New and Emerging Therapies for Lymphedema: Part II

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Introduction

Understanding the intricate roles of inflammation, fibrosis, and adipose deposition in response to lymph stasis is critical to providing excellent medical care for patients with lymphedema. The overarching hypothesis has been that lymph stasis leads to inflammation. This inflammation then leads to progressive tissue fibrosis and adipose deposition, which in turn decreases lymphatic function, creating a pathologic feedback loop [1]. The complex role of inflammation in lymphedema likely explains the phenomenon of late symptom onset in breast cancer-related lymphedema (BCRL), often occurring 1–5 years after initial therapy. Lymph stasis results in the induced expression of danger signals, with upregulated functional gene expression within pathways related to acute inflammation, immunity, complement cascade, wound healing, and fibrosis [2]. Six biomarkers characteristic of lymphatic vascular insufficiency have been identified; these participate in lymphangiogenesis, inflammation, fibrosis, and adipocytokine signaling [3]. In many ways, progressive fibrosis of the superficial tissues can be conceptualized as end-organ failure of the lymphatic system, in analogy with progressive inflammatory disease processes in other organ systems, where parenchymal replacement with scar tissue occurs. Before we discuss new therapies that target inflammation and fibrosis, we must first briefly review our current understanding of the key targets in the inflammatory and fibrotic mechanisms associated with lymphedema. Many of the pathways have been characterized in mouse models of acquired lymphedema; these simulate the histopathology, altered immune trafficking, abnormal lymphoscintigraphic patterns, and volume responses seen in human lymphedema [2, 4].

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The Key Targets of Inflammation and Fibrosis in Lymphedema

Vascular Endothelial Growth Factor (VEGF)-C, Cytokines, and Leukotriene B4 (LTB4)

Vascular endothelial growth factor (VEGF)-C regulates differentiation, survival, and migration of lymphatic endothelial cells (LECs) through VEGF receptor-3 (VEGFR3). Milroy's disease is an autosomal dominant primary lymphedema that occurs when a heterozygous missense mutation of the VEGFR3 gene causes partial inactivation. When VEGF-C is delivered locally or through gene therapy in animal models of primary or secondary lymphedema, the defect is overcome, lymphangiogenesis is increased, and edema diminishes [5]. However, VEGF-C has been demonstrated to play a role in tumor lymphangiogenesis, so there is understandable concern that administration of this growth factor might have the capacity to initiate tumor recurrence or metastasis.

Interleukin (IL)-4 and IL-13 are T helper (T_h) 2 cytokines that participate significantly in allergic diseases such as asthma. IL-4 and IL-13 have been demonstrated to inhibit lymphangiogenesis and diminish LEC survival, proliferation, migration, and tube formation [6, 7]. Tumor necrosis factor (TNF)-α is an inflammatory cytokine and acute phase protein. In a mouse lymphedema model, TNF- α inhibition increases tissue edema, decreases VEGF-C expression, and increases with disease severity [8]. Ketoprofen is a nonsteroidal anti-inflammatory drug (NSAID) that increases TNF-α expression and is discussed later. Leukotriene B4 (LTB4) is an eicosanoid inflammatory mediator. LTB4 production is elevated in preclinical and clinical lymphedema. LTB4 regulates lymphangiogenesis by altering human lymphatic endothelial cell function and survival. Ubenimex inhibits LTB4 production and is discussed below.

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T Cells

CD4+ cells, also known as T helper (T_h) cells, participate actively in the lymphedema inflammatory response. Nearly 70% of all inflammatory cells in lymphedematous tissues are CD4+ positive, and CD4+ cell number correlates positively with increasing disease severity [9]. IL-2 expression is necessary for T cell activation and for the differentiation of CD4+ cells. IL-2 expression is decreased by calcineurin inhibitors such as tacrolimus, discussed below. T_h2 cells predominate in lymphedema-associated inflammation. Blocking T_h2 differentiation has been demonstrated to prevent and reverse lymphedema in animal models [10]. In addition, inhibiting T_h2 differentiation (not generalized inflammation) markedly decreases initiation and progression of fibrosis and improves lymphatic function [10].

In addition to T_h2 cells, regulatory T (Treg) cells are also increased in human lymphedema. Treg cells are immunosuppressive cells that inhibit T cell responses and suppress regional proinflammatory neutrophils. In the mouse model of lymphedema, Treg cells decrease T_h1/T_h2 immune response, fibrosis, and expression of interferon (IFN)-γ, IL-13, IL-4, and transforming growth factor (TGF)-ß1 [11]. T_h1 and T_h17 cells may play a complex role: chronic lymphedema develops through the ability of these cells to promote the excessive generation of leaky neo-lymphatic vessels, mediated by increased macrophage generation of VEGF-C. This has been demonstrated in an axillary lymph node dissection model of lymphedema. Atorvastatin modulates the function of T_h1 and T_h17 as discussed below [12].

Macrophages

Lymphedematous tissues contain increased numbers of macrophages [9, 13]. This increase is mediated, at least in part, by CD4+ cells [10]. Macrophages exercise a complex role in lymphangiogenesis and fibrosis. Microphages can be categorized into two groups. M1 macrophages promote inflammation, whereas M2 macrophages decrease inflammation and promote wound healing. Macrophages contribute significantly to inflammatory lymphangiogenesis. These cells have been demonstrated to promote lymphedema in the acute setting [12]. M2 macrophages predominate in the mouse tail model of acquired lymphedema [13]. M2 macrophages regulate lymphangiogenesis through VEGF-C production and by promotion of tissue remodeling through the regulation of collagen and matrix metalloproteinases [14]. It has been proposed that coumarin, a benzopyrone, increases the proteolytic activity of macrophages; this is discussed below as a therapeutic option [15]. In a lymphedema model, when macrophages are depleted, VEGF-C expression is decreased, fibrosis is increased, and lymphatic function is impaired [13]. Toll-like receptor deficiency results in hindered lymphangiogenesis and lymphatic vascular repair in the mouse tail model of lymphedema; this likely results from decreased recruitment of macrophages [16].

Fibrosis and the Extracellular Matrix

The fibrosis in lymphedema appears to result from T cell inflammation and, more specifically, from T_h2 differentiation, rather than from inflammation in general [10]. Fibrosis proceeds in two stages: fibroblast proliferation and activation. Fibroblasts are regulated by T_h1 and T_h2 cells. T_h2 cells promote fibrosis, whereas T_h1 cells stimulate healing and counteract fibrosis.

The profibrotic factor, TGF-ß1, also plays an interactive and independent role in fibrosis. TGF-ß1 regulates extracellular matrix synthesis and accumulates excessively in the lymphedematous limb in patients with postsurgical lymphedema. Radiation induces fibrosis through TGF-ß1 expression, and this diminishes lymphatic function. Blocking TGF-ß1 results in improved lymphatic function, decreased T cell inflammation, and decreased expression of IL-4 and IL-13 [17]. TGF-ß1 impairs lymphatic endothelial proliferation, migration, and tubule formation.

Hyaluronic acid (HA) is a major component of the extracellular matrix. HA accumulates in lymphedematous tissues. Concentrations have been reported to be approximately eight times greater than in the contralateral limb. HA exists in varying molecular sizes; the function of HA depends on the size of the fragment. Hyaluronidase is the enzyme that breaks down high-molecular-weight (HMW) HA into low-molecular-weight (LMW) HA and is discussed below. HA has a high water-binding capacity and has been used in soft tissue augmentation. The primary HA receptor is CD44; binding of HA to this receptor promotes T_h1 cell differentiation. LMWHA is required for lymphatic growth as it binds lymphatic vessel endothelial HA receptor (LYVE)-1 [18]. LYVE-1 plays a vital role in lymphatic endothelial cell (LEC) biogenesis and in lymphangiogenesis. 4-mer HA upregulates IL-12, which promotes differentiation of T_h1 and TNF-α.

Adipose Deposition

In the later stages of lymphedema, adipose hypertrophy accompanies fibrous tissue deposition. These tissue changes are less likely to respond to conventional therapies. The relationship between lymphatic dysfunction and adipose biology is complex. In the mouse tail model, depletion of T_h2 cell inflammation inhibits adipose tissue deposition [10]. IL-6 has been demonstrated to correlate with the presence of adi-

pose tissue depots in obese patients. Both primary and secondary models of lymphedema demonstrate increased expression of IL-6. Il-6 is increased in lymphedematous tissues and peripheral serum of human lymphedema patients [19]. However, when IL-6 is lost or inhibited, adipose deposition is increased and inflammation is decreased. This suggests that IL-6 decreases adipose deposition and contributes to chronic inflammation in order to maintain adipose homeostasis [19].

Summary of Key Targets

T_h1, T_h2, M1 macrophages, LTB4, IL-4, and IL-13 demonstrate an injurious immune response resulting in decreased lymphatic function. Treg cells, M2 macrophages, and VEGF-C function as a reparative immune response to promote lymphatic function [20].

Anti-inflammatory and Anti-fibrotic Therapies

Ketoprofen

Ketoprofen is an NSAID with a unique dual anti-inflammatory mechanism of action that inhibits both cyclooxygenase and 5-lipoxygenase (5-LO). Systemic administration of ketoprofen in an acquired lymphedema mouse model led to reversal of disease burden and normalization of histopathology [8]. In the murine model, ketoprofen reduced inflammation and tissue edema through induction of TNF-α and an increase in VEGF-C expression [8]. In a prospective, randomized, double-blind, placebo-controlled study, 4 months of ketoprofen treatment markedly improved skin histopathology and reduced skin thickness. However, limb volume and bioimpedance were not significantly altered. One patient withdrew from the study secondary to rectal bleeding from hemorrhoids. Another patient experienced dyspepsia but was able to complete the trial [21]. After patient enrollment was completed, a black box warning for NSAIDs (unrelated to this specific trial) was issued regarding the risk of heart attack and stroke.

Ubenimex

Ubenimex, also known as bestatin, is a competitive, reversible inhibitor of leukotriene A4 hydrolase that converts LTA4 to LTB4. In leukotriene biosynthesis, activation of this enzyme results from the upstream activation of 5-LO expression that is inhibited by ketoprofen. Thus, the therapeutic benefit of ketoprofen is thought to be primarily a result of reduced LTB4 synthesis [22]. High concentrations of LTB4 inhibit lymphangiogenesis and induce human lymphatic endothelial cell (HLEC) death. In the mouse model, ubenimex results in improved lymphatic clearance, diminished tissue inflammation, improved blood vessel integrity, and improved anatomic integrity. Additionally, concentrations of IL-6, IL-4, and IL-13 were significantly decreased after ubenimex treatment. Of note, ibuprofen exacerbated edema in this model [22].

Tacrolimus

Tacrolimus inhibits calcineurin and decreases the IL-2 expression that is necessary for T cell activation and differentiation. A topical formulation of tacrolimus is FDA-approved and used to treat cutaneous inflammatory/fibrotic diseases. In a mouse tail model, topical tacrolimus had a reversing effect on lymphedema without depletion of systemic T cell counts. T cell, CD4+, and macrophage counts in the lymphedematous tissues were decreased. When fibrosis was assessed, type I collagen was decreased in the dermal, subcutaneous, and peri-lymphatic tissues. Lymphangiogenesis was also increased with increased VEGF-C and decreased TGF-β1, IFN-γ, IL-4, and IL-13. Lymphatic function improved with tacrolimus therapy when assessed by near infrared fluorescence and lymphoscintigraphy [23]. However, additional studies will be required to determine the optimal concentration and delivery method for topical tacrolimus for the treatment of lymphedema.

Atorvastatin

HMG-CoA reductase inhibitors (statins) can modulate the function of T cells, including T_h1 and T_h17 cells [24]. In an axillary lymph node dissection mouse model, atorvastatin was demonstrated to suppress early, leaky neolymphangiogenesis by inhibiting the interaction between T_h1 cells, T_h17 cells, and macrophages in lymphedema. Additionally, thickened dermis, fibrosis, and adipogenesis were decreased in later stages in this model [12]. There has been no human, clinical assessment of the lymphedema response to statins.

Hyaluronidase

Subcutaneous injection of hyaluronidase into lymphedematous tissues of a mouse model resulted in a decrease in the total concentration of HA and an increased proportion of LMWHA when compared to control subjects. The group

treated with hyaluronidase demonstrated decreased swelling and improved histologic features. Additionally, the treated group demonstrated increased lymphatic vessel density and increased VEGFR3 expression. Lymphoscintigraphy demonstrated enhanced lymphatic drainage [25]. Pro-fibrotic cytokines, TGF-β and IL-4, are significantly downregulated, whereas anti-fibrotic cytokines, IL-12 and IFN-γ, are upregulated, resulting in suppressed fibrogenesis in mice treated with hyaluronidase [26]. Hyaluronidase has also been demonstrated to decrease lymphedema volume and reduce neutrophils in the mouse tail model [27].

Benzopyrones

The therapeutic mechanism of benzopyrones is poorly understood. However, it has been proposed that α-benzopyrones (e.g., coumarin) activate proteolytic activity of macrophages, and γ-benzopyrones (e.g., diosmin) increase oncotic pressure and the frequency and intensity of lymphatic vessel contraction. Benzopyrones are a group of drugs that have been reported to successfully treat lymphedema, especially when combined with complete decongestive therapy (CDT). However, a Cochrane review found insufficient evidence to support treatment of patients with lymphedema with benzopyrones [28]. A recent prospective randomized controlled trial in BCRL demonstrated that a product containing coumarin, diosmin, and arbutin (a diuretic) combined with CDT was more effective than CDT alone in producing limb volume reduction. While hepatoxicity has been reported with coumarins in the past, this complication is likely dose dependent; no hepatotoxicity was identified in this study [29].

A Few Notes on Non-pharmacologic Therapies

The risk factors for lymphedema, including surgery, radiation, infection, and obesity, are all associated with inflammation and should be minimized whenever possible. Lymphedema therapists should be encouraged to use exercise and other physical techniques to reduce fibrosis. Low-level laser therapy (LLLT) demonstrates anti-inflammatory and anti-fibrotic effects in the mouse model, and a systemic review and meta-analysis of patient with breast cancer-related lymphedema found that patients treated with LLLT, alone or in combination with other treatments, had significantly decreased pain and swelling [30]. Finally, the inflammation associated with lymphedema is decreased by CDT, and this standard of care should be optimized for all patients with lymphedema.

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