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Myeloid cells remodel the mitral valve

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Mitral valve disease is a degenerative cardiac pathology that increases markedly with age in the western hemisphere. Rheumatic heart disease is another frequent cause of valve pathology, particularly in developing countries. In this condition, valvular damage arises from auto-immune responses after Group A streptococcal infection, leading first to mitral valve insufficiency and at later stages to stenosis. Once the disease is established, therapeutic options are currently limited to either symptomatic treatment or invasive surgical procedures[1]. The central importance of the adaptive immune response for developing rheumatic heart disease is well known: B-cells produce antibodies directed against streptococcal epitopes. Those antibodies cross react with antigens such as laminin in the valve endocardium, ultimately leading to endothelial cell activation and VCAM expression. This promotes entry of T-cells, which likely destruct valvular tissue by secretion of proinflammmatory cytokines[1].

In this issue of Circulation[2], Meier et al describe for the first time the causal involvement of innate immune cells in the pathogenesis of mitral valve disease. The authors conducted their studies in K/B.g7 T cell receptor transgenic mice (TCR mice) which express a T-cell receptor that recognizes the self-peptide glucose-6-phosphate isomerase. The resulting autoantibody production causes erosive polyarthritis and mitral valve inflammation. Mitral valves become fibrotic and accumulate myeloid cells that express the fractalkine receptor CX3CR1. Genetic deletion of CX3CR1 protects the mice from valve fibrosis and inflammation, likely because this receptor regulates myeloid cell migration and survival[3].

Using multicolor flow cytometry, the authors identify a CD301b/MGL2 mononuclear cell population as key protagonist of mitral valve disease in TCR mice. They characterize this population as $CXC3CR1^{+}CD64^{+}CD301b^{+}$ mononuclear phagocytes and ascribe them characteristics of macrophages and dendritic cells. The authors report that ablation of $CD301b^{+}$ cells in TCR mice reduces valve inflammation and fibrosis significantly, suggesting a causal pathogenic role for cells expressing this receptor in auto-antibody triggered pathologies of the mitral valve. Using intracellular flow cytometry, the authors

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The authors report no conflict of interest.

found that CD301b$^+$ myeloid cells produce the inflammatory cytokines IL-6 and TNF-α. These preclinical data are accompanied by identification of CD301$^+$ macrophages in human rheumatic valves that were obtained during valve replacement surgery. Mechanistically, employing several genetic mouse models, the authors report that cell type specific ablation of the small tyrosine kinase Syk in mononuclear phagocytes using CX3CR1-cre prevents mitral valve fibrosis and inflammation, including reduced infiltration of CD301b$^+$ myeloid cells which express less IL-6 and TNF-α after Syk deletion. IL1-b is an important inflammatory stimulus of atherosclerosis and a cytokine expressed in leukocytes infiltrating stenotic aortic valves[4]. In the model employed by Meier et al, targeting IL1-b did not prevent recruitment of disease-promoting myeloid cells into mitral valves, arguing for alternative inflammatory pathways.

The presence of macrophages in healthy and diseased myocardium of mice and humans has been recognized in recent years, but the cells' role in tissue homeostasis and cardiovascular disease is not yet fully understood. This is especially true for heart valves. In their present manuscript, Meier et al. convincingly show the importance of myeloid cells for initiation and progression of autoimmune heart valve pathology, which was previously thought to be driven mainly by the adaptive immune system. While the detection of cardiac leukocytes with standard immunohistochemistry techniques was often hampered by limited sensitivity, improvements in imaging technology now enable analysis of leukocyte subsets and phenotypes in much more detail. Using optical tissue clearing procedures prior to whole-mount imaging of mitral valves in combination with myeloid cell-specific expression of fluorescent proteins, the current manuscript[2] visualizes myeloid cells in mitral valves of TCR mice. Their abundance in valve tissues is further corroborated by flow cytometric analysis of inflammatory cells in excised mitral valves.

Macrophages also participate in other valvular pathologies. For example, macrophages were detected in clinical samples of aortic stenosis, where inflammation initiates degenerative valve changes leading to calcification[5]. Although it currently remains unclear if myeloid cells are present in healthy human heart valves, this is likely the case since leukocytes have been visualized with confocal imaging in aortic as well as atrioventricular valves in healthy mice[6]. These cells were described as dendritic cells based on CD11c promoter driven fluorescent protein expression[6]. There is an ongoing debate on how to best phenotypically distinguish macrophages and dendritic cells in the cardiovascular system[7], hence the exact cellular characteristics of leukocytes in heart valves remains somewhat unresolved. The gating strategy used by Meier et al. did not aim to distinguish macrophages from dendritic cells. CD301b, whose expression was used to drive cell ablation in the TCR mouse, is expressed by both, macrophages and dendritic cells (www.immgen.org).

The authors conclude that local myeloid cell proliferation dominates valve pathology in rheumatic heart disease, but also show that cell recruitment is critical, which relies on the cell adhesion molecule VCAM1 expressed on valvular endothelial cells. As long as there are no known organ-specific promotors for macrophages, it is not possible to distinguish the regional impact of the conditional gene knockouts employed by Meier on the valves itself from distant, systemic sites of action. The same is true for systemic treatment with neutralizing antibodies. Remote hematopoietic organs are important coordinators of

leukocyte supply to sites of inflammation in cardiovascular pathologies[8]. Hence, it cannot be excluded that the observed effects after antibody treatment against VCAM-1 or myeloid cell specific deletion of its ligand VLA-4 are due to processes in lymphatic organs, bone marrow or spleen. This also holds true for myeloid cell-specific ablation of Syk, which is highly expressed by all hematopoietic lineage cells[9]. Since hematopoietic organs are likely activated in autoimmune disease, the aspect of localization warrants further studies. Of note, the continued joint inflammation after ablation of $CD301b^+$ cells in TCR mice does support the notion that local valve processes play an important role.

This study highlights the importance of inflammation and specifically myeloid cells in the development of heart valve pathologies triggered by autoimmune disorders. Next, it will be interesting to explore whether the findings translate to the broader collective of patients with degenerative valve disease. Meier et al. reported that the aortic valve is also affected in TCR mice, albeit less frequently than the mitral valve. The presence of macrophages in clinical samples of calcific aortic valve stenosis has been demonstrated with conventional immunohistochemistry; indeed, chronic inflammation is considered a major feature in calcific aortic valve disease, preceding valve calcification[10]. Thus, the pathobiology of calcific aortic stenosis may share parallels to mechanisms observed in mitral valve disease by Meier et al., especially with respect to endothelial damage executed by mechanical stress, resulting in upregulation of adhesion molecules and recruitment of circulating leukocytes[11]. Mitral regurgitation is a frequent complication after myocardial infarction leading to heart failure and associated mortality. Myocardial infarction strongly induces expression of TNFα and IL-6[12], and Meier et al. show that both molecules are necessary for recruitment of myeloid cells in heart valve disease. Albeit the altered post-MI ventricular geometry decisively contributes to mechanical stress on the valve and its insufficiency, leukocyte infiltration may contribute to pathology also in this setting. Mitral valve endothelial cells express higher levels of VCAM-1 after myocardial infarction, which recruits leukocytes[13,14]. Moreover, higher amounts of inflammatory cells have been detected in clinical specimens of myxomatous mitral valve disease[15].

In summary, the work by Meier et al. is a trail blazer because it clearly and elegantly demonstrates causal involvement of innate immune cells during the development of mitral valve disease. The presence of similar cells in murine and human valve pathology provides a strong translational perspective. We will have to add the heart valves to the list of tissues that leukocytes frequent, and yet another cardiovascular disease to the list of conditions myeloid cells contribute to. With the era of immunotherapy dawning, we next have to explore how and when to intervene without compromising host defense, especially critical questions when dealing with chronic disease. The positive experience in non-cardiovascular conditions such as rheumatoid arthritis and the success of immunotherapy in cancer illuminate that this is possible.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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