics are stretched and a net ΔP_{TM} and/or ΔP_{Lymph} favouring fluid entry is provided. Thus, intestinal lymph drainage and propulsion are pulsatile. Lymphatic vessels in the smooth muscle layers are anatomically segregated from submucosal ones; however, both networks merge into larger collectors next to the mesentery, where almost all the lymph is of intestinal origin[46]. Here, the collecting vessels are equipped with intraluminal valves and a proper LM mesh (**Figure 1**) so that intrinsic spontaneous contractions can be identified along the lymphangion chain, allowing lymph propulsion. In rat mesenteric lymphatics, spontaneous contractions arise in the smaller vessels and then propagate to the larger collecting lymphatics, generating progressively higher pressure oscillations from distal (2–4 cmH_2O) to proximal vessels (up to 10–20 cmH_2O)[5]. Those lymphatics display an intrinsic contraction frequency of about of 6.4 ± 0.6 cycles/min and an ejection fraction of about 67% of their resting diastolic volume[47]. Lymph propelled along the mesenteric lymphangions chain passes through mesenteric lymph nodes, then drains into the thoracic duct and, eventually, empties into the blood circulatory system at the level of the subclavian vein (**Figure 1**).

The proper development of a fully functional lymphatic system, essential to guarantee fluid homeostasis, is critically related to the master regulatory gene *Prox1* (Prospero homeobox protein 1), as *Prox1*-null mice are devoid of lymphatic vessels, whose deficiency results in severe oedema and prenatal death at embryonic day E14.5[48]. Heterozygous *Prox1* mice often die at birth or soon after birth, mainly due to lymphatics' defective growth, particularly displaying dilated and dysfunctional submucosal and mesenteric vessels, and impaired lymph drainage, also resulting in chylous ascites and/or chylothorax[49]. However, in surviving haplo-insufficient mice lymph, abnormal leaking from gut lymphatics in the visceral

area accumulates in the surrounding tissues and causes an increase in adipose tissue. This results in adulthood late-onset obesity due to subcutaneous and intra-abdominal dysfunctional lymphatic-related fat accumulation and, eventually, adipocyte proliferation[49][50]. Moreover, *VEGFC* (Vascular endothelial growth factor C) growth factor, which is implicated in prenatal lymphatic system development, is also required for intestinal lymphatics' maintenance during adulthood[51]. As *VEGFC*-null mice die around embryonic day E15.5–17.5 lacking lymphatic vessels differentiation, postnatal deletion of *VEGFC* results in the regression of lacteals. Since, in adulthood, lacteals continue to grow and expand to guarantee proper lipid absorption, smooth muscle fibres located in the villi and within the intestinal inner circular muscle layer may be the prominent *VEGFC* source to maintain proper organisation of intestinal lymphatics[51][52].

4. Maturation and Stability of Lacteals

To date, few mechanisms have been elucidated regarding the development and maintenance of a fully functional lacteal network in the adult subject, and, surprisingly, they all require the presence of a normal *gut microbiota*. Lymph drainage from the interstitial space of the villi represents a balanced mechanism of different needs: while a lymph drainage increase can improve the immune surveillance keeping pathogens under control[53], on the other hand, a lymph drainage reduction can prevent damages caused by the spread of pathogens and/or pro-inflammatory factors coming from nutrients hydrolysis closely in contact with a deteriorated intestinal epithelium[54][55][56].

Lacteals sprout into the villi around postnatal day 7 and continue to develop and remodel after weaning at P21 (in mice), into adult life[57]. The first evidence of the need of gut microbiota for proper lacteals development came from the findings that germ-free (GF) mice, which entirely lack an endogenous microbiota, have decreased lacteal length and a significantly lower number of lymphatic endothelial cells (*Prox1*$^{+}$) in their villi and reduced *VEGFR3* (Vascular endothelial growth factor receptor 3) expression, when compared to same-age mice grown in a controlled, specific pathogen-free condition[58]. Disruption of intestinal lymphatics, in adult mice, leads to immune homeostasis failure and results in rapid lethality, due to the lack of immune surveillance that lacteals and mesenteric lymph nodes are expected to deploy[38][59]. Interestingly, lymphatic regression only affects lacteals, since this phenomenon was not observed in other organs and tissues where lymphatic networks are present, such as diaphragm, skin and trachea.

From a purely phenomenological view, the lacteals wall is not able to selectively avoid the drainage of pathogens, endotoxins and/or pro-inflammatory molecules present in the villi interstitial space. This is due to button-like junctions between adjacent lymphatic endothelial cells, which allow their free, overlapping ends to open and behave similar to unidirectional primary valves[60], favouring interstitial liquid (and all the dissolved and suspended particles) progression into the vessel lumen. Therefore, the prevention of lacteal-draining toxic gut-derived lymph to the rest of the body depends on the maintenance of mucus and epithelial cells' integrity[61]. In healthy individuals, the gut microbiota produces short-chain fatty acids, which stimulate the epithelial cells to produce mucus and antimicrobial peptides, thus increasing the mucosal immune response. Mucus creates a favourable environment, which harbours commensal microbiota, protecting the intestine against colonisation by pathogenic agents[62], and a very hostile environment for pathogens, which are mostly excluded from reaching the epithelial layer[63][64]. Despite the healthy intestine being lined by a monolayer of epithelial cells, it represents a proper selective barrier, thus controlling the movement of different substances and macromolecules. This is due to tight junctions (TJs) and junctional adherens molecules (JAMs) between neighbouring cells, forming a strong seal which regulates the paracellular pathway and prevents the uncontrolled systemic spread of potentially toxic agents[65][66]. In critical illness, TJs homeostasis can be impaired by proinflammatory cytokines, pathogens and lipopolysaccharides, damaging the integrity of the intestinal epithelium. The increase in barrier permeability with the loss of functionality affects not only fat absorption, but also leads to dysbiosis and to an inflammatory-related alteration of immunosurveillance.

5. Closing Remarks

Lymph formation and propulsion are crucial to attain the correct fluid homeostasis of interstitial tissue and serosal cavities. In the peculiar gut microenvironment, this primary requirement is intertwined with the need of lipid transport associated with the absorption of dietary lipids, and the compartmentalised immunosurveillance exerted by dendritic cells (DCs) recirculating between the villi interstitial space and mesenteric lymph nodes. All these factors are mutually coordinated and any small imbalance, in the short or medium time frame, can cause severe illness due to oedema, reduced dietary lipids transport to the blood or even lack of immune surveillance. Most of the research in recent years has been focused on the primary site of potential translocation of bacteria, bacterial-derived or even tissue-derived toxins to the lymph, trying to unveil possible sites of intervention at the first step of this potentially life-threatening process.

Is the influence of the microbiota's density and composition on lacteals development and stability a one-way relationship or is there a mutual exchange and effect by lacteals as well? While the DC-mediated transport of invading intestinal bacteria is well acknowledged, very few research studies are related to the possible alteration of the microbiota in response to a primitive impairment of lymphatic function. Among others, in chronic colitis mice, the supplementation of *VEGFC* causes an increase in lymph drainage from the small intestine, and this, in turn, alters the composition of the intestinal microbiota, causing a net reduction in its amount but not in its diversity. Overall, an increased Bacteroidetes/Firmicutes ratio caused by increased lymphatic drainage closed the gap towards a healthy microbiota profile, thus reducing colitis[67]. Despite the very small amount of data collected so far, it is envisaged that a more efficient lymphatic drainage might exert a positive effect on the composition of gut microbiota, potentially through a better immunological control on the phyla.

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