**Review Article**

# Lipid Biology and Lymphatic Function: A Dynamic Interplay with Important Physiological and Pathological Consequences

**Lim Hwee Ying, Yeo Kim Pin and Angeli Veronique***

*Department of Microbiology, LSI Immunology Programme, Yoon Loo Lin School of Medicine, National University of Singapore, 117456, Singapore*

***Corresponding author:** Véronique Angeli, Immunology Programme Department of Microbiology, National University of Singapore, Centre for Life Sciences, #03-05, 28 Medical Drive, Singapore 117456, Tel: (65) 6516 7207; Fax (65) 6778 2684; E-mail: micva@nus.edu.sg

## Abstract

Lymphatic vessels have been traditionally considered as passive transporters of lipids particularly from the intestine. However, it became apparent from emerging research that lymphatic vessel can play a more extensive role in lipid metabolism than previously realized. Moreover, recent evidence reveal that lipid deposition in the form of white adipose tissue or cholesterol as observed in obesity or hypercholesterolemia, respectively, may perturb lymphatic function. This Review summarizes the evidence supporting a bidirectional relationship between lymphatic function and the deposition of white adipose or cholesterol in peripheral tissue in the context of obesity and hypercholesterolemia. We also discuss potential mechanisms whereby excessive fat or cholesterol accumulation in tissue may account for lymphatic dysfunction. We particularly consider how phenotypic and functional changes in adipose tissue as well as in macrophages accompanying obesity and hypercholesterolemia may affect the lymphatic vasculature. In addition to their transport function, lymphatic vessels play essential roles in regulating inflammatory and adaptive immune responses. Therefore, we highlight how lymphatic dysfunction associated with hypercholesterolemia may influence immunity, inflammation and the significance to atherosclerosis. The emerging importance of the lymphatic system in lipid metabolism and immunity underscores the urgent need to find pharmacological or surgical interventions that can improve lymphatic function which are currently not available.

## Introduction

The lymphatic circulation that runs in conjunction with the blood circulation plays an essential role in maintaining body-fluid homeostasis, immunity, and lipid transport from the intestine [1]. The lymphatic system is composed of an extensive network of lymphatic vessels and interconnected lymph nodes. To accomplish its functions, it must transport lymph from the interstitial spaces into and through the lymphatic vessels, the thoracic duct and back to the blood circulation [1]. This movement of lymph containing lipids, cells, macromolecules and fluid is first collected by initial or capillary lymphatic vessels which are identified by the expression of podoplanin, lymphatic vessel hyaluronan receptor 1 (LYVE-1) and the absence of smooth muscle cells. Lymph is then transported to larger lymphatic vessels, the collecting vessels, in which initial lymphatic vessels converge. Collecting vessels also express podoplanin but down-regulate LYVE-1 expression and exhibit circumferential smooth muscle cell coverage and luminal valves that propel and maintain unidirectional flow. Recent advances in lymphatic biology and functions have demonstrated that lymphatic vessels are not "inert conduits" but rather plastic structures that actively sense and respond to the tissue environment. They also participate in many pathological conditions such as cancer, infectious and chronic inflammatory diseases. For example, inflammation can affect the structure and function of lymphatic vessels and lymph nodes, and such lymphatic remodelling can have important biological consequences including modulation of inflammation and adaptive immune responses [2-4].

Moreover, the relationship between lipids and lymphatic function and its significance in lipid-related pathologies have just recently begun to receive attention [5] although the absorption and transport of fat by intestinal lymphatic vessels have been known for centuries.

Hypercholesterolemia is a condition characterized by high levels of cholesterol in the blood. It is the major risk factor for atherosclerotic disease and is also a common clinical feature in diabetes, obesity, systemic lupus erythematosus, rheumatoid arthritis, and psoriasis. Obesity relates to the accumulation of excess body fat determined by the measurement of the body mass index, and is also a major risk factor for cardiovascular diseases. Even though both medical conditions often overlap, it is important to understand their pathologies as separate entities to elucidate their relative implications. Although obesity is frequently associated with hypercholesterolemia and the combination of both conditions worsened cardiovascular risk status, many obese patients exhibit normal blood lipid profile [6]. Similarly, many hypercholesterolemic patients are not obese [7]. However, the obvious similarity between obesity and hypercholesterolemia is the accumulation of lipids in extravascular tissues. Lipid deposition appears in the form of white adipose expansion or foam cells also known as xanthomas in obesity and hypercholesterolemia, respectively. It is worthy of note that foam cell accumulation has been reported in obesity [8,9] but, it is not known whether adipocyte hypertrophy associated with obesity may also manifest in hypercholesterolemia. This Review summarizes the evidence revealing and supporting a bidirectional relationship between the lymphatic system and lipid accumulation in tissues occurring in the context of obesity and hypercholesterolemia. In addition, the potential mechanisms by which excess white adipose or cholesterol in tissues may alter the function of lymphatic vessels are discussed.

Finally, the possible immunological consequences of lymphatic dysfunction associated with hypercholesterolemia are also considered.

## The Interplay between Lipids and Lymphatic Function

### Evidence connecting lymphatic function with adipose tissue accumulation

Over the past decade, a growing body of evidence from studies in small animal models and human diseases has supported that lymphatic dysfunction often leads to adipose tissue accumulation. Such effect is well illustrated in patients suffering from lymphedema [10]. Lymphedema arises as a consequence of impaired lymph transport which results either from abnormal lymphatic vessel development (primary or hereditary lymphedema) or from lymphatic vascular damage or infection (secondary or acquired lymphedema). If lymphedema is not resolved, patients with primary or secondary lymphedema typically exhibit local edema which follows the retention of interstitial fluid and proteins in the affected tissues. As disease progresses, immune cell infiltration, tissue fibrosis and most relevant to this review, excessive fat accumulation occur [10-12]. In fact, liposuction of lymphedema revealed that fat rather than fluid deposition mainly accounts for tissue swelling [12]. Adipose tissue accumulation has also been described in surgically-induced [13,14] or naturally occurring mouse models of secondary lymphedema. The Chy mice which lacked dermal lymphatic vessels as a result of an inactivating mutation in *Vegfr3* gene exhibited thickening of the subcutaneous fat [13]. Inactivation of *Prox1*, a gene encoding for a transcription factor critical for lymphatic vasculature development [15-17], in mouse leads to lymphatic vasculature leakage and the development of adult-onset obesity [16]. Interestingly, the authors of

this latter study demonstrated that the degree of lymphatic defect correlates with the magnitude of adipose tissue deposition in mice haploinsufficient for *Prox1* and that the lymph leaking from this defective lymphatic vasculature promotes the differentiation of adipocytes. This adipogenic property of lymph was in fact reported in earlier work [18] and has been associated with changes in temporal and spatial expression of CCAAT/enhancer-binding protein-α and peroxisome proliferator-activated receptor-γ which control adipocyte differentiation [19]. Further investigations are warranted to determine whether these mechanisms described in mouse models of lymphedema also account for the fat deposition observed in patients with lymphedema.

Whereas there are strong clinical and experimental data supporting that defects in lymphatic function often lead to fat deposition in peripheral tissues, the impact of adipose tissue accumulation on lymphatic function has only recently begun to be appreciated (Figure 1). In humans, there are some reports showing that obesity is associated with lymphatic impairment [20,21] but not all [22], therefore further confirmation awaits. Two recent studies reported that diet-induced obesity (DIO) in rodents was accompanied by impaired lymphatic flow [23,24] and this impairment was, in part, due to vessel wall dilation and reduced contractile activity of collecting lymphatic vessels [23]. As a result of compromised lymphatic flow, DIO mice developed a more severe phenotype in surgical-induced lymphedema as compared to lean control animals [25]. These findings may provide an explanation as to why obesity or weight gain following sentinel lymph node biopsy or axillary lymph node dissection are predisposing factors for lymphedema although the mechanisms involved are likely more complex [26,27].
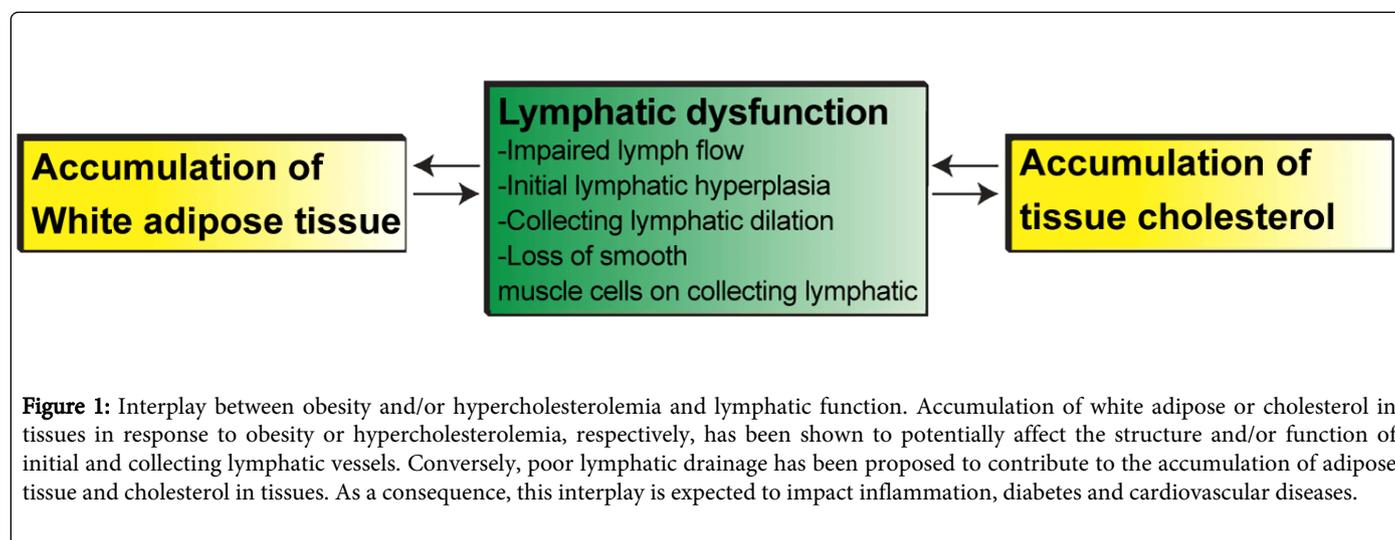


**Figure 1:** Interplay between obesity and/or hypercholesterolemia and lymphatic function. Accumulation of white adipose or cholesterol in tissues in response to obesity or hypercholesterolemia, respectively, has been shown to potentially affect the structure and/or function of initial and collecting lymphatic vessels. Conversely, poor lymphatic drainage has been proposed to contribute to the accumulation of adipose tissue and cholesterol in tissues. As a consequence, this interplay is expected to impact inflammation, diabetes and cardiovascular diseases.

### Evidence connecting lymphatic function with tissue cholesterol accumulation

The impact of lymphatic dysfunction is not limited to adipose tissue expansion and has recently been extended to cholesterol accumulation in tissues (Figure 1). Cells have developed complex mechanisms to regulate the abundance of intracellular cholesterol since an excess can be detrimental. Among these mechanisms, the process referred to reverse cholesterol transport (RCT) has been shown to be critical for the removal of excess cholesterol from peripheral tissues and its transport to the liver via plasma for excretion [28]. RCT is initiated

when cells including macrophages efflux the excess of cholesterol to prevent intracellular over-accumulation. This step is tightly regulated through the action of ATP-binding cassette transporters ABCA1, ABCG1 and scavenger receptor class BI (SR-BI) and high density lipoprotein (HDL) which serves as the major acceptor for cellular cholesterol released in the extrahepatic tissues [29]. Following cholesterol efflux, HDL-cholesterol is transported from the tissue back into the blood circulation. In the final step of RCT, the majority of HDL-derived cholesterol is removed from the liver by secretion into the bile [28,29]. In contrast to the knowledge on the mechanisms by

which cholesterol is effluxed from the cells and its fate after reaching the liver, little information is available on how HDL-cholesterol travels from the peripheral tissues back to the blood circulation. Since one of the major roles of lymphatic vessels is to drain macromolecules from the interstitial space back to the circulation and lymph is rich in cholesterol and HDL [30-32], the implication of lymphatic vessel in the transport of lipoproteins from interstitium to blood was proposed [33] and re-established recently by two separate groups. The study of Martel et al., [34] in Chy mice and our study [35] in wild-type mice in which lymphatic vessels were surgically excised clearly provide the first direct evidence that poor lymphatic drainage significantly decreased the efficiency of RCT. We also further proposed that the transport of effluxed cholesterol from peripheral tissue to the circulation via lymphatics is not only passive as anticipated but can be an active process dependent on HDL and SR-BI [35]. These new findings support the concept that lymphatic vessels tightly regulate the clearance of cholesterol from the peripheral tissues through the transport of HDL-cholesterol [31] (Figure 1). There are some indications that such concept may also hold in humans. Indeed, isolated case reports of xanthomas in primary lymphedema patients have been described in the dermatologic literature since the 60's [36-40]. Cholesterol accumulation in tissues as a result of poor lymphatic function may also increase the adipocyte burden to store more lipids thereby increasing deposition of fat [41,42].

Xanthomas including tendon xanthomas are a clinical feature that often manifest in human familial hypercholesterolemia [43,44]. Notably, hypercholesterolemic mice including mice lacking apolipoprotein E (apoE$^{-/-}$) or low density lipoprotein receptor (Ldlr$^{-/-}$) have been shown to develop peripheral skin edema. The edematous tissues in these mice were found to consist predominantly of cholesterol deposits and formation of foam cells [35,45,46]. Our group has reported that hypercholesterolemia in apoE$^{-/-}$ and Ldlr$^{-/-}$ mice leads to severe skin lymphatic dysfunction as reducing hypercholesterolemia with ezetimibe treatment improves lymphatic function and cholesterol clearance from skin [35,47]. Impairment of lymphatic function in these mice was associated with initial lymphatic vessel hyperplasia, loss of collecting vessel identity as shown by poor smooth muscle cell coverage [47]. These structural changes that likely account for lymphatic dysfunction observed in hypercholesterolemic mice (Figure 1) may result from a decreased in vascular endothelial growth factor-C (VEGF-C), FOXC2 and angiopoietin-2 expression [35], which are important factors to maintain lymphatic vasculature [35,47]. It remains unclear whether lymphatic dysfunction occurs in humans with hypercholesterolemia. A study in patients with familial combined hyperlipidemia reporting lower gene expression of FOXC-2 and Prox-1 in their adipose tissues is consistent with this idea [48].

Collectively, these studies support the link between accumulation of white adipose or cholesterol in tissue and lymphatic function. Moreover, they raise the possibility of a two-way relationship between lipid homeostasis and lymphatic function with the potential to set up a positive feedback: changes in adipose tissue or cholesterol content may impair lymphatic drainage in peripheral tissues, and this loss of lymphatic function may render the tissue susceptible to further and more profound alterations in adipose tissue or cholesterol accumulation (Figure 1). In the following section, we discuss potential mechanisms whereby excessive fat or cholesterol accumulation in tissue may account for lymphatic dysfunction. Although we focus specifically on how alterations in adipose tissue and macrophages accompanying obesity and hypercholesterolemia may impact lymphatic function, we acknowledge that additional pathways may be involved or be uncovered in the near future.

## Possible Mechanisms Leading to Lymphatic Dysfunction under Excessive Fat or Cholesterol Accumulation

### Changes in adipose tissue

The adipose tissue consists of adipocytes, precursor cells, fibroblasts, collagen-rich connective tissues, blood capillaries, nerve fibres, fibroblasts and macrophages. It is no longer perceived as just a reservoir for energy stores but rather as an endocrine organ which releases many bioactive substances termed adipokines. Among them, adiponectin, leptin, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are critical determinants of various metabolic and physiological function [49]. Therefore, the absence of adipose tissue results in detrimental metabolic changes. Similar to humans suffering from lipoatrophy [50], mice lacking white adipose tissue develop insulin resistance, hyperglycemia, hyperlipidemia and fatty liver [51]. Transplantation of wild-type adipose tissue into these lipoatrophic mice completely reversed the phenotype [51]. During obesity, although adipogenesis occurs, the white adipose tissue also undergoes negative remodelling characterized by adipocyte hypertrophy and followed by death [52]. These structural changes in adipose tissue during obesity lead to an alteration in adipokine production which in turn results in insulin resistance, hyperglycemia, endothelium dysfunction and fatty liver [53]. In sum, the adipose tissue is clearly essential for health, and therefore, when it is imbalanced major deleterious effects on the body system arise. The fact that adipose tissue always surrounds the lymphatic vessels and lymph nodes [54] raises the possibility that changes in adipose tissue may account for the lymphatic dysfunction observed in obesity and hypercholesterolemia. Indeed, we detected morphological changes in the subcutaneous adipose tissue of hypercholesterolemic apoE$^{-/-}$ mice similar to those described in obese mice (unpublished data). Pond and colleagues have shown that during chronic inflammation, the adipose tissue surrounding the lymph nodes expands and increases its rate of lipolysis to fuel the inflammation [55,56]. Therefore, the adipose tissue surrounding the lymphatic vessels may also provide energy or adipokines to maintain the lymphatic vasculature (Figure 2). Adiponectin is exclusively expressed by adipocytes, and serum adiponectin concentration inversely correlates with increasing obesity [57,58]. Recently, Shumizu and colleagues [59] demonstrated the importance of adiponectin in resolving lymphedema as adiponectin deficient mice develop a more severe phenotype in surgical-induced lymphedema and the administration of adiponectin in obese mice ameliorates lymphedema [59]. *In vitro*, treatment of adiponectin has been shown to stimulate lymphatic vessel formation and maintain vessel viability via AMPK/Akt/endothelial nitric oxide synthase (eNOS) signalling pathways [59]. Nitric oxide is known to regulate lymphatic pumping through its action on lymphatic smooth muscle cells [60,61]. Therefore, given the effect of adiponectin in the regulation of arterial tone via nitric oxide [62,63], it is also possible that administration of adiponectin could improve collecting vessel structure and function in obese mice (Figure 2).
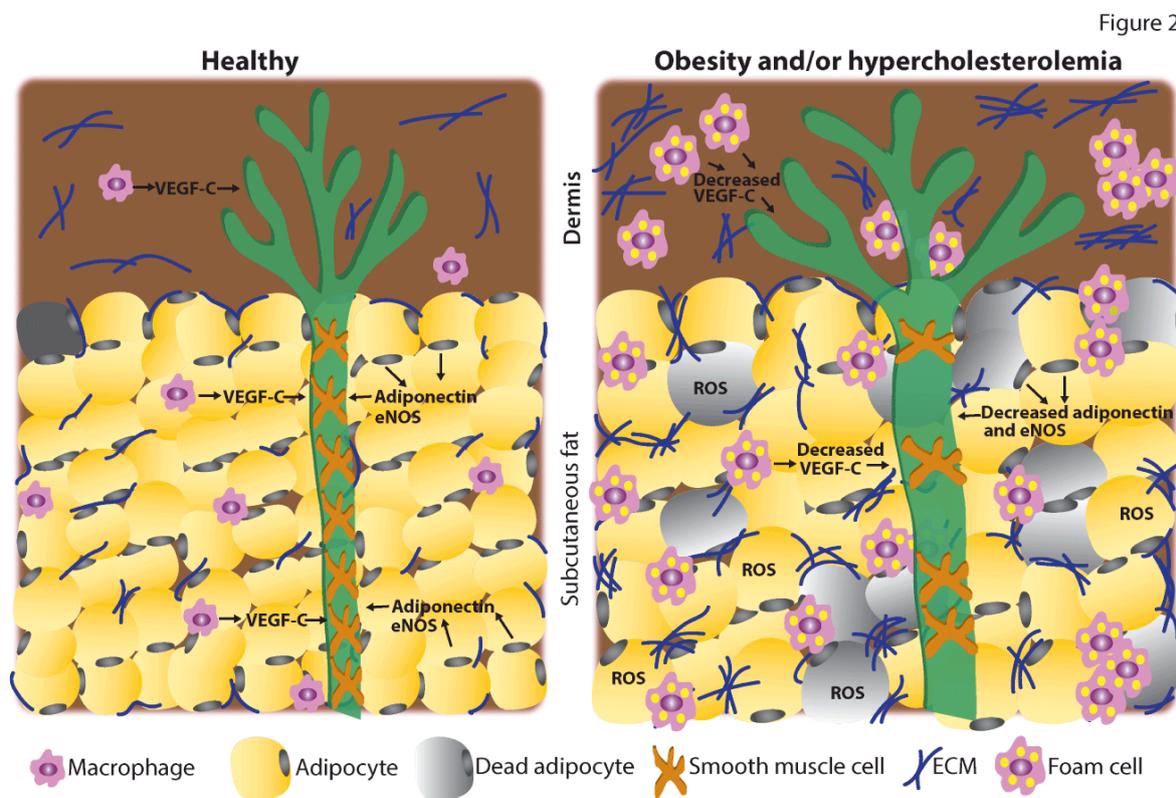
**Figure 2: Possible mechanisms contributing to impaired lymphatic function during obesity and/or hypercholesterolemia.** In skin, dermal blind-ended initial lymphatic vessels converge into collecting vessels, which are covered with smooth muscles and feed into the draining lymph node (not represented here). Under healthy condition, macrophages present in both the dermis and subcutaneous fat and adipocytes may maintain the morphology and the function of lymphatic vessels by secreting vascular endothelial growth factor-C (VEGF-C) and adiponectin as well as controlling the expression of eNOS. In contrast, obesity and hypercholesterolemia have been shown to induce infiltration of macrophages which transform into foam cells upon cholesterol uptake and adipocyte hypertrophy and death. Under these conditions, adipocytes produce reduced amount of adiponectin but increased reactive oxygen species (ROS) which have been shown to reduce lymphatic pumping activity. Lipid-laden macrophages may show reduced capacity to produce VEGF-C. Furthermore, obesity is associated with increased deposition of extracellular matrix (ECM), particularly collagen which may in turn affect lymphatic vasculature. Altogether, these alterations in macrophage and adipocyte along with fibrosis may lead to lymphatic vessel dilation and impaired lymphatic transport.

Obesity in mice and humans has also been reported to be associated with increased oxidative stress in circulation and adipose tissue [64]. Furthermore, lipid-laden cultured adipocytes exhibit an increased content in reactive oxygen species metabolites [65]. Because such metabolites have been shown to inhibit mesentery collecting lymphatic contractility [66], it is likely that the increased oxidative environment in adipose tissue during obesity may also impair lymphatic function (Figure 2). Another possible change in adipose tissue accompanying obesity that may potentially affect lymphatic function is fibrosis (Figure 2). In obesity, the adipose tissue is a major site of fibrosis [67]. Interestingly, fibrosis has been reported to inhibit lymphatic regeneration and function in mouse model of lymphedema [68,69]. During lymphedema, fibrosis in the subcutaneous adipose tissue and collecting lymphatic vessels occurs [14,19,70]. As a consequence of the increase in collagen fibres, collecting lymphatic vessels lose their elastic properties. These pathological changes resemble a condition known as aortic stiffening. In this condition, fractured elastin and increased collagen in the aorta affect the mechanical properties of the aortic wall

ultimately, resulting in vasodilation and vasoconstriction dysfunction [71]. Similarly, fibrosis on the collecting lymphatic vessels may impede their contractility and impair lymph transport. Consistent with this hypothesis, obesity-associated fibrosis has been recently proposed to contribute to the exacerbation of lymphatic dysfunction in a mouse model of lymphedema [25].

## Alterations in macrophage

Obesity is associated with increased macrophage infiltration in adipose tissue [9,72,73]. These macrophages elicit a chronic low-grade inflammation by producing proinflammatory mediators such as TNFα, inducible nitric oxide, IL-6, and IL-1β which, potently impair adipocyte lipid metabolism, augment insulin resistance and promote tissue fibrosis [73,74]. The ablation of macrophages in obese mice reduced inflammation and improved insulin sensitivity [75]. Similarly, in humans, weight loss induced by gastric bypass or low energy diet was accompanied in the adipose tissue by a reduction in macrophage

content and a concomitant decrease in inflammatory markers [72,76]. Whereas all these studies highlight the contribution of infiltrating macrophages in obesity-related complications, the phenotype of these macrophages remain uncertain. Macrophages have been traditionally classified into M1 and M2 macrophages. In this classification, M1 and M2 designation refer to classically activated macrophage and alternatively activated macrophage, respectively [77]. In obese mice, the inflammatory M1 macrophages predominate in the adipose tissues as opposed to the resident non-inflammatory M2 macrophages which are present in lean mice [78,79]. However, one study reported the presence of macrophages with a mixed M1/M2 profile in obese mice [80]. Similarly, in humans, one study described the predominance of M1 macrophage in adipose tissue during obesity [81] whereas another study reported the presence of macrophages expressing M1-M2 surface markers [74]. Furthermore, some studies have shown that despite the expression of M2 surface markers, the majority of these macrophages exhibit both anti-inflammatory and inflammatory features [82,83]. Notably, similar picture has been drawn regarding the phenotype of macrophages that accumulate during hypercholesterolemia associated with atherosclerotic disease [84]. It is possible that the phenotype of macrophages may vary during the course of obesity and hypercholesterolemia as changes in their surrounding tissue are expected over time. This may explain these conflicting results about macrophage phenotype in obesity and hypercholesterolemia. These studies may also highlight the limitations of characterizing macrophage populations during these medical conditions on the basis of M1/M2 classification. A new grouping of macrophage populations based on their functions as proposed [85] should be considered.

So far, research on the involvement of macrophages in regulating lymphatic vessels has mainly focused on their effect on the growth of lymphatic vessels, a process also called lymphangiogenesis, in the context of inflammation and development. Therefore, it is unknown whether they may also maintain lymphatic function and structure under homeostatic conditions. Macrophages have been reported to either act as lymphatic progenitor cells [86-88] or to produce lymphangiogenic factors including VEGF-C and VEGF-D [88-91]. In addition to its lymphangiogenic property, VEGF-C can also control the pumping activity of collecting vessels [92] and dilation of initial lymphatic vessels [93] (Figure 2). Ablation of macrophages inhibit lymphangiogenesis which delays resolution of inflammation [90] and macrophage-deficient mice develop severe lymphatic hyperplasia during development [94]. It is unclear whether these "prolymphangiogenic" macrophages are of M1 or M2 phenotype. Some studies have identified them based on the surface expression of lymphatic vessel endothelial hyaluronic acid receptor-1 (LYVE-1) [94-96]. Notably, LYVE-1+ positive macrophages have been reported in the adipose tissue of rodents [97] and humans [83]. Interestingly, down-regulation of VEGF-C expression was reported in adipose tissue from obese individuals compared to lean controls [98]. Similarly, we demonstrated recently that the lymphatic dysfunction observed in skin of hypercholesterolemic mice is associated with decreased VEGF-C expression. Administration of VEGF-C restores lymphatic function in these mice which in turn promotes cholesterol clearance [35]. Furthermore, analysis of macrophages in apoE$^{-/-}$ mice revealed that they massively accumulate in skin and the majority uptake lipids and become foam cells [35]. Interestingly, these lipid-laden macrophages express negligible amount of VEGF-C compared to resident macrophage in normocholesterolemic skin (unpublished data, Figure 2). Few scenarios may explain the decreased expression of VEGF-C by these macrophages, namely: macrophages may lose their capacity to produce VEGF-C once they become foam cells or the hypercholesterolemic environment may promote the recruitment and/or differentiation of a population of macrophage which does not express VEGF-C. The latter idea may also apply to obesity because adipokines whose production by adipocytes is affected during obesity can modulate macrophage function. Adiponectin seems to be a good candidate since it has been recently reported *in vitro* to induce VEGF-C production by macrophages [99]. Moreover, adiponectin levels are decreased during obese states in which lymphatic function is impaired [57,58]. Taken together, these findings support macrophage dysfunction as a possible contributor to lymphatic impairment during lipid-related disorders which needs further considerations (Figure 2).

## Immunological Consequences of Lymphatic Dysfunction Associated with Hypercholesterolemia

Given the emerging contribution of the lymphatic system in regulating inflammatory [3] and adaptive immune responses [2], we discuss here how lymphatic dysfunction in hypercholesterolemia may alter immunological processes (Table 1). Lymphatic vessels are now recognized by immunologists as important routes for the migration of dendritic cells and lymphocytes from peripheral tissue into the draining lymph nodes and for the egress of lymphocytes from the lymph nodes [4,100-102]. Moreover, lymph fluid drains soluble antigens to the draining lymph nodes [103]. Therefore, lymphatic vessels can regulate immune responses by supporting the interactions between antigen-presenting cells and rare-antigen specific T cells in lymph nodes as well as the recirculation of lymphocytes. In apoE$^{-/-}$ mice, lymphatic dysfunction severely compromises the migration of skin dendritic cells to the draining lymph nodes, and conversely, improving lymphatic drainage restores dendritic cell migration [35,47,104]. It is possible that the trafficking of lymphocytes out of the lymph nodes may also be affected in apoE$^{-/-}$ mice since these mice exhibit hypertrophic lymph node as shown by a significant increase in cellularity [47,104]. Such alterations in immune cell trafficking are expected to impact immune priming and memory responses [2]. This may explain the increased susceptibility of hypercholesterolemic mice to infections [105-107]. Lymphatic endothelial cells have been shown to modulate the activation of T cell through the presentation of antigens via MHC class I [108-110] and class II [111,112] as well as T cell homeostasis through the production of immunoregulatory cytokines [113-115]. Therefore, it would be interesting to evaluate whether these functions of the lymphatic endothelium are affected in hypercholesterolemic mice.

| 1 | Transport of soluble antigens |
|---|---|
| 2 | Immune cell (dendritic cell, lymphocyte) emigration from peripheral tissues |
| 3 | Lymphocyte egress from draining lymph node |
| 4 | Resolution of inflammation |
| 5 | Development of tertiary lymphoid organs |
| 6 | Presentation of antigen by lymphatic endothelium |
| 7 | T lymphocyte activation |

| 8 | T lymphocyte homeostasis |
| --- | --- |

**Table 1:** Immunologic processes potentially altered as a result of lymphatic dysfunction.

Hypercholesterolemia is a major risk factor of atherosclerosis which is a chronic inflammatory disease of medium and large arteries. Arteries are composed of three layers, namely: the intima or inner layer, followed by the media and the most outer layer, the adventitia. Atherosclerosis affecting predominantly the coronary arteries in humans and the aorta in mouse results from the progressive build-up of plaques within the intimal tissue. Accumulation of cholesterol, immune cells and fibrous elements along with inflammation are the hallmark of this chronic inflammatory disease. The intima has been a major focus of research on atherosclerosis and the processes within the vessel wall have been well-characterized. In contrast, changes in the adventitia during atherosclerosis and the physiological and/or pathological role of this layer have been largely neglected. In addition to macrophages, fibroblast, T cells and mast cells, the adventitia contains blood and lymphatic vessels. As other chronic inflammatory diseases affecting the skin, kidney, eye, airways and the gut [3,4], atherosclerosis has also been recently associated with lymphangiogenesis in the adventitia [116-118]. However, further investigations will be necessary to establish whether lymphangiogenesis is an element of the pathology or a productive attempt to resolve atherosclerosis. Since we showed that hypercholesterolemic apoE$^{-/-}$ mice, which also develop atherosclerosis exhibit compromised lymphatic transport in skin, the function of these newly formed or existing adventitial lymphatic vessels may also be altered during atherogenesis. Interestingly, a link between disrupted flow and atherogenesis was postulated as far back as five decades ago [119-123]. It was reported that lymphatic ligation at the level of renal arteries in mongrel dogs increases connective tissue surrounding the obstructed lymphatic vessels and promotes intimal thickening [120]. Similar observations were made after ligating cardiac lymph nodes [119]. Finally, examination of resected colon carcinoma specimens revealed atherosclerotic changes only in those sections of arteries in which tumor infiltrates obstructed lymphatic drainage [123]. Consistent with these early observations, a recent study by Vuorio et al., [124] demonstrated that crossing mice exhibiting lymphatic insufficiency, chy mice and soluble vascular endothelial receptor-3, with atherosclerotic mice enhances cholesterol and triglyceride levels and atherogenesis. Using a surgical model in which the donor aortic arch was transplanted into the abdominal cavity of a recipient mouse, Martel et al., [34] reported that blocking the regeneration of lymphatic vessels after surgery significantly inhibit the clearance of cholesterol by RCT.

Altogether, these studies support the concept that poor lymph flow may contribute to the atherosclerotic process by disturbing the balance of lipoproteins in the arterial wall. HDL, which selectively passes through arterial endothelium and binds cholesterol, may be unable to drain through lymphatic vessels and carry cholesterol to the liver for metabolism as bile acids. As a consequence, cholesterol will accumulate in the arterial wall. LDL which transports lipids into the arterial wall will continue to deposit cholesterol and triglycerides, even in the presence of defective lymph flow. By inducing protein stasis in the arterial wall, impaired lymphatic drainage may also promote local inflammatory reactions as observed during lymphedema [10] which will likely influence plaque progression and stability. As we observed in skin of atherosclerotic mice [104], impaired lymphatic drainage

may also alter the emigration of immune cells from the arteries including dendritic cells [125] and lymphocytes [126,127], which actively participate in atherosclerosis. As discussed above, such alterations in immune cell trafficking will likely affect adaptive immune responses. Finally, defective lymphatic drainage may support the development of tertiary lymphoid organs (TLOs) as suggested in chronic graft rejection [128]. TLOs also named ectopic lymphoid tissues are accumulations of cells emerging in response to chronic inflammation [129,130]. They share similar organization and structure with lymph nodes except the absence of capsule. Interestingly, TLOs have been identified in the aortic adventitia of aged apoE$^{-/-}$ mice during atherosclerosis and shown to exhibit aberrant lymphangiogenesis [131,132]. Similarly, infiltrates of leukocytes in adventitia of coronary arteries of patients with atherosclerosis as well as B cell follicle-like aggregates in human aorta have been described [133,134]. Given the proposed contribution of TLOs in primary immune response and in autoimmune responses in advanced atherosclerosis [132], the functions of these TLOs lymphatic vessels deserve to be investigated.

## Conclusion

There is no doubt that tremendous progress has been made on the role of lymphatic vessels in lipid transport since the first description of milky white vessels in the mesentery after food intake by Gasparo Aselli in 1622, and its association with lipid-associated pathologies. However, many questions remain to be answered in order to establish the link between lymphatic function and fat or cholesterol accumulation in tissues. For example, studies should address whether lymphatic dysfunction is associated with obesity and hypercholesterolemia in humans and whether it can influence the course of obesity and clinical features associated with hypercholesterolemia including xanthomas, atherosclerosis and diabetes. For that, methods, particularly imaging modalities, to assess lymphatic function such as cholesterol transport, need to be further improved or developed. Further basic research is also required to identify the mechanisms by which adipocytes may influence lymphatic function and to better characterize the population of macrophages maintaining lymphatic vessel homeostasis. Answering these questions is essential as it may provide new avenues to treat atherosclerosis which is still a leading cause of death in industrialized countries throughout the world despite the great success of statin drugs and obesity, whose prevalence is expected to continue to augment. Therefore, the importance of the lymphatic system in these lipid-related diseases underscores the urgent need to find pharmacological or surgical interventions that can improve lymphatic function which are currently not available. Finally, a better understanding of the implications of lymphatic endothelium in shaping adaptive immune responses particularly during disease is imperative as lymphatic vessels may be a promising target for immunotherapies.

## Acknowledgements

## References

1. Zawieja D (2005) Lymphatic biology and the microcirculation: past, present and future. Microcirculation 12: 141-150.

2. Card CM, Yu SS, Swartz MA (2014) Emerging roles of lymphatic endothelium in regulating adaptive immunity. J Clin Invest 124: 943-952.

3. Kim H, Kataru RP, Koh GY (2014) Inflammation-associated lymphangiogenesis: a double-edged sword? J Clin Invest 124: 936-942.

4. Tan KW, Chong SZ, Angeli V (2014) Inflammatory lymphangiogenesis: cellular mediators and functional implications. Angiogenesis 17: 373-381.

5. Dixon JB (2010) Lymphatic lipid transport: sewer or subway? Trends Endocrinol Metab 21: 480-487.

6. Miettinen TA (1971) Cholesterol production in obesity. Circulation 44: 842-850.

7. Guerrero-Romero F, Rodríguez-Morán M (2006) Prevalence of dyslipidemia in non-obese prepubertal children and its association with family history of diabetes, high blood pressure, and obesity. Arch Med Res 37: 1015-1021.

8. Shapiro H, Pecht T, Shaco-Levy R, Harman-Boehm I, Kirshtein B, et al. (2013) Adipose tissue foam cells are present in human obesity. J Clin Endocrinol Metab 98: 1173-1181.

9. Xu H, Barnes GT, Yang Q, Tan G, Yang D, et al. (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 112: 1821-1830.

10. Rockson SG (2001) Lymphedema. Am J Med 110: 288-295.

11. Schirger A, Harrison EG Jr, Janes JM (1962) Idiopathic lymphedema. Review of 131 cases. JAMA 182: 14-22.

12. Brorson H, Ohlin K, Olsson G, Nilsson M (2006) Adipose tissue dominates chronic arm lymphedema following breast cancer: an analysis using volume rendered CT images. Lymphat Res Biol 4: 199-210.

13. Rutkowski JM, Markhus CE, Gyenge CC, Alitalo K, Wiig H, et al. (2010) Dermal collagen and lipid deposition correlate with tissue swelling and hydraulic conductivity in murine primary lymphedema. Am J Pathol 176: 1122-1129.

14. Zampell JC, Aschen S, Weitman ES, Yan A, Elhadad S, et al. (2012) Regulation of adipogenesis by lymphatic fluid stasis: part I. Adipogenesis, fibrosis, and inflammation. Plast Reconstr Surg 129: 825-834.

15. Wigle JT, Oliver G (1999) Prox1 function is required for the development of the murine lymphatic system. Cell 98: 769-778.

16. Harvey NL, Srinivasan RS, Dillard ME, Johnson NC, Witte MH, et al. (2005) Lymphatic vascular defects promoted by Prox1 haploinsufficiency cause adult-onset obesity. Nat Genet 37: 1072-1081.

17. Wigle JT, Harvey N, Detmar M, Lagutina I, Grosveld G, et al. (2002) An essential role for Prox1 in the induction of the lymphatic endothelial cell phenotype. EMBO J 21: 1505-1513.

18. Nougues J, Reyne Y, Dulor JP (1988) Differentiation of rabbit adipocyte precursors in primary culture. Int J Obes 12: 321-333.

19. Aschen S, Zampell JC, Elhadad S, Weitman E, De Brot M, et al. (2012) Regulation of adipogenesis by lymphatic fluid stasis: part II. Expression of adipose differentiation genes. Plast Reconstr Surg 129: 838-847.

20. Arngrim N, Simonsen L, Holst JJ, Bülow J (2013) Reduced adipose tissue lymphatic drainage of macromolecules in obese subjects: a possible link between obesity and local tissue inflammation? Int J Obes (Lond) 37: 748-750.

21. Greene AK, Grant FD, Slavin SA (2012) Lower-extremity lymphedema and elevated body-mass index. N Engl J Med 366: 2136-2137.

22. Vasileiou AM, Bull R, Kitou D, Alexiadou K, Garvie NJ, et al. (2011) Oedema in obesity; role of structural lymphatic abnormalities. Int J Obes (Lond) 35: 1247-1250.

23. Blum KS, Karaman S, Proulx ST, Ochsenbein AM, Luciani P, et al. (2014) Chronic high-fat diet impairs collecting lymphatic vessel function in mice. PLoS One 9: e94713.

24. Weitman ES, Aschen SZ, Farias-Eisner G, Albano N, Cuzzone DA, et al. (2013) Obesity impairs lymphatic fluid transport and dendritic cell migration to lymph nodes. PLoS One 8: e70703.

25. Savetsky IL, Torrisi JS, Cuzzone DA, Ghanta S, Albano NJ, et al. (2014) Obesity increases inflammation and impairs lymphatic function in a mouse model of lymphedema. Am J Physiol Heart Circ Physiol 307: 165-172.

26. Helyer LK, Varnic M, Le LW, Leong W, McCready D (2010) Obesity is a risk factor for developing postoperative lymphedema in breast cancer patients. Breast J 16: 48-54.

27. McLaughlin SA, Wright MJ, Morris KT, Giron GL, Sampson MR, et al. (2008) Prevalence of lymphedema in women with breast cancer 5 years after sentinel lymph node biopsy or axillary dissection: objective measurements. J Clin Oncol 26: 5213-5219.

28. Rader DJ, Alexander ET, Weibel GL, Billheimer J, Rothblat GH (2009) The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis. J Lipid Res 50 Suppl: S189-194.

29. Tall AR (2008) Cholesterol efflux pathways and other potential mechanisms involved in the athero-protective effect of high density lipoproteins. J Intern Med 263: 256-273.

30. Nanjee MN, Cooke CJ, Wong JS, Hamilton RL, Olszewski WL, et al. (2001) Composition and ultrastructure of size subclasses of normal human peripheral lymph lipoproteins: quantification of cholesterol uptake by HDL in tissue fluids. J Lipid Res 42: 639-648.

31. Randolph GJ, Miller NE (2014) Lymphatic transport of high-density lipoproteins and chylomicrons. J Clin Invest 124: 929-935.

32. Reichl D, Simons LA, Myant NB, Pflug JJ, Mills GL (1973) The lipids and lipoproteins of human peripheral lymph, with observations on the transport of cholesterol from plasma and tissues into lymph. Clin Sci Mol Med 45: 313-329.

33. Reichl D, Miller NE (1989) Pathophysiology of reverse cholesterol transport. Insights from inherited disorders of lipoprotein metabolism. Arteriosclerosis 9: 785-797.

34. Martel C, Li W, Fulp B, Platt AM, Gautier EL, et al. (2013) Lymphatic vasculature mediates macrophage reverse cholesterol transport in mice. J Clin Invest 123: 1571-1579.

35. Lim HY, Thiam CH, Yeo KP, Bisoendial R, Hii CS, et al. (2013) Lymphatic vessels are essential for the removal of cholesterol from peripheral tissues by SR-BI-mediated transport of HDL. Cell Metab 17: 671-684.

36. Wu JJ, Wagner AM (2003) Verruciform xanthoma in association with milroy disease and leaky capillary syndrome. Pediatr Dermatol 20: 44-47.

37. Woolling KR, Jenkins RE, Dolan PA, Evans PV (1970) Localized xanthomas in lymphedema praecox. JAMA 211: 1372-1374.

38. Goldrick RB, Ahrens EH Jr (1964) Unilateral Chylous Lymphedema and Xanthomatosis: A Study of Factors Governing the Flow of Intestinal Lymph. Am J Med 37: 610-622.

39. Romaní J, Luelmo J, Sáez A, Yébenes M, Sábat M, et al. (2012) Localized xanthomas associated with primary lymphedema. Pediatr Dermatol 29: 113-114.

40. Berger BW, Kantor I, Maier HS (1972) Xanthomatosis and lymphedema. Arch Dermatol 105: 730-731 passim.

41. Krause BR, Hartman AD (1984) Adipose tissue and cholesterol metabolism. J Lipid Res 25: 97-110.

42. Toh SA, Millar JS, Billheimer J, Fuki I, Naik SU, et al. (2011) PPARÎ³ activation redirects macrophage cholesterol from fecal excretion to adipose tissue uptake in mice via SR-BI. Biochem Pharmacol 81: 934-941.

43. Goldberg AC, Hopkins PN, Toth PP, Ballantyne CM, Rader DJ, et al. (2011) Familial hypercholesterolemia: screening, diagnosis and management of pediatric and adult patients: clinical guidance from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Clin Lipidol 5: S1-S8.

44. Luttun A, Tjwa M, Moons L, Wu Y, Angelillo-Scherrer A, et al. (2002) Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. Nat Med 8: 831-840.

45. van Ree JH, Gijbels MJ, van den Broek WJ, Hofker MH, Havekes LM (1995) Atypical xanthomatosis in apolipoprotein E-deficient mice after cholesterol feeding. Atherosclerosis 112: 237-243.

46. Ishibashi S, Goldstein JL, Brown MS, Herz J, Burns DK (1994) Massive xanthomatosis and atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice. J Clin Invest 93: 1885-1893.

47. Lim HY, Rutkowski JM, Helft J, Reddy ST, Swartz MA, et al. (2009) Hypercholesterolemic mice exhibit lymphatic vessel dysfunction and degeneration. Am J Pathol 175: 1328-1337.

48. Horra A, Salazar J, Ferré R, Vallvé JC, Guardiola M, et al. (2009) Prox-1 and FOXC2 gene expression in adipose tissue: A potential contributory role of the lymphatic system to familial combined hyperlipidaemia. Atherosclerosis 206: 343-345.

49. Singla P, Bardoloi A, Parkash AA (2010) Metabolic effects of obesity: A review. World J Diabetes 1: 76-88.

50. Seip M, Trygstad O (1996) Generalized lipodystrophy, congenital and acquired (lipoatrophy). Acta Paediatr Suppl 413: 2-28.

51. Gavrilova O, Marcus-Samuels B, Graham D, Kim JK, Shulman GI, et al. (2000) Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. J Clin Invest 105: 271-278.

52. Sun K, Kusminski CM, Scherer PE (2011) Adipose tissue remodeling and obesity. J Clin Invest 121: 2094-2101.

53. Lafontan M (2014) Adipose tissue and adipocyte dysregulation. Diabetes Metab 40: 16-28.

54. Harvey NL (2008) The link between lymphatic function and adipose biology. Ann N Y Acad Sci 1131: 82-88.

55. Mattacks CA, Sadler D, Pond CM (2003) The cellular structure and lipid/protein composition of adipose tissue surrounding chronically stimulated lymph nodes in rats. J Anat 202: 551-561.

56. Mattacks CA, Sadler D, Pond CM (2002) The effects of dietary lipids on adrenergically-stimulated lipolysis in perinodal adipose tissue following prolonged activation of a single lymph node. Br J Nutr 87: 375-382.

57. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, et al. (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 257: 79-83.

58. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, et al. (2003) Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 46: 459-469.

59. Shimizu Y, Shibata R, Ishii M, Ohashi K, Kambara T, et al. (2013) Adiponectin-mediated modulation of lymphatic vessel formation and lymphedema. J Am Heart Assoc 2: e000438.

60. Chatterjee V, Gashev AA (2012) Aging-associated shifts in functional status of mast cells located by adult and aged mesenteric lymphatic vessels. Am J Physiol Heart Circ Physiol 303: H693-702.

61. Liao S, Cheng G, Conner DA, Huang Y, Kucherlapati RS, et al. (2011) Impaired lymphatic contraction associated with immunosuppression. Proc Natl Acad Sci U S A 108: 18784-18789.

62. Fésüs G, Dubrovska G, Gorzelniak K, Kluge R, Huang Y, et al. (2007) Adiponectin is a novel humoral vasodilator. Cardiovasc Res 75: 719-727.

63. Xi W, Satoh H, Kase H, Suzuki K, Hattori Y (2005) Stimulated HSP90 binding to eNOS and activation of the PI3-Akt pathway contribute to globular adiponectin-induced NO production: vasorelaxation in response to globular adiponectin. Biochem Biophys Res Commun 332: 200-205.

64. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, et al. (2004) Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 114: 1752-1761.

65. Subauste AR, Burant CF (2007) Role of FoxO1 in FFA-induced oxidative stress in adipocytes. Am J Physiol Endocrinol Metab 293: E159-164.

66. Zawieja DC, Greiner ST, Davis KL, Hinds WM, Granger HJ (1991) Reactive oxygen metabolites inhibit spontaneous lymphatic contractions. Am J Physiol 260: H1935-1943.

67. Sun K, Tordjman J, Clément K, Scherer PE (2013) Fibrosis and adipose tissue dysfunction. Cell Metab 18: 470-477.

68. Avraham T, Clavin NW, Daluvoy SV, Fernandez J, Soares MA, et al. (2009) Fibrosis is a key inhibitor of lymphatic regeneration. Plast Reconstr Surg 124: 438-450.

69. Avraham T, Daluvoy S, Zampell J, Yan A, Haviv YS, et al. (2010) Blockade of transforming growth factor-beta1 accelerates lymphatic regeneration during wound repair. Am J Pathol 177: 3202-3214.

70. Mihara M, Hara H, Hayashi Y, Narushima M, Yamamoto T, et al. (2012) Pathological steps of cancer-related lymphedema: histological changes in the collecting lymphatic vessels after lymphadenectomy. PLoS One 7: e41126.

71. Zieman SJ, Melenovsky V, Kass DA (2005) Mechanisms, pathophysiology, and therapy of arterial stiffness. Arterioscler Thromb Vasc Biol 25: 932-943.

72. Cancello R, Henegar C, Viguerie N, Taleb S, Poitou C, et al. (2005) Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. Diabetes 54: 2277-2286.

73. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, et al. (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112: 1796-1808.

74. Wentworth JM, Naselli G, Brown WA, Doyle L, Phipson B, et al. (2010) Pro-inflammatory CD11c+CD206+ adipose tissue macrophages are associated with insulin resistance in human obesity. Diabetes 59: 1648-1656.

75. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, et al. (2008) Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab 8: 301-309.

76. Clément K, Viguerie N, Poitou C, Carette C, Pelloux V, et al. (2004) Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. FASEB J 18: 1657-1669.

77. Gordon S (2003) Alternative activation of macrophages. Nat Rev Immunol 3: 23-35.

78. Lumeng CN, DelProposto JB, Westcott DJ, Saltiel AR (2008) Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. Diabetes 57: 3239-3246.

79. Lumeng CN, Bodzin JL, Saltiel AR (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117: 175-184.

80. Shaul ME, Bennett G, Strissel KJ, Greenberg AS, Obin MS (2010) Dynamic, M2-like remodeling phenotypes of CD11c+ adipose tissue macrophages during high-fat diet--induced obesity in mice. Diabetes 59: 1171-1181.

81. Aron-Wisnewsky J, Tordjman J, Poitou C, Darakhshan F, Hugol D, et al. (2009) Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. J Clin Endocrinol Metab 94: 4619-4623.

82. Zeyda M, Farmer D, Todoric J, Aszmann O, Speiser M, et al. (2007) Human adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. Int J Obes (Lond) 31: 1420-1428.

83. Bourlier V, Zakaroff-Girard A, Miranville A, De Barros S, Maumus M, et al. (2008) Remodeling phenotype of human subcutaneous adipose tissue macrophages. Circulation 117: 806-815.

84. Wilson HM (2010) Macrophages heterogeneity in atherosclerosis - implications for therapy. J Cell Mol Med 14: 2055-2065.

85. Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8: 958-969.

86. Hall KL, Volk-Draper LD, Flister MJ, Ran S (2012) New model of macrophage acquisition of the lymphatic endothelial phenotype. PLoS One 7: e31794.

87. Kerjaschki D, Huttary N, Raab I, Regele H, Bojarski-Nagy K, et al. (2006) Lymphatic endothelial progenitor cells contribute to de novo lymphangiogenesis in human renal transplants. Nat Med 12: 230-234.

88. Maruyama K, Ii M, Cursiefen C, Jackson DG, Keino H, et al. (2005) Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. J Clin Invest 115: 2363-2372.

89. Baluk P, Tammela T, Ator E, Lyubynska N, Achen MG, et al. (2005) Pathogenesis of persistent lymphatic vessel hyperplasia in chronic airway inflammation. J Clin Invest 115: 247-257.

90. Kataru RP, Jung K, Jang C, Yang H, Schwendener RA, et al. (2009) Critical role of CD11b+ macrophages and VEGF in inflammatory lymphangiogenesis, antigen clearance, and inflammation resolution. Blood 113: 5650-5659.

91. Kim KE, Koh YJ, Jeon BH, Jang C, Han J, et al. (2009) Role of CD11b+ macrophages in intraperitoneal lipopolysaccharide-induced aberrant lymphangiogenesis and lymphatic function in the diaphragm. Am J Pathol 175: 1733-1745.

92. Breslin JW, Gaudreault N, Watson KD, Reynoso R, Yuan SY, et al. (2007) Vascular endothelial growth factor-C stimulates the lymphatic pump by a VEGF receptor-3-dependent mechanism. Am J Physiol Heart Circ Physiol 293: H709-718.

93. Kajiya K, Sawane M, Huggenberger R, Detmar M (2009) Activation of the VEGFR-3 pathway by VEGF-C attenuates UVB-induced edema formation and skin inflammation by promoting lymphangiogenesis. J Invest Dermatol 129: 1292-1298.

94. Gordon EJ, Rao S, Pollard JW, Nutt SL, Lang RA, et al. (2010) Macrophages define dermal lymphatic vessel calibre during development by regulating lymphatic endothelial cell proliferation. Development 137: 3899-3910.

95. Maruyama K, Asai J, Ii M, Thorne T, Losordo DW, et al. (2007) Decreased macrophage number and activation lead to reduced lymphatic vessel formation and contribute to impaired diabetic wound healing. Am J Pathol 170: 1178-1191.

96. Kubota Y, Takubo K, Shimizu T, Ohno H, Kishi K, et al. (2009) M-CSF inhibition selectively targets pathological angiogenesis and lymphangiogenesis. J Exp Med 206: 1089-1102.

97. Cho CH, Koh YJ, Han J, Sung HK, Jong Lee H, et al. (2007) Angiogenic role of LYVE-1-positive macrophages in adipose tissue. Circ Res 100: e47-57.

98. Tinahones FJ, Coín-Aragüez L, Mayas MD, Garcia-Fuentes E, Hurtado-Del-Pozo C, et al. (2012) Obesity-associated insulin resistance is correlated to adipose tissue vascular endothelial growth factors and metalloproteinase levels. BMC Physiol 12: 4.

99. Hu D, Fukuhara A, Miyata Y, Yokoyama C, Otsuki M, et al. (2013) Adiponectin regulates vascular endothelial growth factor-C expression in macrophages via Syk-ERK pathway. PLoS One 8: e56071.

100. Johnson LA, Jackson DG (2008) Cell traffic and the lymphatic endothelium. Ann N Y Acad Sci 1131: 119-133.

101. Girard JP, Moussion C, Förster R (2012) HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes. Nat Rev Immunol 12: 762-773.

102. Martín-Fontecha A, Lanzavecchia A, Sallusto F (2009) Dendritic cell migration to peripheral lymph nodes. Handb Exp Pharmacol : 31-49.

103. Clement CC, Rotzschke O, Santambrogio L (2011) The lymph as a pool of self-antigens. Trends Immunol 32: 6-11.

104. Angeli V, Llodrá J, Rong JX, Satoh K, Ishii S, et al. (2004) Dyslipidemia associated with atherosclerotic disease systemically alters dendritic cell mobilization. Immunity 21: 561-574.

105. de Bont N, Netea MG, Demacker PN, Verschueren I, Kullberg BJ, et al. (1999) Apolipoprotein E knock-out mice are highly susceptible to endotoxemia and Klebsiella pneumoniae infection. J Lipid Res 40: 680-685.

106. Ludewig B, Jäggi M, Dumrese T, Brduscha-Riem K, Odermatt B, et al. (2001) Hypercholesterolemia exacerbates virus-induced immunopathologic liver disease via suppression of antiviral cytotoxic T cell responses. J Immunol 166: 3369-3376.

107. Netea MG, Demacker PN, de Bont N, Boerman OC, Stalenhoef AF, et al. (1997) Hyperlipoproteinemia enhances susceptibility to acute disseminated Candida albicans infection in low-density-lipoprotein-receptor-deficient mice. Infect Immun 65: 2663-2667.

108. Lund AW, Duraes FV, Hirosue S, Raghavan VR, Nembrini C, et al. (2012) VEGF-C promotes immune tolerance in B16 melanomas and cross-presentation of tumor antigen by lymph node lymphatics. Cell Rep 1: 191-199.

109. Cohen JN, Guidi CJ, Tewalt EF, Qiao H, Rouhani SJ, et al. (2010) Lymph node-resident lymphatic endothelial cells mediate peripheral tolerance via Aire-independent direct antigen presentation. J Exp Med 207: 681-688.

110. Nichols LA, Chen Y, Colella TA, Bennett CL, Clausen BE, et al. (2007) Deletional self-tolerance to a melanocyte/melanoma antigen derived from tyrosinase is mediated by a radio-resistant cell in peripheral and mesenteric lymph nodes. J Immunol 179: 993-1003.

111. Tewalt EF, Cohen JN, Rouhani SJ, Guidi CJ, Qiao H, et al. (2012) Lymphatic endothelial cells induce tolerance via PD-L1 and lack of costimulation leading to high-level PD-1 expression on CD8 T cells. Blood 120: 4772-4782.

112. Dubrot J, Duraes FV, Potin L2, Capotosti F2, Brighouse D, et al. (2014) Lymph node stromal cells acquire peptide-MHCII complexes from dendritic cells and induce antigen-specific CD4(+) T cell tolerance. J Exp Med 211: 1153-1166.

113. Hara T, Shitara S, Imai K, Miyachi H, Kitano S, et al. (2012) Identification of IL-7-producing cells in primary and secondary lymphoid organs using IL-7-GFP knock-in mice. J Immunol 189: 1577-1584.

114. Onder L, Narang P, Scandella E, Chai Q, Iolyeva M, et al. (2012) IL-7-producing stromal cells are critical for lymph node remodeling. Blood 120: 4675-4683.

115. Miller CN, Hartigan-O'Connor DJ, Lee MS, Laidlaw G, Cornelissen IP, et al. (2013) IL-7 production in murine lymphatic endothelial cells and induction in the setting of peripheral lymphopenia. Int Immunol 25: 471-483.

116. Xu X, Lin H, Lv H, Zhang M, Zhang Y (2007) Adventitial lymphatic vessels -- an important role in atherosclerosis. Med Hypotheses 69: 1238-1241.

117. Kholová I, Dragneva G, Cermáková P, Laidinen S, Kaskenpää N, et al. (2011) Lymphatic vasculature is increased in heart valves, ischaemic and inflamed hearts and in cholesterol-rich and calcified atherosclerotic lesions. Eur J Clin Invest 41: 487-497.

118. Nakano T, Nakashima Y, Yonemitsu Y, Sumiyoshi S, Chen YX, et al. (2005) Angiogenesis and lymphangiogenesis and expression of lymphangiogenic factors in the atherosclerotic intima of human coronary arteries. Hum Pathol 36: 330-340.

119. Jellinek H, Gabor G, Solti F, Veress B (1967) The problem of the coronary changes due to disturbance of vascular wall permeability. Angiology 18: 179-187.

120. Nakata Y, Shionoya S (1979) Structure of lymphatics in the aorta and the periaortic tissues, and vascular lesions caused by disturbance of the lymphatics. Lymphology 12: 18-19.

121. Lemole GM (1981) The role of lymphstasis in atherogenesis. Ann Thorac Surg 31: 290-293.

122. Sacchi G, Weber E, Comparini L (1990) Histological framework of lymphatic vasa vasorum of major arteries: an experimental study. Lymphology 23: 135-139.

123. Sims FH (1979) The arterial wall in malignant disease. Atherosclerosis 32: 445-450.

124. Vuorio T, Nurmi H, Moulton K, Kurkipuro J, Robciuc MR, et al. (2014) Lymphatic vessel insufficiency in hypercholesterolemic mice alters lipoprotein levels and promotes atherogenesis. Arterioscler Thromb Vasc Biol 34: 1162-1170.

125. Llodra J, Angeli V, Liu J, Trogan E, Fisher EA, et al. (2004) Emigration of monocyte-derived cells from atherosclerotic lesions characterizes regressive, but not progressive, plaques. Proc Natl Acad Sci U S A 101: 11779-11784.

126. Galkina E, Kadl A, Sanders J, Varughese D, Sarembock IJ, et al. (2006) Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis is partially L-selectin dependent. J Exp Med 203: 1273-1282.

127. Galkina E, Ley K (2009) Immune and inflammatory mechanisms of atherosclerosis (*). Annu Rev Immunol 27: 165-197.

128. Thaunat O, Kerjaschki D, Nicoletti A (2006) Is defective lymphatic drainage a trigger for lymphoid neogenesis? Trends Immunol 27: 441-445.

129. Drayton DL, Liao S, Mounzer RH, Ruddle NH (2006) Lymphoid organ development: from ontogeny to neogenesis. Nat Immunol 7: 344-353.

130. Ruddle NH (2014) Lymphatic vessels and tertiary lymphoid organs. J Clin Invest 124: 953-959.

131. Gräbner R, Lötzer K, Döpping S, Hildner M, Radke D, et al. (2009) Lymphotoxin beta receptor signaling promotes tertiary lymphoid organogenesis in the aorta adventitia of aged ApoE-/- mice. J Exp Med 206: 233-248.

132. Mohanta SK, Yin C, Peng L, Srikakulapu P, Bontha V, et al. (2014) Artery tertiary lymphoid organs contribute to innate and adaptive immune responses in advanced mouse atherosclerosis. Circ Res 114: 1772-1787.

133. SCHWARTZ CJ, MITCHELL JR (1962) Cellular infiltration of the human arterial adventitia associated with atheromatous plaques. Circulation 26: 73-78.

134. Houtkamp MA, de Boer OJ, van der Loos CM, van der Wal AC, Becker AE (2001) Adventitial infiltrates associated with advanced atherosclerotic plaques: structural organization suggests generation of local humoral immune responses. J Pathol 193: 263-269.

J Clin Cell Immunol
Role of Lymphatics in Immunity
ISSN:2155-9899 JCCI, an open access journal