# Pathophysiology of Lymphedema

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**Key Points**

- Lymphedema is a pathophysiologic process resulting from injury, infection, obstruction, or congenital defects in the lymphatic system.
- Primary lymphedemas occur as a result of genetic or developmental abnormalities in the lymphatic system; secondary lymphedemas are caused by secondary insults to the lymphatic system
- The histological hallmarks of lymphedema are lymphatic fluid stasis, chronic inflammation, fibroadipose tissue deposition, and hyperkeratosis.
- Risk factors for development of lymphedema include genetic abnormalities, obesity, radiation, and infection.
- Cellular mechanisms of lymphedema are unknown; however, recent studies have demonstrated a critical role for CD4+ cells in the regulation of fibrosis in animal models and correlative clinical studies.

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## Introduction

The lymphatic system is an essential component of the circulatory system whose main roles are maintaining fluid homeostasis, acting as a conduit for migration and transport of immune cells, regulation of inflammatory responses, and enabling dietary absorption of fat. Networks of lymphatic vessels begin as blind-ended lymphatic capillaries and transport interstitial fluid unidirectionally back to the heart. Disruption of lymphatic vasculature secondary to chronic parasitic infections, during the course of cancer treatment, after trauma, or as a consequence of genetic mutations results in stasis of protein rich interstitial fluid and chronic inflammation. These pathologic changes, over time, lead to lymphedema; a progressive disease characterized by adipose deposition and tissue fibrosis. It is well established that patients with lymphedema have significant impairments in quality of life, recurrent infections, and in some cases deadly secondary tumors.

Broadly speaking, lymphedema can be categorized as either primary or secondary. Primary lymphedema, as the name implies, is caused by abnormal development of the lymphatic system or pathological changes intrinsic to the lymphatic vasculature. These developmental abnormalities may be related to genetic defects that either directly or indirectly regulate lymphatic differentiation and function. In contrast, secondary lymphedemas occur as a consequence of postnatal iatrogenic, infectious, or traumatic insults to the

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lymphatic system. Although both primary and secondary lymphedemas share similar pathologic features including chronic swelling, inflammation, adipose deposition, and fibrosis, there important pathologic distinctions remain between patient responses, disease course, and response to treatments. In addition, although recent studies have improved our understanding of the pathology of lymphedema in general, the mechanisms that regulate these disease specific responses remain unknown and are an important area of research.

## Classification of Lymphedema

### Primary Lymphedema

Primary lymphedemas can be classified either by the timing of presentation, mode of inheritance (genetic linked or sporadic), or region of pathology (e.g., systemic or visceral). Traditionally, the timing of presentation has been used most commonly to categorize patients as having congenital lymphedema (i.e., present at birth), developing lymphedema around the time of puberty (lymphedema praecox), or presenting in adults older than 35 years (lymphedema tarda). The vast majority of patients present with either congenital lymphedema or lymphedema praecox; lymphedema tarda is diagnosed in less than 10 % of patients [1].

Classification of lymphedema based on timing of presentation is not particularly useful since there is no reference to the pathological causes. More recently, Connell et al. published a classification system and diagnostic algorithm that subcategorizes congenital lymphedemas as syndromic, systemic/visceral, disturbed growth, congenital onset, and late onset [2]. This classification system is helpful because it takes a more functional approach to lymphatic development and will likely aid in identifying genetic mutations due to its more inclusive nature.

Primary lymphedemas, in general, affect females twice as often as males and tend to more frequently involve the lower extremities. It is estimated that nearly 30 % of patients with primary lymphedema have an identifiable genetic mutation (often in the signaling pathway for vascular endothelial growth factor-C (VEGF-C)). The most studied example of this scenario is **Milroy's disease**, a form of congenital primary lymphedema that is caused by a heterozygous inactivating mutation of FLT4, the gene that encodes for the receptor for VEGF-C (Vascular Endothelial Growth Factor Receptor-3 (VEGFR-3). Milroy's disease is a familial, sex-linked condition and accounts for approximately 2 % of all lymphedemas. These patients most commonly have bilateral lower extremity lymphedema that is, in some cases, accompanied by hydroceles. Another common genetic cause of lymphedema is an autosomal dominant condition known as *lymphedema–distichiasis syndrome.* These patients have an autosomal dominant mutation in the FOXC2 gene, resulting in a combination of lower extremity lymphedema and an extra row of eyelashes.

Sporadic (i.e., not familial) cases are the most common causes of primary lymphedema accounting for ~60 % of all patients with lymphedema. The most common form of sporadic primary lymphedema is **Meige's disease**. Patients with this disorder present with symptoms usually around the time of puberty with a female to male ratio of 4:1. These facts have led some authors to suggest that female sex hormones may play a role in the development of lymphedema.

### Secondary Lymphedema

Secondary lymphedema is the most common cause of lymphedema and develops secondary to either direct or indirect injury to the lymphatic system. The most common form of secondary lymphedema worldwide is filariasis, a condition in which parasitic roundworms *Wuchereria bancrofti* occupy the lymphatic vasculature, thereby blocking the flow of lymph from the extremity. Although the true incidence of filariasis remains unknown, estimates ranging between 140 and 200 million are commonly cited among patients residing primarily in third world countries [3]. In contrast, this form of lymphedema is very rare in developed countries.

In developed countries secondary lymphedema occurs most commonly as a complication of cancer treatment with breast cancer being the most common cause. It is estimated that nearly one in three patients who undergo axillary lymph node dissection for staging or treatment of breast cancer go on to develop lymphedema. Lymphedema, however, is not limited to breast cancer survivors, as a recent study demonstrated that nearly one in six patients who undergo treatment for other solid tumors such as melanoma, sarcoma, and gynecological malignancies also go on to develop lymphedema [4]. Even relatively minor disruption of the lymphatic system with sentinel lymph node biopsy, a procedure in which only a few lymph nodes are sampled for cancer staging, can result in lymphedema in 5–7 % of patients [5]. Lymphedema can also develop in patients who do not have lymph node biopsy but rather wide skin excisions (for example during the course of treatment for sarcoma or melanoma) particularly when these procedures are combined with radiation therapy suggesting that extensive injury of either the deep (i.e., lymph nodes) or superficial (i.e., dermal) lymphatic system can result in lymphedema development.

Importantly, the development of lymphedema after lymphatic injury usually occurs in a delayed fashion. Thus, although virtually all patients experience minor swelling immediately following surgery, in the vast majority of cases the swelling resolves within the first 4–6 weeks. However, a subset of patients develop recurrent swelling at a later point, typically 6–12 months postoperatively (77 % within the first 3 years) that does not resolve. In these cases, the diagnosis of lymphedema can be made when other causes of swelling (e.g., recurrent disease, deep venous thrombosis, systemic fluid overload,) are ruled out. This diagnosis is often confirmed with physiologic studies such as lymphoscintigraphy or indocyanine green near infrared angiography demonstrating diminished lymphatic transport capacity, dermal back flow, and dysfunctional lymphatic vessels. Lymphedema may even develop after very prolonged periods of time in at-risk patients with the longest reported case occurring 30 years after the initial surgery. In these cases, often an inciting event such as an infection or additional injury precedes the development of progressive limb swelling and lymphedema.

Recent studies have suggested that progression of lymphedema may be retarded during its early stages through the use of conservative measures such as compression garments or manual lymphatic massage therapy. Although there is some debate regarding the efficacy or timing of these treatments in preventing lymphedema development, the fact that measures aiming to decrease interstitial fluid stasis can alter disease progression/development is interesting and suggests that lymph stasis (rather than lymphatic injury alone) is necessary for development of lymphedema. However, once lymphedema develops it is usually progressive and chronic in nature although there is wide variability in the rate at which pathologic changes occur. Thus, in some cases lymphedema has a smoldering course with relatively mild changes in limb volume or tissue changes over very long periods of time (often years), while in other cases there is rapid progression of disease with disabling swelling and physiologic changes.

## Risk Factors for Lymphedema

A large number of epidemiologic studies have analyzed genetic and comorbid factors that increase the risk of developing lymphedema. A clear understanding of these risk factors is important in preoperative surgical consultation and can be used as a means of tailoring surgical procedures to individual patients in an effort to decrease the risk of lymphedema development.

### Genetics

The discovery of lymphatic markers and mechanisms that regulate lymphangiogenesis has led to an attempt by numerous researchers to test the hypothesis that mutations in the coding or non-coding regions of these genes increase the risk of developing primary or secondary lymphedema.

The majority of these studies have been performed in patients with primary lymphedema; however, recent studies have also targeted genetic risk factors for secondary lymphedema. The study of genetic risk factors for development of secondary lymphedema is interesting and based at least partially on the observation that some patients with breast cancer-related lymphedema exhibit abnormalities in lymphatic transport even in their unaffected normal extremity.

An interesting report by Mendola and colleagues from the lymphedema research group analyzed genetic mutations in 78 patients with familial (i.e., inherited) and 149 patients with sporadic primary lymphedemas. The investigators found that mutations in seven genes encoding molecules regulating VEGFR3 and its downstream pathways were responsible for 36 % of inheritable forms of primary lymphedema. In contrast, only 8 % of patients with sporadic primary lymphedema exhibited these mutations [6]. These findings are important and demonstrate that complex pathways regulate development of hereditary lymphedema. More importantly, these studies highlight the need for additional study since the majority of hereditary and sporadic primary lymphedema patients did not exhibit known genetic mutations.

Recent studies provide support for the theory that genetic mutations may also increase the risk of secondary lymphedema after surgery. For example, Feingold et al. sequenced the coding and flanking noncoding regions of the hepatocyte growth factor (HGF) and its high affinity receptor MET in 59 women with breast cancer-related upper extremity lymphedema, and 159 unrelated matched controls. This analysis was based on previous studies demonstrating that this signaling pathway is an important regulator of lymphangiogenesis in a number of physiologic settings [7]. Interestingly, the authors identified an increased rate of mutations in HGF/MET pathways in patients with lymphedema suggesting that impairments in lymphatic regeneration after injury due to dysfunctional HGF/MET signaling contributes to an increased risk of developing lymphedema. In a follow-up case–control study (80 patients with breast cancer-related lymphedema compared with 108 breast cancer controls), Feingold and colleagues found mutations in CJC2 (encoding connexin 47), a gap junction protein that is thought to regulate dermal lymphatic transfer, in four patients with lymphedema but not in any of the controls. Similar mutations have been observed in cohorts of patients with primary lymphedema [8]. Interestingly, in contrast to their previous report the authors identified only one patient with a MET mutation, suggesting that larger samples of patients are needed for these studies.

Newman and colleagues used a nested case–control approach to study genetic changes in 22 patients who developed breast cancer-related lymphedema within 18 months of surgery (case) as compared to 98 patients who did not develop lymphedema. The authors reported that single nucleotide polymorphisms (SNPs) within VEGFR2, VEGFR3, and RORC were associated with development of lymphedema [9]. SNPs are variations in DNA sequence that occur normally in the general population. These variations can occur in both coding and noncoding regions of the DNA and although gene function may be normal under physiologic conditions, some SNPs may lead to gene function changes that increase the risk of pathology. Future studies in this arena should focus on identifying the functional gene changes that occur in patients with SNPs that increase the risk of lymphedema after breast surgery.

## Obesity

The increased risk of developing lymphedema in obese patients is well described in previous epidemiologic studies. In a seminal study in 1957, Treves followed over 1,000 patients after treatment for breast cancer and found that obese patients were at significantly increased risk of developing lymphedema [10]. This observation has been confirmed in numerous subsequent studies. For example, Werner et al. reviewed 282 patients with upper extremity lymphedema after treatment for breast cancer and showed that patients with a higher body mass index (BMI) also had a higher incidence of lymphedema. Another prospective study examined the risk of

developing lymphedema in the upper extremity in 137 patients with breast cancer and showed that patients with a BMI >30 had a threefold greater risk than patients with a BMI < 25 [11]. The best supporting evidence is a randomized controlled trial in which patients with upper arm lymphedema underwent 12 weeks of dieting and nutritional counseling as compared with patients who did not. The results showed that patients who lost weight had significant reductions in arm volumes and upper arm lymphedema when compared to the control patients who did not diet [12]. These results suggest that lymphatic impairment in obesity is reversible. More importantly, these studies show that obesity and lymphedema are intricately linked. This is not surprising since a defining feature of chronic lymphedema is progressive adipose tissue deposition. This observation has led some authors to conclude that lymphedema may be a form of "regional" obesity in which tissues are more prone for depositing fat in the setting of caloric excess. This hypothesis is supported by the fact that lymphatic fluid has been shown to increase adipocyte proliferation and differentiation and by other studies demonstrating that even mild lymphatic injury activates expression of genes necessary for adipocyte activation.

Obesity, independent of surgery, has been shown to decrease lymphatic function. For example, previous studies have shown that obese patients have decreased clearance of macromolecules from the skin and subcutaneous tissues as compared with lean individuals [13, 14]. In addition, Greene and colleagues have shown that morbidly obese patients (BMI > 59) develop primary lower extremity lymphedema (i.e., without antecedent trauma or injury) characterized by decreased lymphatic transport and dermal back flow on lymphoscintigraphy [13]. Interestingly, the upper extremity seems to be less susceptible to obesity-induced lymphedema as the obese patients in Greene's series who developed upper extremity lymphedema tended to be significantly more obese (BMI > 65) than those who had just lower extremity lymphedema.

In order to study the molecular mechanisms that regulate obesity induced lymphatic function, Weitman et al. and Blum and colleagues have used a mouse model of diet induced obesity and have found that increasing obesity results in impaired transport of interstitial fluid, decreased migration of immune cells, decreased pumping capacity of collecting lymphatics, and abnormal lymph node architecture [15, 16]. Further investigation with this model showed that obese mice have heightened inflammatory responses following lymphatic injury and that these promoted increased adipose deposition and fibrosis.

## Radiation

Radiation therapy is frequently used as an adjunct to the treatment of a variety of cancers. Although these treatments are highly effective in some settings, they also are known to cause significant tissue damage and fibrosis. Not surprisingly, numerous studies have shown that radiation therapy delivered to lymph node basins is a significant contributor to the development of lymphedema particularly when radiation follows surgical injury. Thus, radiation in isolation is rarely associated with development of lymphedema; however, the combination of surgery and radiation significantly increases risks. For example, Hinrich et al. analyzed 105 patients who received postmastectomy radiotherapy and found that total dose, posterior axillary boost, and overlapping techniques were significantly associated with development of lymphedema [17]. Other studies have suggested that radiation increases the risk of lymphedema by as much as tenfold.

Although the precise mechanisms by which radiation increases the risk of lymphedema remain unknown, preclinical studies suggest that radiation-induced fibrosis is a major contributor. For example, using a mouse tail model of radiation injury, Avraham and colleagues demonstrated that lymphatic endothelial cells are sensitive to radiation and that this injury can induce apoptosis and subclinical lymphatic dysfunction [18]. These findings were corroborated by a clinical study demonstrating that radiation treatment decreases the density of small vessel lymphatics [19]. Interestingly, however, in the mouse studies protection of lymphatic endothelial cells from

apoptotic death did not decrease lymphatic dysfunction even though the lymphatic architecture was largely preserved. In contrast, inhibition of radiation induced fibrosis markedly improved lymphatic function suggesting that changes in the extracellular matrix independently regulate lymphatic function. Therefore, clinical strategies that decrease fibrosis after radiation treatment may be a novel means of decreasing the risk of lymphedema in cancer survivors.

### Infection

Patients who undergo lymph node dissection are at increased risk for infections. Unfortunately, infections often precede the development of lymphedema and may cause progressive damage to the lymphatic system. This concept is supported by numerous studies examining the association between cellulitis and development of lymphedema after treatment for gynecological or breast malignancies. For example, Gould et al. assessed complications associated with inguinal lymphadenectomy in vulvar cancer and found that patients who developed early cellulitis were at a significantly increased risk for the development of subsequent lymphedema [20]. Another cross sectional study evaluating 807 patients with lymphedema secondary to breast cancer treatment found that a past history of cellulitis was a significant factor associated with increased upper extremity volume [21]. This finding led the authors to conclude that avoidance of cellulitis through meticulous skin care is an effective means of preventing development or progression of lymphedema.

## Lymphedema Staging

### Clinical Staging

Whether it is primary or secondary lymphedema, the timeline by which symptoms present themselves is highly variable and difficult to predict. Likewise, staging systems for lymphedema are numerous and inconsistent. Many traditional classifications rely on clinical findings and physical exam to diagnose lymphedema. The most commonly used staging system is The International Society of Lymphology staging system that divides lymphedema into four stages. Briefly, a patient is classified as having **Stage 0**, or *latent*, lymphedema when their lymphatic vasculature has been damaged but they have no clinically measurable swelling or edema. These patients may present with subjective symptoms of heaviness, discomfort, or early fatigue in the affected extremity with activity. **Stage I** lymphedema, or *spontaneously reversible lymphedema*, occurs when measureable swelling starts to occur and is manifested by pitting edema. Patients with stage I lymphedema primarily have accumulation of interstitial fluid in the limb, and as a result, may have an excellent response to conservative treatments such as compression or complete decongestive therapy. **Stage II** lymphedema, or *spontaneously irreversible lymphedema*, is described as non-pitting swelling of the limb. At this point, adipose deposition and fibrosis prevent conservative therapies from being highly effective (hence the lack of pitting) and, as a result, patients have relatively modest improvements secondary to compression. The most advanced stage of lymphedema, **Stage III** lymphedema, is also known as lymphostatic elephantiasis, which is characterized by significant non-pitting swelling, fibroadipose deposition, hyperkeratosis, and acanthosis. These patients, in general, do not respond to conservative measures and typically have progression of disease.

Campisi et al. have proposed another alternative, albeit less commonly used staging system for lymphedema; stage I is defined as initial or irregular edema, stage II is persistent lymphedema, stage III is persistent lymphedema with lymphangitis, stage IV is fibrolymphedema ("column" limb), and stage V is elephantiasis [22].

Other studies have classified lymphedema based on circumference measurements or changes in excess volume relative to the contralateral normal limb (or preoperative) measures [23]. A change in circumference of less than 2 cm is considered to be mild lymphedema, a change in 2–4 cm is considered to be moderate

lymphedema, and a change in circumference of over 4 cm is considered to be severe lymphedema. One problem with this method is that it does not take into account differences in relative size of the upper and lower limb (for example, a 2 cm change in an arm is much more significant than a 2 cm change in a leg) or the effect of BMI (a given change in circumference is more severe in a thin person than an obese person). Other studies measure differences in limb volume either by water displacement or the use of the truncated cone formula and multiple measurements. In these studies an excess volume of 200 $cm^3$ is typically used to make the diagnosis of lymphedema. These measurements may be more accurate than circumference measurements; however, a complete classification systems using changes in volume has not been proposed.

## Functional/Physiological Staging

While most staging methods have used physical measurements to categorize and diagnose lymphedema, more recently developed methods have advocated the use physiological studies for diagnosis and staging. These systems quantify and analyze lymphatic function using imaging techniques with compounds that are selectively taken up by the lymphatic system. For example, in a study of 72 consecutive patients with lower limb lymphedema secondary to treatment for gynecological malignancies, a prolific group from Japan published an interesting study subclassifying patients into 12 subtypes based on patterns of indocyanine green lymphangiography (ICG) flow in the superficial and collecting system. However, while this work is interesting and worthy of further pursuit, the classification system is complicated and requires refinement [24]. More importantly, future studies should address the implications of these findings on surgical or medical treatment options

Finally, recent efforts have proposed the use of histological methods to classify lymphedema. For example, in an interesting study by Mihara et al. published in 2012, the authors reviewed macroscopic and microscopic findings in 114 lymphatic collector histological specimens from 37 patients who had lower limb lymphedema after treatment for gynecological malignancies [25]. Based on their detailed histological analysis demonstrating progressive fibrosis and obliteration of the lymphatic vessels, the authors defined lymphedematous changes as normal, ectasis, contraction, and sclerosis types (NECST) and attempted to correlate clinical/pathological degrees of lymphedema staging with these outcomes. Although these histological classification schemes are invasive and therefore less likely to be useful clinically, the implications of these findings on the pathology of lymphedema are extremely important and worthy of additional future study (see below).

## Pathologic Changes in Lymphedema

Numerous studies have analyzed histologic changes in lymphedema and have shown that characteristic features of this disease include fibrosis, hyperkeratosis, chronic inflammation, and adipose deposition. Although the cellular and molecular mechanisms that regulate these responses remain unknown, this area has been a focus of intense study in recent years and important advancements have been made both clinically and in animal models of lymphedema. For purposes of discussion, it is helpful to think of lymphedema as a progression from a disease of interstitial fluid accumulation to a disease of fibroadipose tissue deposition. This model of lymphedema pathophysiology is reflected in the current clinical staging systems of lymphedema. For example, **stage 0** (latent lymphedema) reflects symptomatic changes related to lymphatic injury and impaired drainage; **stage I** (spontaneously reversible) reflects *initial accumulation of interstitial fluid*; **stage II** (spontaneously irreversible) patients have *progressed from fluid accumulation to fibroadipose deposition*; **Stage III** (elephantiasis) is the end stage of disease with *massive fibroadipose deposition*. Clearly this is somewhat simplistic and there are likely to be important clinical parameters that

regulate the progression of disease, however, this model serves as a simple starting point for studies aiming to elucidate the pathological mechanisms of lymphedema.

## Interstitial Fluid Accumulation

A major function of the lymphatic system is to transport protein rich interstitial fluid. Conservative estimates suggest that the venous system is responsible for absorbing more than 90 % of extracellular fluid produced as a consequence of cellular metabolism and capillary perfusion. The remaining 10 % is transported by the lymphatic system. Ordinarily, the lymphatic system has a large reserve for fluid transport and mild disturbances in function (or baseline variability between individuals) do not result in noticeable fluid accumulation. However, when the system is damaged or overloaded, then interstitial fluid accumulation can occur and manifest as pitting edema. This fluid can have major effects on the cellular behavior of the affected limb, resulting in activation of inflammatory cascades and adipose cell differentiation. Chronic fluid accumulation, as can occur in patients with early stage lymphedema, leads to progressive inflammation. These changes are important because recent studies have shown that inflammatory reactions play a major role in the pathology of lymphedema. In addition, recent studies have shown that control of interstitial fluid accumulation by complete decongestive therapy significantly decreases inflammatory reactions lending support to the concept that early intervention with compression and prevention of fluid accumulation can prevent progressive lymphatic injury and development of overt lymphedema.

## Chronic Inflammation and Fibrosis

Chronic inflammation and fibrosis are histological hallmarks of lymphedema. For example, Koshima et al. biopsied lymphatics in patients with lymphedema and found lymphatic vessels became progressively fibrosed and occluded due to proliferation of surrounding smooth muscle cells [26]. Similarly, Suami et al. studied lymphatic vessels in a cadaver that had undergone unilateral axillary lymph node dissection and found that this treatment was associated with chronic inflammation and collagen deposition around collecting lymphatic vessels [27]. More recent studies have shown that although a variety of inflammatory cells are present in lymphedematous tissues, the vast majority of these cells (>70 %) express the cell surface receptor CD4. CD4+ cells constitute a large number of different mature cell types but can be broadly categorized as T-helper cells, natural killer cells, and T-regulatory cells. T helper, in turn can be subclassified into T helper type 1 (TH1), T helper type 2 (Th2), among others. Th1 reactions occur in response to acute inflammation and help defend against bacterial pathogens by producing cytokines such as interferon gamma. In contrast, Th2 responses play an important role in responses to parasites and have been shown to promote tissue fibrosis in other organ systems by producing profibrotic cytokines such as interleukin 4 (IL4), interleukin 13 (IL13), and transforming growth factor beta-1 (TGF-B1).

Interestingly, comparison of normal and lymphedematous tissues obtained from patients with lymphedema has shown that the degree of CD4+ cell infiltration correlates positively with the severity or stage of lymphedema. In addition, these tissues demonstrate a mixed Th1/Th2 inflammatory response. This finding is important since fibrosis is a major pathological component of lymphedema and suggests that CD4+ cell responses contribute to this end result. In fact, preclinical studies in mice have shown that depletion of CD4+ cells in general, or inhibition of IL4, IL13, or TGF-B1 in particular potently decreases fibrosis after lymphatic injury and this response is associated with improved lymphatic function. More importantly, these preclinical studies have shown that these approaches can successfully treat established lymphedema and may therefore represent a novel means of treating lymphedema. Thus, these approaches may be used to prevent the development of lymphedema in high-risk patients, treat patients with early

stage lymphedema, or be used in conjunction with surgical management to improve outcomes. This is an exciting development and a paradigm shift in the treatment of lymphedema away from the use of conservative measures or experimental interventions that aim to increase lymphatic repair/regeneration.

## Adipose Deposition

The cellular and molecular mechanisms that regulate adipose deposition in lymphedema remain unknown. However, recent studies have begun to decipher these pathways and shed light into this area. For example, using mouse models of lymphedema and axillary lymph node dissection, Zampell et al. have shown that lymphatic injury and interstitial fluid stasis rapidly and significantly activates differentiation of local adipocytes [28]. Similarly, Harvey et al. have shown that lymphatic fluid in culture is a potent activator of adipocyte differentiation and lipid storage [29]. In addition, these authors found that mice bearing a heterozygous inactivating mutation of Prox-1, a transcription factor that is required for differentiation of lymphatic, were prone to development of adult onset obesity suggesting that impaired lymphatic function and chronic leakage of lymphatic fluid may regulate differentiation of preadipocytes and be a predisposing factor to the development of obesity.

Adipose deposition after lymphatic injury requires activation and accumulation of CD4+ cells since depletion of these cells or inhibition of Th2 differentiation potently decreases adipose deposition after lymphatic injury. In addition, recent studies have suggested that inflammatory responses can indirectly contribute to adipose deposition by regulating adipocyte breakdown/turnover. For example, Cuzzone et al. found that expression of interleukin 6 (IL6) is significantly increased in the tissues and serum of patients with lymphedema. IL6 is known to be produced by adipose tissues and is an important regulator of adipose cell metabolism. Interestingly, these authors found that inhibition of IL6 paradoxically increased adipose deposition in lymphedema suggesting that the expression of this cytokine may play a homeostatic role in lymphedema aiming to decrease adipose deposition. This hypothesis is supported by previous studies demonstrating that IL6 may play a lipolytic role depending on its source and pattern of expression [30].

## Summary

The lymphatic system plays an essential role in fluid homeostasis in the body. Disruption of the lymphatic system can occur as a result of a number of causes including inherited or sporadic genetic mutations, surgical injury, or chronic infection. These pathologic states result in progressive interstitial fluid accumulation, chronic inflammation, fibrosis, and adipose deposition. The progression from a disease of fluid accumulation to fibroadipose deposition limits the potential for conservative treatments to improve lymphedema clinically since fibroadipose tissues are not compressible.

Although the cellular and molecular mechanisms that regulate pathological changes in lymphedema remain unknown, recent studies suggest that infiltration of lymphedematous tissues by CD4+ cells and differentiation along the Th2 lineage plays a key role in this process. These cells are thought to regulate fibrosis by producing profibrotic cytokines and either directly or indirectly regulate adipose deposition. Inhibition of these responses may therefore represent a novel means of preventing or treating lymphedema. It is hoped that advances in the understanding of the pathophysiology of lymphedema will help identify future diagnostic tests and therapies in a previously neglected field.

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