

Immunity and Gastrointestinal Disease: A Role for Lymphatic Vessels

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Abstract

The lymphatic system comprises a series of vessels that operate together to maintain tissue fluid homeostasis as well as traffic nutrients and immune cells throughout the body. Immune cell trafficking is an important and often overlooked function of the lymphatic system and over the years, data has amassed which supports its vital role in a healthy immune system. An intact lymphatic system is not only involved in activation of the immune system in response to a pathogen, it is also involved ensuring that the body does not exaggerate responses to otherwise benign molecules encountered every day. Nowhere is this pivotal role for the lymphatics more apparent than in the gastrointestinal system, an organ system that is assaulted daily with antigens and potential disease-causing pathogens. Dysfunctional lymphatic systems have been reported in patients in which uncontrolled inflammation and exaggerated immune responses occur; inflammatory bowel disease is one such disease. Animal models lend support to the role the lymphatic system plays in modulating inflammation and trafficking of immune cells to lymph nodes. New evidence even suggests lymphatics may play a role in food allergies. This review aims to highlight some of the recent evidence emerging with respect to lymphatic system involvement in intestinal inflammation, immune cell trafficking and food allergies.

Keywords: Lymphatic system; Lymphatic pumping; Lymph flow; Inflammation; IBD; Crohn's disease; Dendritic cells

Introduction

First discovered in the 1600s and initially confused with veins, we now know how important lymphatic vessels are in transporting nutrients, maintaining tissue fluid balance, facilitating an immune response and even promoting cancer metastasis. While lymph nodes and lymphatic vessels, the two main components of the lymphatic system, are found throughout the body, they are especially important in the gut where they mediate immune-homeostasis. Our gastrointestinal system is subject to a barrage of microbial antigens, and a functional lymphatic vasculature is vital to ensuring these antigens are appropriately dealt with. By impairing transport via the lymphatic vessels in animals, researchers have shown the importance of this system in maintaining tissue health and immunity; recent studies on human tissue samples have enforced this. This review aims to highlight the functions of the lymphatic system in the gut in the context of inflammation and immunity.

Immune System Activation

Lymph nodes are small, bean-shaped organs of the lymphatic system that act as a garrison for naïve lymphocytes and other immune cells; in lymph nodes antigens are presented to lymphocytes either freely flowing or via antigen-presenting cells (APCs). This critical interaction between APCs such as dendritic cells (DCs) and naïve lymphocytes is fundamental to maintaining immune homeostasis and can be the site of dysfunction in immune conditions such as Inflammatory Bowel Disease (IBD). DCs are specialized APCs with superior antigen presentation functions that are intimately involved in

the immune response. DCs are myeloid cells that have two major functions: acquisition of antigens and stimulation of lymphocytes [1]. Acquisition of antigens occurs in the peripheral tissues where naïve DCs express low levels of MHC Class II and co-stimulatory molecules (CD80, CD40, CD86). These naïve cells are weak stimulators of T lymphocytes, however, after maturation (provoked by an insult such as infection or tissue damage), they upregulate their expression of CCR7 and co-stimulatory molecules to gain the ability to stimulate naïve T cells in the lymph nodes [2]. DCs prime specific effector T cell responses, which include TH1, TH2 and immunoregulatory T lymphocytes (Treg) responses. Once activated, T cells undergo clonal expansion and acquire specific tissue homing patterns allowing them to return to the tissue of interest. Within the gastrointestinal mucosa DCs are abundantly located in the lamina propria and Peyer's patches, small aggregates of lymphoid tissue. Here, DCs are able to sample luminal antigens of the bowel via dendritic projections, expressing tight junction proteins to prevent compromise of intestinal barrier integrity [3]. In addition, DCs can also acquire antigen through phagocytosis of antigens and apoptotic bodies [4].

Lymphatics and Immunity

The lymphatic system makes up a network of vessels, which provide a conduit for immune cells to activate and recruit further lymphocytes to respond to a particular immune environment. Lymph and cells enter the lymphatic system at the level of the initial lymphatics, blind-ended tubes composed of a single layer of non-fenestrated endothelial cells [5]. From here, smooth muscle and one-way valves in the larger collecting lymphatics [6], allow lymph to be propelled towards the lymph nodes where an immune response can be mounted [7] and the appropriate immune cells and nutrients can be returned to the circulation via efferent lymphatics. There is significant evidence to

suggest that the ability of DCs to reach lymph nodes and actually interact with both B and T lymphocytes largely depends on functional lymphatic vessels. Studies in mice with underdeveloped lymphatic vasculature showed an absence of DC emigration to lymph nodes in both resting and stimulated conditions [8,9]. Furthermore, it is now better understood how DCs enter lymphatic capillaries; this is done by signalling between CCR7 which is expressed on the surface of DCs and its chemokine ligands, CCL19 and CCL21, both of which are expressed by lymphatic capillaries and lymph nodes [10]. The current model of trafficking through lymphatics is that the DCs enter lymphatics via CCL19/21 expression on initial lymphatic vessels and subsequently respond to a downstream ligand gradient to enter lymph nodes [11]. Expression of CCR7 can be transiently increased under the influence of prostaglandin E2 and administration of inflammatory cytokines correlated with an increased expression of CCL21 in lymphatic capillaries [12,13].

There is also a strong link between the lymphatic system and bacterial translocation, in which passage of viable bacteria occurs from the intestinal lumen into the mucosa and muscle layer to extra-intestinal sites. These instances can be promoted by bacterial overgrowth in the intestine, host immune deficiencies and in situations of increased permeability of the intestinal wall such as inflammatory bowel diseases [14,15]. The importance of the lymphatic system here is evident, as an intact vasculature is necessary for bacteria to travel to a lymph node where the bacterial antigens can be processed and an acute response and memory can be initiated. Evidence shows that CCL21-positive lymphatic vessels within the small intestine express Toll-like receptors (TLRs) [16], a family of receptors critical in mediating innate immunity. These data suggest that the lymphatic system may be directly involved in innate immunity by recognition of pathogen-associated molecular patterns (PAMPs) via TLRs.

In addition to some of the molecular changes described during inflammation, it is well documented that chronic inflammation leads to significant lymphangiogenesis, a complex process mediated by many factors including Vascular Endothelial Growth Factor (VEGF) C and VEGF-D [17,18]. The function of this inflammation-induced lymphangiogenesis remains unknown.

The strong link between inflammation, increased lymphatic vasculature and increased expression of molecules involved in immune trafficking to lymphatics, and expression of innate immune sensing proteins lends support to the importance of the lymphatic vasculature in infective or inflammatory disease. It stands to reason, therefore, that dysfunctional lymphatic vessels could cause a dysfunction in immune cell trafficking.

Lymphatic Function and Inflammation

The lymphatic system, like most tissues in the body, responds to inflammatory mediators during an acute or chronic inflammatory response. One of the hallmark effects of inflammatory mediators is to stimulate vascular leakage, which increases interstitial fluid volumes. Furthermore, the various inflammatory mediators released during the inflammatory response have also been implicated in directly influencing lymphatic contractility [19]. Some prostanoids - namely prostaglandin E2 and I2 - have been shown to inhibit lymphatic contractility, and blockade of cyclooxygenases (involved in the production of these products) reversed pumping inhibition [20-22]. Furthermore, studies have shown that nitric oxide, a vasoactive molecule implicated in the inflammatory response, also

limits lymphatic contractile activity [19,23]. Histamine, an inflammatory mediator that is crucial for increasing microvascular permeability has also been shown to have a direct stimulatory effect on lymphatic contractility and function [24-26]. This potent effect of histamine on lymphatic function has implications on the pathogenesis of food allergies, as large amounts of histamine are released during mast-cell degranulation. This has been shown to increase lymphatic contraction frequency [27]. Other inflammatory mediators, such as serotonin and as well as neuropeptides, have impacts on lymphatic contractility [19] but are beyond the scope of this review.

Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD) represents a spectrum of disorders characterised by chronic, relapsing and remitting symptoms, including abdominal pain and diarrhea [28]. IBD presents as two major forms: Crohn's disease and ulcerative colitis, both of which are associated with an abundant production of inflammatory cytokines and chemokines [29]. Crohn's disease is characterized by aggregation of epithelioid histiocytes causing granulomas, whereas ulcerative colitis features significant numbers of neutrophils in the *lamina propria* and crypts where they form abscesses [30]. The granulomatous nature of Crohn's disease has prompted recent speculation regarding the involvement of lymphatic vessels in the pathogenesis of this disease [31]. While much is known about the pathology surrounding IBD, little is known about its precise etiology, which is believed to be multifactorial, involving environment, genetics and immune dysregulation [32]. DCs isolated from the lamina propria of Crohn's disease patients showed elevated expression of co-stimulatory molecules CD40, CD80, CD83 and CD86, involved in T cell stimulation by DCs [33-36].

Lymphatic Dysfunction and Intestinal Inflammation

While the lymphatic system is clearly pivotal for immunity and fluid homeostasis in the entire body, it is even more closely linked with the gastrointestinal system because of its role in nutrient absorption and immune cell trafficking to this area. The relationship between the gut and the lymphatic system has also been shown to be critical during intestinal inflammation. IBD patients presenting with oedema were reported to have enlarged lymphatic vessels, which appeared obstructed [37-40]. The enlarged and stenotic lymphatic vessels suggest poor lymphatic drainage, which would further exacerbate the tissue oedema. Interestingly, animal studies where mesenteric lymphatic drainage was experimentally obliterated led to Crohn's disease-like macroscopic and microscopic patterns [40-42]. These animal models of lymphatic obstruction are considered by some to best reproduce Crohn's disease symptoms without contribution from genes, environment or bacterial makeup in the gut [31]. Importantly, these animal models of lymphatic obstruction recapitulate transmural inflammation and intestinal fistulae characteristic of Crohn's disease [42].

Another feature seen in histological samples from IBD patients is the presence of lymphoid aggregates. Although the mechanisms surrounding the formation of these structures are not well understood, the hypothesis of lymphoid neogenesis proposes that the obstruction of lymphatic drainage promotes ectopic germinal centres to form in the tissues [43]. Further involvement of the lymphatic system in the development of intestinal inflammation relates to lymphangiogenesis, the formation of new lymphatic vessels, which is commonly seen in biopsy samples from IBD patients [44-47]. This lymphangiogenesis

may function to increase the delivery of dendritic cells and antigen-presenting cells to the lymph nodes to enhance the adaptive immune response, however this has not been proven.

Whether these new lymphatic vessels improve drainage to the lymph nodes is still under debate. A study reporting that a large number of DC and proliferating T cells were found in the lamina propria of Crohn's disease patients, suggests that they are unable to be drained away by the lymphatic system and are trapped at the site of inflammation forming lymphoid aggregates, and creating a self-perpetuating feedback loop that prolongs the local immune response [48]. On the other hand, Jurisic et al. [49] found a significant increase in the severity of colonic inflammation in IL-10-deficient mice (that spontaneously develop IBD-like symptoms) treated with an antibody directed against the vascular endothelial growth factor receptor 3 (VEGFR-3), a major lymphangiogenic receptor [50]. The study demonstrated that when lymphatic function in an inflamed gut was inhibited or reduced in anti-VEGFR-3-treated mice, inflammation was exacerbated. This was attributed to the possibility that decreased lymphatic drainage promotes increased leukocyte numbers in, and poor cell transport out of, tissue. They also suggested that increased inflammatory mediators in the local tissue allow extravasation of leukocytes out of blood vessels, thus further exacerbating the tissue inflammation. It was suggested that the episodic relapsing-remitting nature of IBD might be due to the periodic dysfunction of local tissue lymphatic vessels. Data in other studies of rheumatoid arthritis (a disease characterised by chronic inflammation with flare and remission periods similar to IBD) in mice showed that inhibition of lymphangiogenesis and lymphatic drainage via VEGFR-3 blockade exacerbated the inflammatory response [50]. Other studies have shown the relationship between dysfunctional immune responses and lymphangiogenesis in other organ systems. Lymphangiogenesis has been observed in kidney transplant rejection as well as in psoriatic skin lesions, both involving altered immune and inflammatory responses in their pathogenesis. These new studies highlight the potential importance of intact lymphatic vasculature as well as the propagation of new lymphatic vessels in the resolution of inflammation or maintenance of remission in a chronically inflamed gut.

Granulomas are a hallmark histological feature of Crohn's disease and a recent study has found that the granulomas in Crohn's patients show lymphangiectasia (pathological dilation of lymphatic vessels), as well as lymphocytic perilymphangitis. This supports the association between inflammation, granulomas and tertiary lymphoid follicles in the lymphatic vasculature of Crohn's disease patients [51]. Animal models of ileitis have shown that the contractile function of mesenteric lymphatic vessels can be altered both *in vivo* and *in vitro* with an increasing level of dysfunction correlated with an increasing degree of mucosal damage [52]. This dysfunction was significantly reduced in the presence of cyclooxygenase and nitric oxide synthase inhibitors, suggesting the involvement of arachidonic acid products and nitric oxide in the inflammation-induced dysfunction [52,53]. These studies lend support to the possibility that the lymphatic system is intimately involved in the pathogenesis and damage done to the tissues in patients with inflammatory bowel disease. One study has even suggested that Crohn's disease is caused by a congenital lack of mesenteric lymphatic vessels which leads to lymph and bacterial stasis, lymphangitis and eventual inflammatory change [54]. While this is not the currently accepted aetiology for IBD, it is important not to dismiss the lymphatic system as a potential contributor to the pathogenesis of IBD and, importantly, as a potential therapeutic target in the management of intestinal inflammation. Altogether, these studies

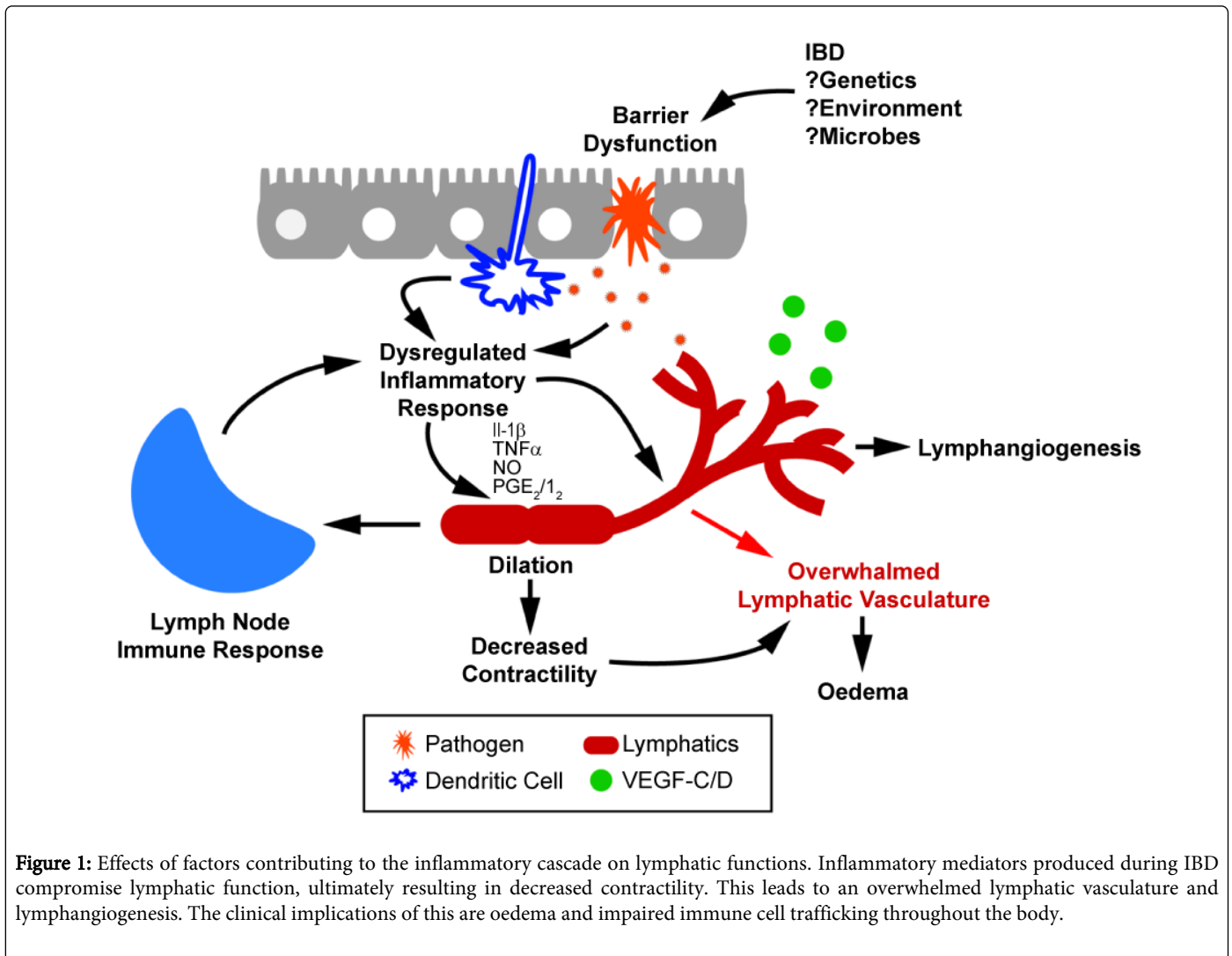
suggest a very strong link between the integrity of lymphatic function and the exacerbation or resolution of instances of intestinal inflammation.

Lymphatic Function and Food Allergies

Food allergies represent a growing problem in developed countries. At present, over 90% of food allergies are caused by only a few foods, namely cow's milk protein, soy, shellfish, nuts and wheat [55,56]. For most people, tolerance to these food antigens is mainly mediated by Tregs. Abnormalities in the development of these cells can elicit an allergic response. Once again DCs play an important role, as CD103⁺ DCs trafficking from the lamina propria of the gut to the lymph nodes preferentially activate FoxP3⁺ cells, driving their differentiation into Treg lymphocytes, which then return to the lamina propria. This interaction is vital to the development of oral tolerance to many of these allergenic food antigens. An animal model in which guinea pigs were sensitized to cow's milk protein showed that lymphatic contractility was altered secondary to mast cell degranulation [27]. While contraction frequency was indeed increased, the amplitude of contractions was not, and therefore the total calculated volume of lymph was not significantly increased. This then has implications for the development of oral tolerance. Recent evidence has shown that histamine in high - but physiological - doses in conjunction with nitric oxide is able to relax mesenteric lymphatic vessels and alter lymph flow [57,58]. For some time now we have known that the mucosa of patients with food allergies contain larger than normal amounts of resident mast cells [59]. It is possible that the higher number of mast cells causes a massive bolus of histamine to be released into the local tissue as part of the immune response. This, in addition to nitric oxide being released as part of the inflammatory cascade, causes lymphatic contractile inhibition, delayed or absent antigen delivery to Tregs and a perpetuation of oral food intolerance. The studies of histamine, nitric oxide and lymphatic function are still emerging and further characterization of their role in allergy pathophysiology is still needed.

Conclusions

The lymphatic system has gained much attention in recent years thanks to evidence that highlights its important involvement in immunity. Studies have highlighted its role in a functional immune system and how damaged lymphatic vessels are associated with inflammatory change and tissues that are similar to those in disease processes. Recent evidence has demonstrated the effects of products of the inflammatory cascade on the function of lymphatic vessels (Figure 1) and this has implications for their role in disease progression or disease initiation; the specifics of this are yet to be elucidated. While it is clear that the lymphatic vessels are important in the disease progression in intestinal inflammation, what is unclear is whether a dysfunctional lymphatic system is the initiating step in disease progression or whether dysfunction represents an adaptation of the body during disease. These specifics remain to be known but represent an exciting area in the field. Lymphangiogenesis has been shown in other fields, such as organ transplant, rheumatology and dermatology to be involved in the pathogenesis of inflammation and disease progression. These areas complement the data being produced in gastrointestinal research regarding the importance of an intact lymphatic vasculature. Furthermore, recent evidence implicating the function of lymphatic vessels in the progression of the development of food allergies poses an exciting new frontier in lymphatic research and further studies in this area will be important in determining how the lymphatic system is so intimately involved in the immune system in both health and disease.



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