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Granulomatous Inflammation in Tuberculosis and Sarcoidosis: Does the Lymphatic System Contribute to Disease?

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A striking and unexplained feature of granulomatous inflammation is its anatomical association with the lymphatic system. Accumulating evidence suggests that lymphatic tracks and granulomas may alter the function of each other. The formation of new lymphatics, or lymphangiogenesis, is an adaptive response to tumor formation, infection, and wound healing. Granulomas also may induce lymphangiogenesis which, through a variety of mechanisms, could contribute to disease outcomes in tuberculosis and sarcoidosis. On the other hand, alterations in lymph node function and lymphatic draining may be primary events which attenuate the risk and severity of granulomatous inflammation. This review begins with an introduction of granulomatous inflammation and the lymphatic system. A role of the lymphatic system in tuberculosis and sarcoidosis is then hypothesized. With a focus on lymphangiogenesis in these diseases, and on the potential for this process to promote dissemination, parallels are established with the well-established role of lymphangiogenesis in tumor biology.

1. Introduction: Infectious and Sterile Granulomatous Diseases

The formation of granulomas is a distinct and striking response of the immune system. Hubs of activity, granulomas engage in immune surveillance and direct local immune responses. While various types of cells, including CD4+ T cells, CD8+ T cells, and B cells are associated with granulomas, macrophages are the most conspicuous and fundamental cellular element of this lesion.[1] Cytokines such as Tumor Necrosis Factor-alpha (TNF-α) promote the functional integrity of granulomas,[2] which can wax and wane in concert with disease activity. Resolution of the granulomatous response presumably occurs when antigen is eradicated or the local immune response is altered and inflammation resolved.

Granulomas are an important part of the immune response to certain infections that can eventually result in restriction of microbial spread. They may also contribute to microbial killing, although the scope and mechanisms of this process are poorly understood. On the other hand, vigorous granulomatous inflammation can lead to fibrotic and necrotic reactions which damage the host.[1] The range of possible outcomes of granulomatous inflammation is exemplified in leprosy, a common granulomatous disease caused by infection with *Mycobacterium leprae*. An exuberant inflammatory response in tuberculoid leprosy is associated with low microbial burden but extensive damage of the host, whereas weak granulomatous responses in lepromatous leprosy are associated with rampant bacterial growth yet minimal collateral damage.[3] In this review, we focus on granulomatous responses in tuberculosis and sarcoidosis, and explore the association of granulomas and the lymphatic system.

Tuberculosis remains one of the deadliest human infectious diseases, accounting for up to 2 million deaths annually.[4] In addition, a third of the global population is considered to be latently infected and at risk for re-activation disease. Therefore, understanding the pathogenesis of microbial latency and re-activation is a critical global health agenda. Human infection with mycobacteria, including the etiologic agent of tuberculosis, *Mycobacterium tuberculosis*, is a potent trigger of granuloma formation. Tuberculoid granulomas are typically highly organized structures, composed of a mix of macrophage phenotypes.[5] Macrophage populations in the granuloma include epithelioid histiocytes, marked by abundant cytoplasm, and foamy macrophages with intracellular lipid accumulation. Alternatively, macrophages can coalesce into multi-nucleated giant cells, also called Langhans' cells. Beyond the bevy of macrophages, collections of lymphocytes in the outer border are common, as is a fibrotic layer beyond that in mature granulomas (**Figure 1**).[6] Granulomas in tuberculosis can be necrotic or non-necrotic, and often both types of lesions are

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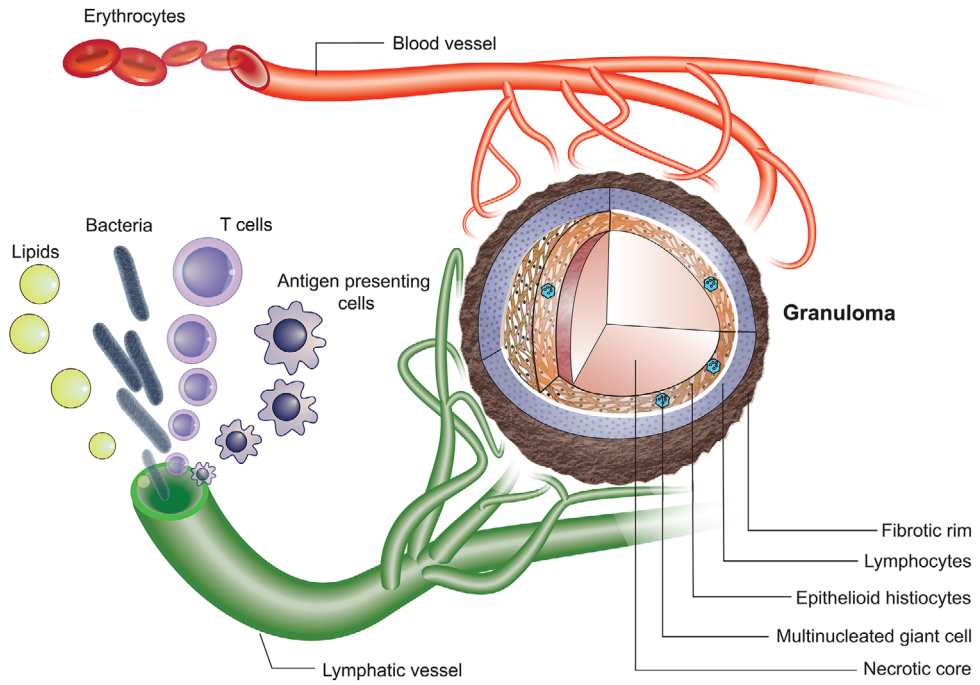

Figure 1. The relationship between granulomas, lymphatic, and blood vessels. Granulomas in tuberculosis and sarcoidosis occur along lymphatic tracks. In this model, a well-organized granuloma is hypothetically associated with both lymphatic and blood vessels. In the granuloma, the necrotic core is surrounded by a layer of macrophages, with a slimmer outer ring of lymphocytes. The phenotype of macrophages in granulomas include foamy macrophages, epithelioid histiocytes, and multi-nucleated giant cells. The potential function of lymphatic vessels in the delivery of antigen presenting cells, other immune cells, and lymph to secondary lymphatic structures as well as the relationship with the blood vessels is also depicted.

present in infected tissues. When it occurs, central necrosis can be expansive, leading to effacement of normal structures.

In contrast to tuberculosis, sarcoidosis is a sterile granulomatous disease of unknown aetiology. The lungs and thoracic lymph nodes are the most commonly affected sites, although many patients have widespread, multi-organ disease. Similar to tuberculosis, macrophages are the primary cellular component of sarcoid granulomas, which are typically large, well-formed, and non-necrotic.[7]

Many of the salient clinical features of re-activation tuberculosis and sarcoidosis are similar. This includes an overlap of presenting symptoms and chest imaging findings. Circulating biomarkers and gene expression profiles also are similar, reflecting shared immune response programs.[8–10] Given the pro-inflammatory nature of these diseases, cutaneous anergy is a seemingly paradoxical finding observed in both sarcoidosis and tuberculosis. Necrotic granulomas on biopsy are most suggestive of tuberculosis. However, in some cases the distinction between tuberculosis and sarcoidosis can only be definitively established via microbial studies which rule in or out the presence of mycobacterial species.

Granulomatous inflammation in both tuberculosis and sarcoidosis has a distinct predilection for lymphatic tissue. In patients with widespread tuberculosis or sarcoidosis, lymph nodes are the most common site of disease, although spleen and liver involvement is frequently observed. In sarcoidosis, granulomatous inflammation develops specifically along lymphatic tracks, especially in the lung.[7,11,12] In an animal model of mycobacterial disease, granulomatous inflammation also primarily occurred along the lymphatic tracks of the lung.[13] In spite of this close anatomical relationship, the potential contribution of the lymphatic system is not typically recognized in the disease models of granulomatous inflammation, which emphasize the role of antigen, macrophages, T cells, and pro-inflammatory cytokines. Yet, accumulating evidence suggests that the lymphatic network may have a role in the disease process of tuberculosis and sarcoidosis. In the following sections, we explore this hypothesis, including the potential impact of granuloma-mediated lymphangiogenesis. We begin with a concise review of lymphatic development and post-embryonic lymphatic remodeling.

2. Lifelong Lymphangiogenesis Is Supported by Dynamic Lymphatic Endothelial Cells

The maintenance of a robust and dynamic lymphatic system is essential to life. Embryonic lymphangiogenesis occurs via the establishment of lymphatic sacs which bud and separate from early venous channels.[14] Centrifugal sprouting from these lymph sacs results in a network of lymphatic vessels, constituting a mix of blind-ended capillary beds and larger collecting vessels. From the time of their initial development, lymphatic vessels are anatomically coupled to the venous network. In this way, they are well-positioned to remove excess interstitial fluid which accumulates as a result of vascular hydrostatic forces.

A seemingly invisible network, the daily circulation of lymph through human lymphatic vessels parallels that of blood flow through the blood vascular system. Yet, lymphatic clearance is

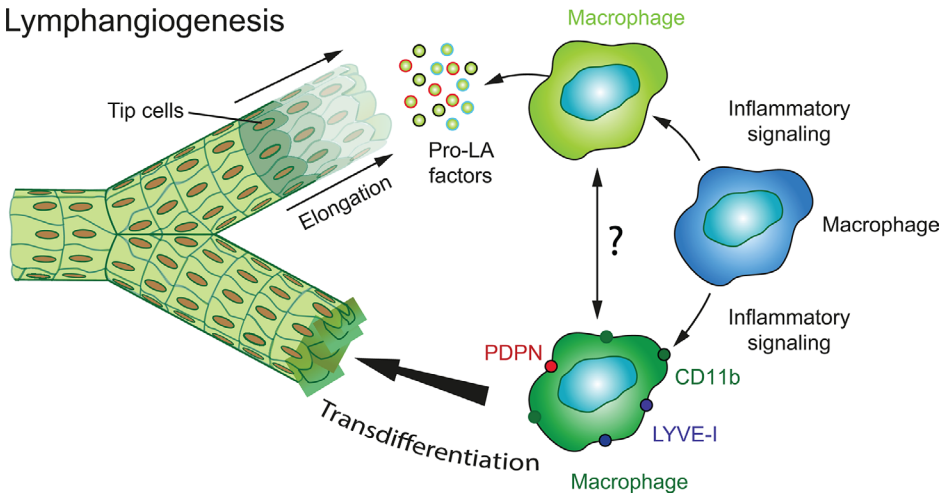

Figure 2. Contribution of macrophages to the process of lymphangiogenesis. The replication of specialized LECs (tip cells) appears to be the predominant mechanism by which lymphatic networks elongate. Macrophages support post-embryonic lymphangiogenesis through the production of pro-lymphangiogenic cytokines, or via transdifferentiation into lymphatic endothelial cells which integrate into developing lymphatic vessels.

not uniform throughout the body. In contrast to blood flow, lymphatic flow is not supported by a single systemic pump. External muscle contractions promote the movement of fluid through lymphatic capillaries. In contrast, movement through larger vessels is aided by one-way valves and the contraction of smooth muscle cells investing lymphatic vessels.[15] Beyond reclaimed interstitial fluid, central lymphatic vessels deliver intestinal fats and associated nutrients to circulating blood.[14] They are also a conduit for immune cell trafficking, serving the migration of immune cells to secondary lymph organs where adaptive immune responses develop.

Lymphatic endothelial cells (LECs) are the fundamental cellular structure of lymphatic vessels. Functionally distinct from blood endothelial cells, human LECs are characterized by the expression of specific markers such as lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) and podoplanin, as well as the Prospero homeobox protein 1 (PROX-1) transcription factor. PROX-1 is essential for the lymphatic identity of LECs and drives expression of pro-lymphangiogenic genes.[16–21] LECs are also characterized by specific metabolic features. Endothelial cells have relatively low mitochondrial counts[22] and cytoplasmic glycolysis is the primary mechanism of adenosine triphosphate (ATP) generation. Glycolysis is inefficient for ATP generation compared to mitochondrial oxidative phosphorylation.[23–26] Yet, it favors the ability of LECs to generate sufficient amounts of ATP even when oxygen tensions are low, as can occur in tumor or granuloma environments.[27] During lymphangiogenesis, LECs also engage in fatty acid β-oxidation. This supports mitosis, where by-products of fatty acid oxidation become precursor molecules for nucleotide synthesis. Inhibition of fatty acid oxidation in LECs has a minor effect on energy production and redox homeostasis, but strongly impairs the deoxynucleotides synthesis required for DNA replication during lymphatic vessel growth.[28] Additionally, fatty acid derived acetyl-CoA is a cofactor for enzymes which epigenetically regulate genes, including those expressed under control of the transcription factor PROX-1. In this way, acetyl-CoA promotes the selective transcription of pro-lymphangiogenic genes such as vascular endothelial growth factor receptor (VEGFR) 3 which helps maintain LEC identity.[29]

The ability to regenerate or remodel lymphatic vessels is essential to life. The expansion and reconstruction of vessels contribute to the dynamic maintenance of a functional lymphatic network.[30] Distal cells in budding branches develop a "tip cell" phenotype, providing directional guidance during growth of lymphatic vessels.[31] Signaling through the VEGFR3-VEGF ligand pathway is implicated in the transformation of distal cells to a tip cell phenotype.[31] Stabilization of budding vessels is achieved by adhesion molecules which mechanically couple LECs with the extracellular matrix.

The replication of LECs enables post-embryonic lymphangiogenesis.[32] Circulating progenitor cells also may incorporate into lymphatic vessels undergoing repair or growth (**Figure 2**). In explanted human kidneys, circulating (male) progenitor cells were found to be integrated into the lymphatic vessels of (female) donor kidneys.[33] A variety of progenitor cell types have been implicated in lymphangiogenesis.[32] In a study of labeled donor stem cells in irradiated mice, only haematopoietic stem cells, and not more lineage committed stem cells, served as precursor cells for LECs.[34] However, findings from a variety of other studies most strongly implicate myeloid derived cells as the primary source, with the assumption that circulating progenitor cells can access inflamed tissues and acquire a mature macrophage phenotype. For example, in a study of irradiated mice, donor bone marrow derived CD11b+ macrophages adopted a LEC phenotype and integrated into growing lymphatic vessels at sites of wound inflammation.[35]

3. Macrophages Are Critical for Post-Embryonic Lymphangiogenesis

In a variety of ways, macrophages play a critical role in post-embryonic lymphatic remodeling. In animal models of mechanical injury and infection, macrophage suppression is associated with decreased lymphangiogenesis.[35,36] Similarly, in tumor

models the depletion of macrophages is associated with a decrease in lymphatic vessel density.[37] Several mechanisms have been invoked to account for the role of macrophages in lymphangiogenesis (Figure 2). Macrophages are a source of VEGF ligands, which promote the growth of sprouting lymphatic vessels.[36,38,39] In one study, a reduction in lymphangiogenesis was achieved equally by suppression of the VEGF-C/VEGFR3 axis directly or by macrophage depletion.[40] However, the paramount contribution of macrophages to VEGF ligand production has been questioned, and in tumor models may be less critical.[32,37] Similar to what has been observed in angiogenesis,[41] macrophages also may have an organizational role in lymphangiogenesis. In a study of diaphragm lymphatics, the deletion of macrophages prevented their co-localization at the tips of developing lymphatic vessels, and was associated with more disorganized vessel branching.[42] The mechanisms of how macrophages regulate sprouting are still poorly characterized. Nonetheless, these findings highlight the potentially multifaceted contribution of macrophages to a dynamic lymphatic network.

Crucially, a variety of findings indicate that macrophages maintain a high degree of plasticity and are able to transdifferentiate (undergo transcriptomic reprogramming) into LECs. Cells expressing both macrophage and LEC markers were present in the vicinity of and within lymphatic vessels in an animal model of wound inflammation.[35] Similarly, human monocytes expressed lymphatic markers and developed morphologic features of LECs when cultured with VEGF-containing media.[33] VEGF signaling appears to be a strongly determining step in the transdifferentiation process, at least initially, and VEGFR-3 suppression with either a decoy receptor or RNA silencing abrogated the transdifferentiation of macrophage to LECs.[32] VEGFR-3 expression on macrophages corresponds with up-regulation of VEGF-C, representing the potential for a self-amplifying autocrine loop that supports transdifferentiation.

Transdifferentiated macrophages appear capable of integrating into spouting lymphatic vessels. In an in vivo corneal injury mouse model, cells expressing both lymphatic and macrophage markers were integrated into newly generated lymphatic vessels.[35,43] When Green Fluorescent Protein (GFP)-labeled transdifferentiated macrophages were cultured with immortalized murine lymphatic cells, GFP+ macrophages integrated into developing vessel-like structures.[37] Furthermore, the integration of macrophages occurred specifically at the tips and branching points, suggesting that these cells support sprouting from pre-existing branches. Tet, transdifferentiated macrophages may also participate in the de novo formation of lymphatic tracks. Ex vivo CD11b+ alveolar macrophages from patients with idiopathic pulmonary fibrosis demonstrated a propensity to aggregate and form structures resembling nascent lymphatic vessels.[44] Similarly, transdifferentiated murine macrophages in tissue culture clustered together and formed early vessel-like structures.[35] The clinical importance of these findings remains unclear, as de novo vessel formation is generally considered a minor contribution to lymphatic remodeling. However, in kidneys affected by rejection-induced inflammation, in which lymphatic vessel density is substantially increased, recipient-derived progenitor cells accounted for, on average across several samples, 13% of vessels. In contrast to recruited myeloid progenitor cells, the role of long-term tissue resident macrophages in lymphangiogenesis is less clear.

After a phase of a dual-cell phenotype, transdifferentiated macrophages may evolve toward a fully differentiated LEC state. In a study of chronic kidney rejection, in which tissue sampling occurred years after the initial inflammatory events, recipient progenitor cells which had integrated into the lymphatic vessels of donor kidneys were negative for myeloid markers.[33] The authors hypothesized that cells had undergone programmed loss of myeloid markers. In one of the only studies to capture this phenotypic progression, transdifferentiated bone marrow-derived macrophages in culture not only began to express lymphatic markers but also down-regulated their expression of macrophage markers.[37]

4. Lymphangiogenesis Is a Double-Edged Sword in Disease

A variety of diseases trigger lymphangiogenesis, and cancer has been the most widely studied in this context. The dissemination of tumor cells via peritumoral lymphatic vessels and the upregulation of lymphangiogenesis in regional lymph nodes appear to be important mechanisms of tumor spread.[45,46] Animal models and human studies strongly link tumor-associated lymphangiogenesis with poor outcomes, including reduced survival.[46] Lymphangiogenesis also is observed in a variety of chronic infections, including Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV), and leishmaniasis.[47–49] In contrast to the detrimental relationship between lymphangiogenesis in cancer, an expanded lymphatic network during infection facilitates immune cell trafficking to infected tissues and helps mobilize the associated increase in interstitial fluid. In this way, lymphangiogenesis also contributes to tissue regeneration, leading to improved outcomes following wound and ischemic injury events.[43,50]

Granulomatous diseases also appear capable of promoting lymphangiogenesis. In mice infected with the attenuated vaccine strain Bacillus Calmette-Guerin (BCG), lymphangiogenesis was observed in infected liver.[51] Moreover, lymphatic vessels co-localized to areas of granulomatous inflammation. Similar findings were observed in lungs infected with *Mycobacterium tuberculosis*.[51] Lymphangiogenesis also appears to be upregulated in sarcoidosis. In one study, over half of granulomas from lung biopsy samples were associated with one or more lymphatic vessels.[52] In contrast, blood capillaries were infrequently observed near granulomas, suggesting that lymphangiogenesis was not merely a by-product of angiogenesis. Lymphatic vessels were significantly and selectively associated with large granulomatous lesions, raising the question of a possible role for lesion-triggered hypoxia in promoting lymphangiogenesis. In another study, 67% of lung sarcoid granulomas had a lymphatic vessel in close proximity to the granuloma border.[53] Most of these vessels had an irregular morphology consistent with post-embryonic lymphangiogenesis. In a study of cardiac lymphatics, biopsy samples from patients with cardiac sarcoidosis demonstrated an increase in the number of lymphatic vessels compared to non-sarcoidosis controls.[54] Again, vessel morphologies in sarcoidosis samples were suggestive of post-embryonic lymphangiogenesis. As a mechanistic link for these findings, elevated levels of circulating and tissue VEGF have been observed in both tuberculosis and sarcoidosis.[53,55–57]

5. More than a Passive Conduit: Lymphatic Tracks May Modulate Granulomatous Disease

The anatomical relationship between granulomas with the lymphatic system and recent findings linking granulomatous inflammation with lymphangiogenesis underpin the hypothesis that the lymphatic network may contribute to the pathophysiology of granulomatous diseases. In this section, we explore two questions about the specific functional impact of the lymphatic system in tuberculosis and sarcoidosis. In the first, we also speculate about the role of macrophages linking granulomas to the lymphatic system.

5.1. Does Lymphangiogenesis Promote the Dissemination of Granulomatous Disease?

The impact of lymphangiogenesis in granulomatous disease is unclear. However, tumors may serve as an informative model from which to draw inferences for granuloma biology. Like tumors, oxygen tension is low in the center of expanding granulomas,[27] and anaerobic metabolism which supports the proliferation of LECs during tumor-associated lymphangiogenesis may also support lymphangiogenesis associated with expanding granulomas. Also similar to cancer, the extent of "metastatic" disease in tuberculosis and sarcoidosis is associated with long-term outcomes. These and other parallels between malignant tumors and granulomas, and between cancer and granulomatous inflammation more broadly, raise the question of whether lessons from lymphangiogenesis in cancer apply to granulomatous inflammation. Specifically, does an expanded network of lymphatic vessels contribute to the dissemination of tuberculosis and sarcoidosis? Haematogenous spread is commonly invoked for multi-organ tuberculosis.[58] However, several factors argue against a simple model of haematogenous spread. In non-miliary re-activation tuberculosis, a strong predilection for upper lung zone involvement is observed despite low blood flow rates relative to lower lung zones. Even in miliary pulmonary tuberculosis,[59] in which disease is attributed to haematogenous spread, the lymphatic tracks are often affected. These observations suggest that regional spread of tuberculosis via lymphatics needs to be considered during disease progression.[12] While biologically plausible, the role of lymphatics during dissemination remains to be demonstrated experimentally. In contrast to tuberculosis, the mechanism(s) of multi-focal sarcoidosis are virtually unexplored. It is likely, though, that mechanisms of disease spread are similar, particularly as the internal organs most at risk of multi-organ involvement in tuberculosis and sarcoidosis are nearly identical.

Another similarity between tumors and granulomas is the central role of macrophages in orchestrating local immune responses. While tumor cells are a source of VEGF-C, the development of a population of tumor-associated macrophages (TAMs) also promotes lymphangiogenesis. TAMs derive from circulating macrophages[60] and have been linked to an increase in peritumoral lymphatic vessels. In the tumor microenvironment, a subset of TAMs acquire the ability to express VEGF-C.[60] TNF-α is one of the key tumor-associated cytokines driving TAMs toward a VEGF-C-expressing phenotype. In addition to pro-lymphangiogenic factors, TAMs can produce and secrete extracellular matrix remodeling mediators (metalloproteinases) which further support tumor vascularisation, including lymphangiogenesis.[60–64] Several studies have demonstrated that the secretion of factors such as matrix metalloproteinase-9 or VEGF by multi-nucleated giant macrophages is associated with vascular invasion of bone tumors during cancer metastasis.[65–67] However, the involvement of multi-nucleated giant cells in the vascularization of granuloma is still not clearly established. Finally, while the integration of TAMs directly into peritumoral lymphatic vessels has not been demonstrated, this possibility is suggested by the dual expression of macrophage and LEC markers, such as LYVE-1 and VEGF-R3.[60]

5.2. Does the Lymphatic System Impair Antigen Clearance and Microbial Eradication?

Lymph nodes are the most common site of active tuberculosis, and granulomas persist within lymph nodes in latent tuberculosis. These key clinical features support the notion that mycobacterial infection is primarily a lymphatic disease.[12] In this model, re-activation reflects failure of microbial eradication and lymph node containment. This failure may be due to lymph node specific factors. In a macaque model of infection, lymph node granulomas were less protective against *M. tuberculosis* than lung granulomas, containing a larger number of live bacteria and serving as a reservoir for the long persistence of bacilli.[68] The reasons for this are unclear, although findings in cancer may be relevant, where immunosuppressive cytokines are elevated in draining lymph nodes, while lymphocyte and dendritic cell populations are reduced.[69] Whether altered immunocompetence in lymph nodes also occurs in tuberculosis and contributes to the persistence of infected granulomas remains to be determined. Other factors specific to the local environment, including local hypoxia and metabolic pressures, could also contribute to regional and tissue differences in lymphatic function.

Another possibility is that the lymphatic vasculature contributes to microbial persistence. Recently, *M. tuberculosis* bacteria were found within nodal LECs.[70] It remains unclear if these LECs can harbor tuberculosis bacilli in the long-term. If LECs are infected during latent tuberculosis, these cells may contribute to the phenomenon of higher post-treatment relapse rates in extrapulmonary tuberculosis.[71]

The function of non-nodal lymphatic vessels may also have a role in granulomatous disease. In pulmonary sarcoidosis, the formation of granulomas occurs nearly exclusively along lymphatic tracks. This suggests that antigen is found within or around lymphatic vessels, and that T cells and macrophages are recruited to these areas. Sluggish lymphatic flow rates may contribute to the retention of immune cells.[72,73] Flow rates are largely determined by the volume of reclaimed interstitial fluid, which is a function of pulmonary vascular (hydrostatic) pressures. These pressures, and thus lymphatic flow rates, are lowest in the upper lung zones. Regional differences in lymphatic flow in the lung have been invoked to account for the upper zone predominance of tuberculosis.[74] However, rather than enhanced immunity from the retention of immune cells, low flow conditions could facilitate lymphatic vessels becoming a site of immune privilege.

Alternatively, peri-lymphatic granulomatous inflammation could obstruct flow, converting low flow states into no-flow states.

Pathological conditions which alter lymphatic clearance attenuate the risk of tuberculosis, and support lymphatic congestion as a risk factor for granulomatous disease. For example, silicosis is associated with an elevated risk of pulmonary tuberculosis. The fibrosis of draining lymph nodes affected by silicosis eventually leads to the obstruction of afferent lymphatic vessels in the lungs.[75,76] Pulmonary (valve or artery) stenosis similarly results in reduced lymphatic flow through the lung, and is associated with an increased risk of pulmonary tuberculosis. Conversely, aortic stenosis raises pulmonary arterial pressures, which leads to increased lymphatic flow, and is associated with a reduced risk of pulmonary tuberculosis. As the prevalence of sarcoidosis is substantially lower than that of tuberculosis, data correlating the impact of valvular disease on disease risk are not available. However, similar to tuberculosis, sarcoidosis is a strikingly upper lung zone predominant disease, and lymphatic flow rates may contribute to this phenomenon.

6. Important Considerations and Future Challenges

To further elucidate the role of the lymphatic system in granulomatous disease, several specific research agendas are worth considering. Perhaps the most urgent need is to determine the clinical relevance of lymphangiogenesis in granulomatous inflammation, particularly in regard to its role in sustaining or abrogating immune responses, and in potentiating disease spread. Lessons from angiogenesis should be highly relevant to this pursuit. Through the discovery of VEGF signaling and endothelial cell metabolism, advances in understanding inflammatory vascularization in angiogenesis have provided new therapeutic targets in cancer.[77,78] Similarly, inhibition of VEGF signaling promotes the normalization of blood vessels, and improves the bioavailability drugs and efficacy of treatment in inflammatory angiogenesis associated with tuberculoid granulomas.[57,79] Accumulating evidence suggests that understanding the consequences of lymphatic remodeling holds similar therapeutic potential. Drugs that promote or impair lymphangiogenesis could complement the action of anti-microbial drugs in tuberculosis, or immune-suppressing medications in pathogen-free sarcoidosis. The discovery of pro-lymphangiogenic factors in the past 20 years has facilitated a surge in lymphatic research. Drawing on those results, the potential to target lymphangiogenesis for disease control is a very attractive possibility. While there are currently no drugs approved which target lymphangiogenesis in cancer or other diseases, early animal studies are underway and human studies are eagerly anticipated. VEGF, epidermal growth factor receptor, fibroblast growth factor, and platelet-derived growth factor pathways have been identified as potential targets.[80] Similar to challenges encountered with cancer treatment, the problem with common pathways implicated in angiogenesis will need to be addressed and avoided.

The relationship between granulomas and lymphangiogenesis may depend on the specific tissue environment. For example, granuloma-driven lymphangiogenesis may not be the same in lymph nodes versus lung tissue. The cytokine milieu, extracellular matrix components, and local immune cells vary across tissue microenvironments, and may affect lymphangiogenesis potential. Human studies can be difficult, although lymphatic-focused histopathology and tissue culture studies have been fruitful methodologies to begin to understand the impact of tissue environment.

The role of macrophage phenotype is relatively unexplored in granulomatous inflammation. In cancer, TAMs have immunosuppressive properties similar to alternatively activated macrophages.[81] TNF-α helps further polarize TAMs to acquire a lymphangiogenesis-promoting phenotype.[82] TNF-α is one of the signature cytokines in granulomatous inflammation, and TNF-α inhibitors are used therapeutically in sarcoidosis. Weakened granuloma integrity is often invoked as the mechanism of action, but this assumption has been challenged.[83] Given the role of TNF-α in cancer-related lymphangiogenesis, it will be instructive to determine the effect of TNF-α on lymphangiogenesis in sarcoidosis and tuberculosis.

Further clarification of the role of macrophage transdifferentiation in lymphangiogenesis is needed. The process of macrophage transdifferentiation shares features with epithelial transdifferentiation,[84] in which epithelial cells undergo transcriptional reprogramming and transition to a mesenchymal phenotype. Transdifferentiated epithelial cells lose cell-cell adhesion properties and gain the capacity to replicate and migrate. This process, triggered by cytokines released from matrix cells in the setting of tissue injury, serves tissue repair needs. Lessons from epithelial transdifferentiation[84] may be relevant to macrophage function in granulomatous inflammation. Specifically, it will be instructive to define the extent of macrophage reprogramming, and determine the contribution of macrophage transdifferentiation to lymphangiogenesis, wound repair, and fibrotic responses.

Beyond their role in lymphangiogenesis, understanding how granuloma macrophages interface with lymphatic structures is also important. Macrophages do not typically traffic through lymphatic vessels, yet granulomatous inflammation in tuberculosis and sarcoidosis occurs in the anatomical distribution of lymphatic tracks. The spatial relationship of granulomas to lymphatic vessels needs to be more precisely determined. Localizing granulomas to the inside or outside of the lymphatic lumen is important for understanding how lesion micro-environments affect pathophysiology and may have implications for drug delivery.

Another unmet need is a better understanding of the role of LECs in granulomatous inflammation. The view of LECs as elements of a passive conduit has significantly changed in the last few years. Accumulating evidence suggests that LECs modulate immune responses in response to infection.[85] Defining the role LECs in trans-endothelial leukocyte migration[86] and cytokine signaling in granulomatous inflammation is needed. In addition, determining whether LECs participate in microbial sequestration in tuberculosis has important therapeutic ramifications. It is tempting to speculate that LECs could also participate in the sequestration of antigen in sarcoidosis. In a viral infection model, LECs were found to harbor antigen long after viral eradication, and participated in the delivery of antigen to antigen presenting cells.[87] Antigen presentation by LECs promotes anergic responses,[87] which may be relevant to the clinical phenomenon of anergy observed in sarcoidosis and tuberculosis.

7. Conclusion

The role of the lymphatic system in the pathogeneses of granulomatous diseases such as tuberculosis and sarcoidosis remains understudied. In this review, we have explored several fundamental and unresolved questions regarding this relationship, highlighting findings which support the hypothesis that the lymphatic system contributes to the pathogenesis of granulomatous disease. Recent studies suggest that LECs may have direct microbial contact in tuberculosis, and lymphatic vessel function may contribute to the promotion and spread of granulomatous diseases. Understanding the relationship between lymphatics and granulomatous inflammation has important therapeutic implications, and further research in this domain is eagerly anticipated.

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Conflict of Interest

The authors declare no conflict of interest.

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