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Lymphedema and Lymphatic-dependent Immune Dysfunction

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Abstract

The lymphatic system is increasingly appreciated as an essential circulatory system whose dysfunction is associated with many immune dysregulated conditions. The lymphatics unquestionably fulfill a vital role in immune surveillance. Lymphatic insufficiency, as a consequence of developmental lymphatic vascular defects, injury, obstruction, or infection, results in the accumulation of protein-rich interstitial fluid in affected tissues-a disease known as lymphedema. At later states of this disease, the condition is often characterized by inflammation, recurrent infection, fat deposition and fibrosis. Although relatively under-investigated, mechanisms associated with the development of lymphedematous pathophysiology and immune dysfunction are starting to be elucidated. In this article, we will discuss the most recent developments in lymphedema biology and the ways in which lymphatic insufficiency contributes to various immune dysregulated conditions.

Keywords: Lymphedema; Inflammation; Pathogenesis; Immune dysfunction

Introduction

The lymphatic system consists of linear networks of lymphatic vessels and secondary lymphoid organs. Although historically relatively under-investigated, the lymphatic system is now increasingly appreciated as a circulatory system that plays critical roles in tissue fluid homeostasis, lipid absorption, host defenses against pathogen invasion and tumor metastasis [1]. In contrast to the traditional conception of the microcirculation, in which it was believed that the preponderance of the interstitial ultrafiltrate would be reabsorbed at the venous end of the capillary, the most recent evidence suggests that more than 90% of the capillary ultrafiltrate re-enters the blood vascular circulation via the lymphatic route. Therefore, tissue fluid balance relies heavily on the functionality of the lymphatic vascular system [2]. The lymphatic vasculature is comprised of lymphatic capillaries, larger collecting lymphatic vessels and lymphatic ducts. Lymphatic capillaries are blind-ended microvascular structures that lack an investment of either basement membrane or mural cells [3], and in contrast, collecting lymphatic vessels are characterized by the presence of basement membrane, pericytes, and smooth muscle that help to propel the lymph back to the central circulation [4].

Along with tissue fluid transport, the lymphatic vasculature also serves as a conduit for the traffic of lymphocytes and antigen presenting cells to the regional lymph nodes, thereby sub-serving the process of immune surveillance [5]. Moreover, the lymphatic endothelium actively participates in modulating the immune response through secretion of chemokine CCL21, thereby facilitating the recruitment of $CCR7^+$ dendritic cells to the lymphatic vessel [1,6]. The mesenteric lymphatic vessels (lacteals) are responsible for the absorption of fat and fat-soluble vitamins [5]. Lymph fluid, with its content of chylomicrons, has the capacity to promote adipocyte differentiation [7]. The vital link between lymphatic dysfunction and inflammation-associated adipose deposition has evoked scientific interest for many decades [8].

Lymphedema is broadly categorized into primary or secondary forms, based upon the underlying etiology. Primary lymphedema can be recognized at birth or appear, unprovoked, at various timepoints in childhood or later. Congenital lymphedema (Milroy's disease) is an uncommon autosomal dominant heritable disorder that is has been linked to missense mutations of *Vegfr3* [1]. Secondary, or acquired, lymphedema appears after a provoking stimulus. Globally, the most common cause of secondary lymphedema is filariasis, a result of direct lymphatic vascular invasion by mosquito-borne filarial nematodes such as *Wuchereria bancrofti* and *Brugia malayi/timori* [9]. In developed countries, however, cancer therapy is the leading cause of secondary lymphedema. Although lymphedema is rarely life-threatening, it is a disabling and disfiguring condition which has a profound impact upon physical and psychosocial functioning, and thereby erodes the individuals' quality-of-life [10]. Unfortunately, therapeutic options for lymphedema management are still limited [11,12]. Whether the origin is congenital or acquired, chronic lymph stasis in lymphedema impairs local immune surveillance by disrupting trafficking of immunocompetent cells in the lymphedematous regions, stimulates inflammation-associated angiogenesis and engenders adipose abnormalities. In this review, we attempt to highlight current concepts regarding the pathogenesis of these disturbances, with a focus on lymphedema-induced immune dysfunction.

Pathophysiology of Lymphedema

The natural history of lymphedema can be considered to evolve through two distinct phases: the first phase reflects disturbances limited to the vasculature, and is associated with impairment in fluid transport; the latter phase is characterized by overt pathological changes in the soft tissues [5]. In the first period of the disease, with

regard to underlying etiology, an increase of intraluminal pressure within the lymphatic vasculature leads to structural changes within the lymphatic vessels, including impaired contractility, incompetence of the lymphatic valves and vascular dilation [5,13]. As the disease progresses, the pathological changes are no longer restricted to the vasculature: the soft tissues undergo remodeling, with progressive cellular overgrowth, fibrosis, and adipose hypertrophy. Clinically, there is a progression from pitting to non-pitting edema, often accompanied by a propensity to recurrent infection and increasing resistance to successful therapeutic intervention [11].

Both chronic and acute inflammation have been intimately linked to lymphatic dysfunction [14]. In preclinical studies, cells of both the adaptive and innate immune systems, including lymphocytes and monocytes/macrophages, have been described in the lesions of chronic lymphedema [5,15]. Therapies targeting the inflammatory response appear to reverse both edema and disease pathology [16]. In human secondary lymphedema, IL-4, IL-10, IL-6 and TNF-α have been reported to be directly related to the pathogenesis of lymphatic vascular insufficiency [17-19]. Chronic inflammation can be a result of sterile inflammation induced by tissue injury, or due to ineffective pathogen clearance that is caused by dysfunctional interstitial fluid clearance in lymphedema [8,20,21]. Taking together, the available evidence suggests that chronic tissue inflammation is a prominent pathological contribution to the natural history of lymphedema. Chronic inflammation may also represent a factor that is associated with lymphedema exacerbation [8]. In addition, fat deposition and adipocytokine-mediated pathways are also known to be implicated in chronic lymphedema [5,13,22]. While the mechanism of adipose tissue deposition in chronic lymphedema remains poorly understood, adipocyte proliferation and differentiation induced by lymph originated factors may promote this process [23].

Lymphangiogensis in Lymphedema

Lymphangiogenesis and lymphatic vessel remodeling are common attributes of inflammation. Accumulating evidence suggests that inflammation-associated lymphangiogensis is not merely an endpoint phenomenon of inflammation but, rather, a dynamic, context-dependent reaction that can regulate the natural course of inflammation and tissue repair [24]. Both the extranodal lymphatic vessels within the peripheral tissues, and those that are intranodal, exhibit exuberant growth and vigorous expansion in response to inflammatory stimuli. In this process, lymphangiogensis is largely governed by lymphangiogenic growth factors, including VEGF-C, VEGF-A, and VEGF-D, secreted by macrophages and binding to their corresponding receptors [3,25-27]. This association has been studied in several experimental and preclinical models of inflammation, including the suture-induced corneal inflammation model [28], the corneal transplantation model [29], the *Mycoplasma pulmonis*–induced chronic respiratory tract inflammation model [30], the renal transplantation model [31], the dermal wound healing model [32], and the lipopolysaccharide (LPS)-induced dermal and peritoneal inflammation model [33,34]. Moreover, in the mouse embryo, $CD45^+$, $F4/80^+$, $LYVE\text{-}1^+$ and $Prox\text{-}2^+$ mesenchymal cells are in close contact with growing lymphatic vessels [35], suggesting that macrophages might have the capacity to transdifferentiate into lymphatic endothelial cells or to integrate within lymphatic vessels during development.

Further evidence can be extrapolated from the attribute of human pathology. For example, the intestinal lymphatics have been observed to be extremely dilated in Crohn's disease [36] and the dermal lymphatic microvasculature is reported to be quite dilated in diseased regions from cutaneous specimens derived from lymphedema patients [37,38]. Thus, in inflammation, lymphangiogenesis can be construed to represent a mechanism that compensates for structurally impaired lymphatic drainage and thereby helps to maintain clearance of interstitial fluid and inflammatory infiltrate. In fact, impaired lymph drainage and persistent lymph stasis might contribute to chronic inflammation by fostering the accumulation of activated dendritic cells and antigens in lymphoid follicles without adequate drainage to the lymph nodes. Further investigation is required to determine whether inflammation-associated lymphangiogenesis in the setting of lymphedema is pathological or protective.

Immune Surveillance and Lymphedema

The lymphatic vasculature, in addition to its central role in interstitial fluid clearance, also functions as conduits for the migration of immune cells from peripheral tissues to draining lymph node and, subsequently, to the blood vascular circulation. There are more than 400 lymph nodes dispersed throughout the human body; they serve as the site of residence for circulating naïve lymphocytes and destination for antigen/antigen presenting cell complexes, such as the dendritic cells (DC) that actively migrate from peripheral tissues in the lymph [39,40]. Lymphocytes first migrate rapidly into lymph nodes by traversing the specialized blood vessels known as high endothelial venules (HEV) [41]. After crossing the HEVs, T cells migrate to the T cell areas in the lymph node paracortex, whereas B cells enter the B cell follicles in the cortex. The intranodal migration and positioning of B, T and dendritic cells are tightly regulated by chemokine ligands and receptors: CCL21, CCL19, CXCL13 and CCR7 [40,42]. If lymphocytes do not recognize their specific antigen in the lymph node, they exit through efferent lymphatic vessel and return to the circulation. This continuous recirculation of lymphocytes between lymphoid organ and blood provides an effective immune surveillance mechanisms for the detection of foreign invaders, such as virus, and bacteria, as well as for alterations in self-antigens [43].

In lymphedema, accumulation of protein-rich interstitial fluid is accompanied by an increased cellularity resulting, at least in part, from a massive infiltration of immunocompetent cells, including neutrophils, macrophages, and DCs. Concurrently, defective lymphatic conduits prevent the homing of leukocytes and DCs from the peripheral tissue to the recipient lymph nodes. Eventually, inadequate lymphatic drainage, chronic lymph stasis, and impaired lymphocyte and DC trafficking erode the process of immune surveillance: the lymphedematous region becomes an immunologically vulnerable area, where neoplasms, infections and immune-related disorders, such as bullous pemphigoid, toxic epidermal necrolysis and neutrophillic dermatosis, occur with greater than expected frequency [44-46].

Cutaneous Infection and Lymphedema

In patients with long-standing lymphedema, bacterial, fungal and viral infections in the lymphedematous limb are the most common complications [2,47]. In addition, various malignant tumors, including lymphangiosarcoma, basal cell carcinoma, squamous cell carcinoma, lymphoma, malignant melanoma and Kaposi's sarcoma have been described in affected limbs [48-50]. A recent study has examined the differences in symptoms and infection status among 723 patients with upper extremity lymphedema and 1114 individuals with lower

extremity lymphedema [51]. The data indicate that individuals with lower extremity lymphedema experience a higher symptom burden and more frequent infectious episodes, complications and hospitalizations than those with upper extremity lymphedema. In sum, these clinical observations suggest that interruption of normal lymphatic function through obstructive vascular lesions is likely to have a knock-down effect upon regional immunocompetency. This notion has been supported by the clinical and experimental observations on skin homografts [52], where defective cell-mediated immunity in the lymphedematous extremity delays transplant rejection. Furthermore, investigation of cutaneous cell-mediated immunity in 35 women with post-mastectomy lymphedema demonstrates that, when compared to an unaffected limb, the lymphedematous limb manifestes a suppressed allergic contact sensitivity response with exposure to dinitrochlorobenzene [53]. The proposed mechanism is that impairment in dendritic cell and lymphocyte trafficking leading to the presumed ineffective removal of foreign antigen [54,55]. However, the underlying mechanisms for the loss of immunocompetence in lymphedema are not yet fully understood.

Bacterial infections, especially cellulitis, may be the most commonly occurring medical emergencies associated with lymphedema [56]. Cellulitis, or erysipelas, is an acute infection of the skin and deeper tissues characterized by painful swelling, cutaneous erythema, and increased cutaneous temperature. Cellulitis may become life-threatening when the infection disseminates to the lymphatic vasculature (lymphangitis) and, thereby, to other vital organs and to the bloodstream [57]. The rapid dissemination of bacterial pathogens through the superficial lymph vessels can contribute to the advent of fibrosis in the affected tissues [58]. Each infection suffered by lymphedema patients may further increase skin fibrosis and lead to additional loss of lymphatic vascular function.

In addition to the immune dysregulation that is present in lymphedema, additional physical factors may predispose these patients to recurrent soft tissue infection. The skin in lymphedema tends to be dry and scaly, causing a disruption of the physical barrier to the entry of pathogens. The enlarged limbs, with their redundant deepened skin folds present a warm and humid environment for the overgrowth of bacteria. Most importantly, the accumulation of tissue fluid enriched with protein and cellular debris provides an internal milieu that is ideal for pathogen replication and growth.

Adipose Deposition and Lymphedema

A functional link has emerged between lymphatic malfunction and the pathogenesis of obesity as well as atherogenesis. Lymphatic vessels are functionally important to lipid transport and metabolism. Lymph nodes, the organizing centers of immune surveillance and response, are always found in proximity to surrounding adipose tissue. Subcutaneous fat lies in close proximity to the dermal lymphatic vasculature, while visceral adipose tissue surrounds the collecting lymphatic vessels of the mesentery, cisterna chyli and thoracic ducts, as well as the efferent and afferent lymphatic vessels of the intra-abdominal lymph nodes. This perinodal adipose tissue gives rise to a chronic, low-grade inflammation, which is known to contribute to obesity-related metabolic and cardiovascular disease [59].

As early as the 19th century, the German dermatologist Paul Unna proposed that the stagnation of lymph with lymphatic vascular interruption resulted in fat accumulation [60]. The lymphedematous limb accumulates fat at an enhanced rate and, when weight loss occurs, the limb loses fat more slowly than the other, non-involved tissues of the body. A relatively observation of the murine lymphatic vascular defects caused by Prox-1 haploinsufficiency suggests that lymph leakage from mispatterned and ruptured lymphatic vessels is associated with adult-onset obesity and inflammation [23]. The $Prox1^{+/-}$ mice weighed two to three times more than their wild-type counterparts. Extensive characterization of food intake, energy expenditure or mediators of appetite and lipid metabolism failed to reveal changes in any of these parameters that could account for the onset of obesity of the $Prox1^{+/-}$ mice. The weight gain was explained by subcutaneous and intra-abdominal fat accumulation, and the magnitude of adipose tissue accumulation correlated with the degree of lymphatic vascular disorganization and dysfunction [23]. Adipose tissue deposition has also been described in a murine model with a heterozygous mutation in *Vegfr3* [61]. The so-called *Chy* mouse develops lymphedema spontaneously as a result of hypoplastic cutaneous lymphatic vascular development. While the mechanism of adipose accumulation due to lymphatic vascular dysfunction has not yet been elucidated in either of these models, it is notable that chronic tissue inflammation is associated with abnormal fat deposition in both. In a mouse-tail lymphatic ablation model of lymphedema, sustained lymphatic fluid stasis in the tail causes marked subcutaneous fat deposition as a result of adipocyte hyperplasia and hypertrophy, a pathologic finding evident in clinical lymphedema [62]. Adipogenesis in response to lymphatic fluid stasis is also associated with marked mononuclear cell and macrophage inflammatory responses and increased expression of individual inflammatory proteins, including adiponectin, CCAAT/enhancer binding protein-alpha (CEBP-α) and PPAR-γ [62].

Patients with lymphedema have significantly elevated numbers of $CD4^+$ cells within the dermis of the affected regions, as well as tissue and serum expression of IL-6 and p-Stat3 [63]. The increased expression of IL-6 correlates with adipose deposition and T cell-dependent inflammation. While impaired lymph drainage seems to predispose to fat disposition and obesity, the reverse relationship may also be relevant. This notion is supported by the observation that weight gain is a strong risk factor for the development of breast cancer-related lymphedema [64].

Low-grade inflammation is increasingly recognized to be associated with, and to augment, obesity and obesity-associated metabolic complications, such as insulin resistance, type 2 diabetes, and cardiovascular disease. The adipose tissue of obese mice and humans produces pro-inflammatory cytokines, chemokines, and peptides including TNF-α, TGF-β, IL-6, monocyte chemoattractant protein-1, and leptin, all of which are able to recruit and stimulate cells of the immune system. Many of these pro-inflammatory mediators also have been documented exhibit pro-lymphangiogenic potential. Multiple lines of evidence suggest that inflammation-associated adipogenesis is, in fact, a pathological attribute of the disease state of lymphedema. Nevertheless, the exact mechanisms linking lipid pathologies with lymphatic function remain to be elucidated.

Concluding Remarks

Research in the last few decades has dramatically enhanced our understanding of the mechanisms associated with the development of the pathophysiology of secondary lymphedema. Compelling evidence now suggests that the chronic inflammation elicited by lymph stasis is an integral pathological attribute that likely promotes the evolution of

the disease. Paradoxically, this inflammatory response is also often accompanied by an increased propensity to infection, driven in part by the defective immunosurveillance that arises as a consequence of the circulatory abnormality. Newer lines of evidence in preclinical models suggest that abrogating the inflammation can effectively reduce lymphedema pathology [16]; similar approaches may be effective in the human disease (unpublished data). Additional intensive, mechanistic studies of inflammation and infection will be required before lymphedema can be effectively reversed. The mechanisms through which lymphatic dysfunction and altered adipose biology promote one another represent another compelling avenue for future research. Elucidation of this link may also provide novel therapeutic targets for human lymphedema.

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