Arteriosclerosis, Thrombosis, and Vascular Biology

EDITORIAL

Lymphangiogenesis in Rheumatic Heart Valve Disease: A New Factor in the Pathogenic Conundrum

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Rheumatic heart valve disease (RHVD) is a major global health concern, affecting over 40 million individuals and resulting in 300 000 reported annual fatalities worldwide. RHVD primarily impacts children and young adults in low- and middle-income countries, with a strikingly higher prevalence in women.[1] The origin of RVHD lies in untreated *Streptococcus pyogenes* pharyngitis, culminating in acute rheumatic fever in a subset of patients. Repeated acute rheumatic fever episodes set off a persistent immune response, resulting in cardiovascular damage, particularly affecting mitral valve leaflets in up to 60% of the reported cases. This immune reaction manifests as valve thickening, fibrosis, and calcification, ultimately leading to mitral valve stenosis or regurgitation.[2]

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Despite significant efforts, our knowledge of the genetic, cellular, and molecular underpinnings of RVHD progression remains limited. The present understanding of how *S pyogenes* instigates a host-directed autoimmune response is based on molecular mimicry, involving epitopes of the pathogen, notably its surface-anchored M protein antigen or the *N*-acetylglucosamine side chain of its cell wall carbohydrate, and targets of cross-reactivity in the host, for example, collagen and myosin.[2,3] Nevertheless, the mechanism that triggers specific reactions in the mitral valve years after acute rheumatic fever remains unknown, and the emergence of lymphangiogenesis could provide valuable mechanistic insights into this phenomenon, as illustrated in Figure 1. Furthermore, neovascularization has been described as a central feature of fibrotic valve disease, including RHVD,[4] and preventing angiogenesis could serve as a potential therapeutic startegy.[5] This emphasizes the crucial role of valvular endothelium, and previous reports on endothelium pluripotential differentiation capacity playing a pathogenetic role in valve disease[6] described its ability to generate lymphatic vessels.

A critical aspect of the lymphatic system is its essential role in both draining interstitial fluid and supporting adaptive immune responses. This dual function is achieved by transporting antigens and activated antigen-presenting cells to the lymph nodes, while simultaneously exporting immune effector cells and factors integral to humoral responses to the bloodstream. Notably, de novo lymphangiogenesis has been implicated in chronic inflammation, observed in conditions such as psoriasis, rheumatoid arthritis, and allograft transplantation.[7] In these instances, inhibiting VEGFR3 (vascular endothelial growth factor receptor 3) has been shown to attenuate lymphangiogenesis and reduce CCL21 (C-C motif chemokine ligand 21)-mediated adaptive immune responses.[7,8]

The recent study by Osinski et al,[9] featured in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, describes the emergence of lymphatic capillaries in the mitral valves during autoimmune valvular disease in K/B.g7 mice. These mice spontaneously produce autoantibodies that target the widely expressed glycolytic enzyme GPI (glucose-6-phosphate isomerase), leading

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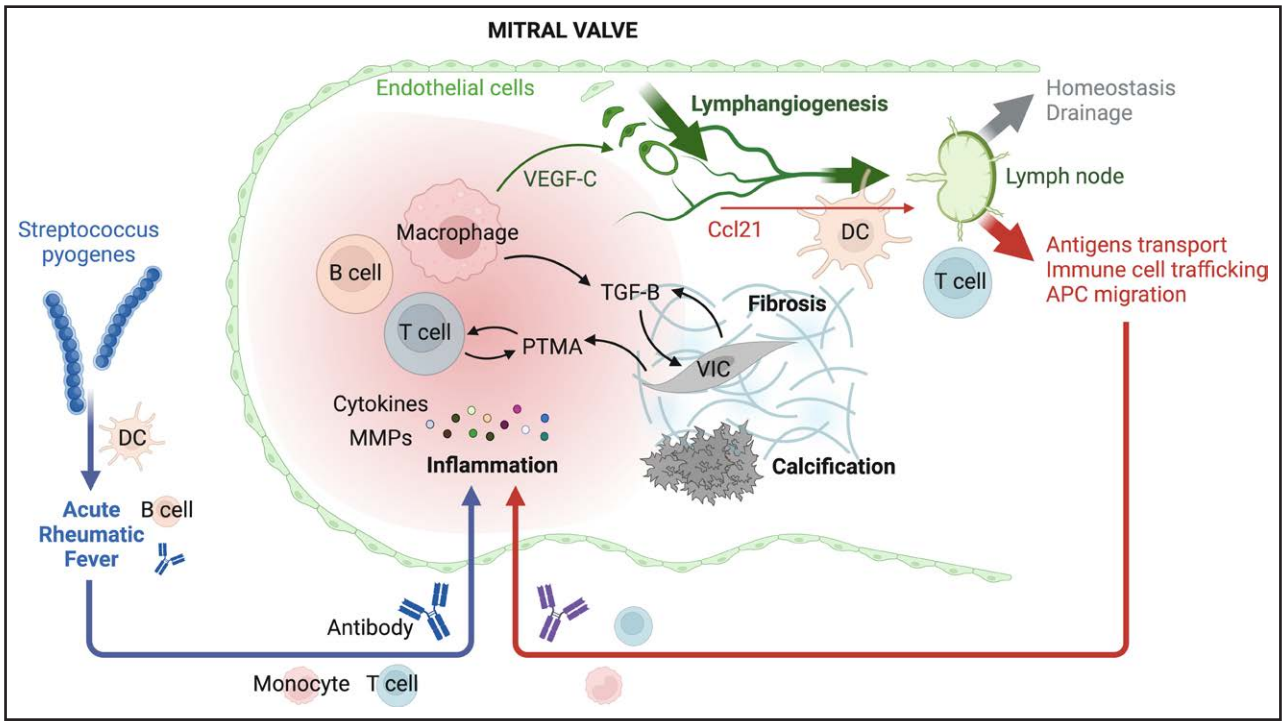

Figure 1. Contribution of lymphangiogenesis in the pathogenesis of rheumatic heart valve disease (RVHD).
The schematic illustrates the pathogenesis of RVHD, initiated by *Streptococcus pyogenes* throat infection that triggers an adaptive immune response targeting bacterial antigens. This process involves monocyte/macrophage, B-cell, and T-cell activation, leading first to acute rheumatic fever (ARF). Subsequently, in the mitral valve, cross-reactive T cells and antibodies expanded through repeated ARF episodes act against valve components, causing intense inflammation that results in valve fibrosis and calcification. The emergence of lymphatic capillaries within the inflamed leaflet suggests a potential role in maintaining tissue homeostasis by draining excess interstitial fluid. However, these new lymphatic capillaries may also play a role in sustaining inflammation by facilitating the transport of immune cells, antigens, and antigen-presenting cells (APCs) to the lymph node, thereby intensifying the adaptive immune response. Ccl21 indicates C-C motif chemokine ligand 21; DC, dendritic cell; MMP, matrix metallopeptidase; PTMA, prothymosin-alpha; TGF-B, transforming growth factor-beta; VEGFC, vascular endothelial growth factor C; and VIC, valvular interstitial cell.

to the development of severe autoimmune erosive arthritis and valvular carditis.[10]

While the development of lymphatic vessels in mitral valves of patients with RHVD has been previously observed[3,11] (Figure 2), this article represents the first exploration into the origin of these lymphatic structures, coupled with an examination of the associated transcriptional regulation at the single-cell level. Employing endothelial lineage tracing with a Cdh5 (cadherin 5) promoter-dependent reporter, the study unveils the emergence of novel lymphatic capillaries originating from the endothelial cells on the leaflet surface. Remarkably, as the disease progressed, Lyve1+ (lymphatic vessel endothelial hyaluronan receptor 1) cells first appeared on the atrial endothelial layer, seemingly migrating into the interstitium. This suggests that some valvular endothelial cells (VECs) have the potential to differentiate into Lyve1+ lymphatic VECs, migrate, and subsequently connect to facilitate neolymphangiogenesis through a sprouting-independent mechanism (Figure 1). The understanding and characterization of this noncanonical form of lymphangiogenesis requires further investigation. Additionally, Osinski et al observed that the capillary network became connected to the annulus region in the valvular interstitium at an advanced disease stage. This suggests a potential association with the cardiac lymphatic system, essential for functionality. However, it is important to note that the study did not evaluate the functionality and connection of the newly formed mitral lymphatic network. Further investigations are necessary to characterize these features.

Mitral valve single-cell RNA sequencing data further identified distinct populations of lymphatic VECs characterized by a high expression level of Lyve1 and VEGFR3 (Flt4 [fms-related receptor tyrosine kinase 4]), and these populations demonstrated expansion with the disease progression. Two major subtypes of lymphatic VECs exhibited a unique transcriptional profile, marked by increased expression of profibrotic (Col1a1 [collagen, type I, alpha 1], Vim [vimentin], Timp2 [TIMP metallopeptidase inhibitor 2], Dcn [decorin], Fbln2 [fibulin 2]) and proinflammatory (Ccl21a) genes during disease advancement. The lymphatic expression of the chemokine CCL21—a marker of activated lymphatic vessels—plays a pivotal role in attracting effector immune cells and antigen-presenting cells through the binding of the

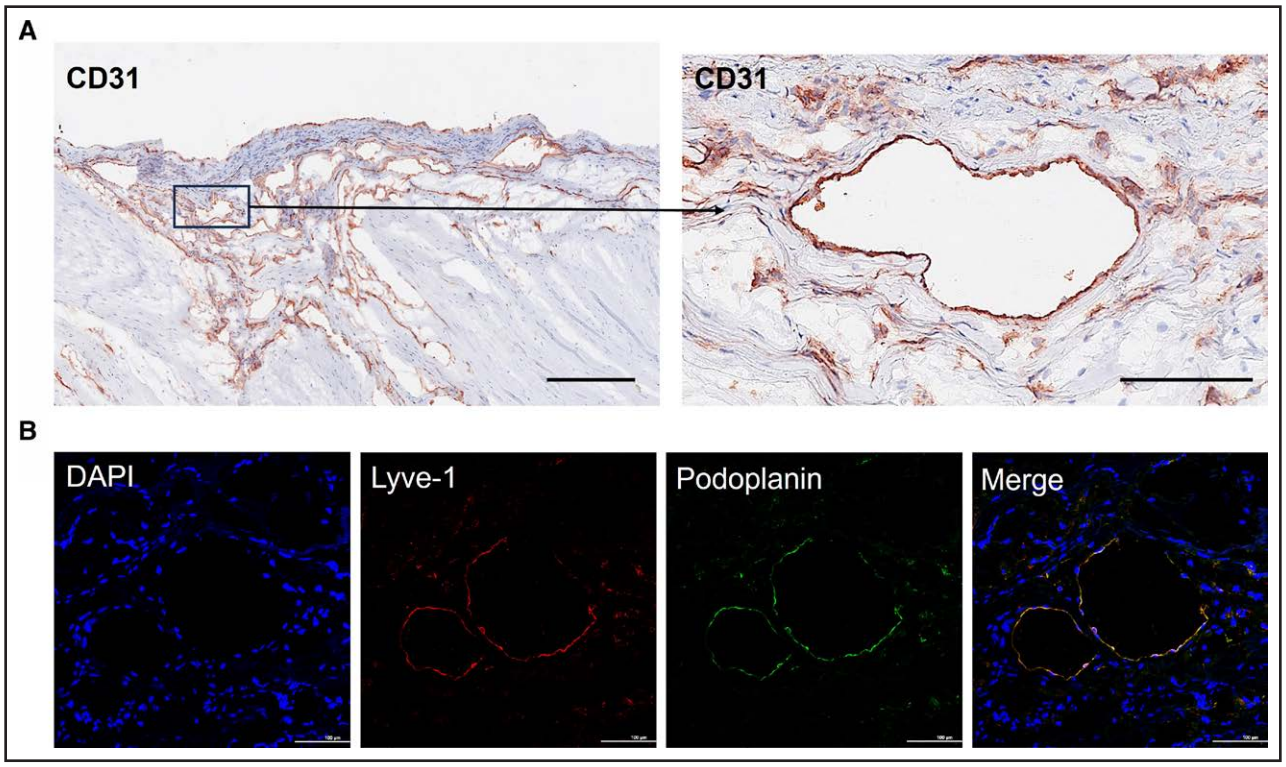

Figure 2. Lymphatic vessel in rheumatic heart valve disease (RHVD).
A, Representative images for CD31 staining evidencing the presence of extensive vascular networks in RHVD human mitral valves. Scale bars=300 µm (**left**) and 100 µm (**right**). **B**, Representative immunofluorescence images showing coexpression of Lyve1 (lymphatic vessel endothelial hyaluronan receptor 1; red) and podoplanin (green) demonstrating the presence of lymphatic vessels in human rheumatic mitral valve. Scale bars=100 µm. DAPI indicates 4′,6-diamidino-2-phenylindole. Data derived from Passos et al.[3]

chemokine receptor CCR7 (C-C motif chemokine receptor 7). Subsequently, these cells are transported to lymphoid organs, supporting adaptive immune responses.[7] The activation of lymphatic VECs indicates a function in supporting inflammation, warranting additional investigation to thoroughly elucidate this role.

While these results significantly expand our insight into the understanding of RHVD, they simultaneously raise additional questions regarding whether the observed process is a cause or a consequence. Further studies are needed to clarify the relationship. Indeed, the strategy of blocking the VEGFC (vascular endothelial growth factor C)-VEGFR3 pathway for 4 weeks efficiently reduced lymphangiogenesis during autoimmune valvular disease in K/B.g7 mice. However, it failed to reduce the thickening of the valve. This outcome suggests that lymphatic capillaries may have a limited impact at the early stage of the disease. Consequently, it remains crucial to ascertain the long-term effects of the lymphangiogenesis blockade. This understanding is essential for identifying potential therapeutic opportunities, especially for patients diagnosed at the early phase of RHVD.

Epidemiological data on RHVD clearly indicate a 2-fold higher prevalence in women, aligning with patterns observed in many autoimmune diseases. Sex hormones, particularly estrogen, possess the potential to impact the immune response. Notably in their work, Osinski et al did not discern any sex differences in K/B.g7 mice. This lack of differentiation could be attributed to either the absence of a sex-specific phenotype for this particular animal model or the limited statistical power resulting from the limited number of mice allocated for each sex.

The question of the cellular and molecular mechanisms rendering women more susceptible to RHVD remains enigmatic. However, new insights into the sex-specific pathobiology of RHVD are gradually emerging. Recent findings highlight the protein PTMA (prothymosin-alpha) and indicate its potential contribution to CD8+ T-cell cytotoxicity, linked with estrogen receptor-alpha activity, thus proposing a plausible role in the sex predisposition in RHVD.[12] Moreover, the same study underscores that PTMA can facilitate CD8+ T-cell recognition of human type 1 collagen, exhibiting molecular mimicry with *S pyogenes* and the capacity to elicit autoimmune responses in these cells. Altogether, it is conceivable that these processes are contingent on the development of valvular lymphatic capillaries, supporting the adaptive immune response through the transport of PTMA, antigens, antigen-presenting cells, and effector cells to the lymph node. Unraveling this intricate interplay may furnish a fresh understanding of the complex pathology inherent in RHVD.

Demonstrating the significance of this often-neglected disease, the American Heart Association has recently issued a call to action aimed at diminishing the global impact of RHVD.[13] Addressing the challenges to reduce the burden of RHVD necessitates a multifaceted approach. Along with public health campaigns, a crucial aspect involves enhancing access to the sole existing prevention method, relying on the early and preventive administration of antibiotics to limit repeated exposure to *S pyogenes*.[2,14] Addressing severe cases of RHVD demands invasive and expensive interventions, typically encompassing cardiac surgery for valve repair or replacement, or percutaneous valve interventions. Currently, no pharmaceutical interventions exist for treating RHVD, and bridging this gap requires international partnerships for resource mobilization, monitoring, and evaluation. Such initiatives were set in motion by the Cairo Accord on Rheumatic Heart Disease[15] and are actively pursued by other organizations including the recently launched Leducq Foundation Consortium on Rheumatic Heart Disease.

ARTICLE INFORMATION

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Disclosures

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