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Monocytes and Macrophages in Heart Valves: Uninvited Guests or Critical Performers?

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Abstract

Monocytes and macrophages are critical components of the myeloid niche of the innate immune system. In addition to traditional roles as phagocytes, this subsection of innate immunity has been implicated in its ability to regulate tissue homeostasis and inflammation across diverse physiological systems. Recent emergence of discriminatory features within the monocyte/macrophage niche within the last 5 years has helped to clarify specific function(s) of the subpopulations of these cells. It is becoming increasingly aware that these cells are likely implicated in valve development and disease. This review seeks to use current literature and opinions to show the diverse roles and potential contributions of this niche throughout valvulogenic processes, adult homeostatic function, valve disease mechanisms, and tissue engineering approaches.

Introduction

Monocytes and macrophages are well known components of the innate immune system that can regulate inflammation, tissue homeostasis, wound repair, and disease¹. Their ability to regulate not only cytokine/chemokine-mediated inflammatory events, but also actively remodel tissue matrices and regulate fibrosis has been implicated in many disease pathologies^{2–4}. The heart valve has shown to be a tissue that is readily associated with hematopoietic-mediated maintenance at homeostasis. While fibrosis and macrophage infiltration during degenerative disease processes are well known hallmarks of valve disease, the specific roles of macrophages in driving disease and facilitating homeostatic function remain elusive⁵. Yet, there is a growing body of literature highlighting an increased awareness of these cells in valvular development and disease. This niche also has great importance in the current and future engineering strategies for valve replacement^{6,7}. This review seeks to highlight the contributions of monocytes and macrophages throughout valvulogenic processes and tissue engineering approaches.

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Monocytes

Monocytes are circulatory precursors to macrophage populations, originating either from the yolk sac or the fetal liver⁸. These cells arise from myeloid progenitors, such as common monocyte progenitors (cMoP) or monocyte and dendritic progenitors (MDP)^{9,10}. Beyond their involvement in phagocytic processes, they undergo both inflammatory and anti-inflammatory cytokine production and contribute to endothelial regulation¹¹. Monocytes are currently categorized into three functional states: classical, non-classical, and intermediate¹². Classical, or inflammatory, monocytes (IMs) are functionally considered to be CD14⁺ (lipopolysaccharide [LPS] receptor) and CD16⁻ (Fcγ receptor III) in humans, CCR2^{high} (chemokine receptor 2), Ly6C^{high}, and CX3CR1^{low} (chemokine receptor of CX3CL1) in mice, and CD43^{low} in rat^{1,13}. These cells account for 80-90% of all circulating monocytes in blood¹⁴. Non-classical, or anti-inflammatory, monocytes (AMs) are considered to be CD14⁺ and CD16⁺ in humans, CCR2^{low}, Ly6C^{low}, and CX3CR1^{high} in mice, and CD43^{high} in rat¹. Unlike their inflammatory counterparts, these cells are implicated in their ability to regulate matrix remodeling and anti-fibrotic activity¹⁵. CD14⁺CD16⁺ monocytes have also been shown to be CD32⁺ (Fcγ receptor II), CD64⁺ (Fcγ receptor I). Some literature points to a third monocyte subset that is CD16⁺ but CD14⁻¹⁶. These are referred to as intermediate monocytes, with low Fc receptor expression, low phagocytic activity, and decreased antigen presentation capacity¹⁷. Although no studies have demonstrated the roles of these monocyte subtypes in pathology, some have shown that these cells are upregulated during sepsis¹⁸.

Macrophages

In mice, embryonic macrophages in the heart arise from two hematopoietic events. During primitive hematopoiesis (E7.5-E11.5), yolk sac progenitors develop into macrophages that populate throughout the embryo⁸. By means of definitive hematopoiesis (E11.5 and E16.5), fetal liver hematopoietic stem cells (HSCs) differentiate into monocytes that circulate, take residence, and give rise to tissue-specific macrophages¹⁹. In the heart, yolk sac-derived macrophages are present as early as E9.5¹⁹. At E13.5, fetal liver monocytes begin to invade cardiac tissue, differentiate into a small contingent of resident macrophages, and proliferate²⁰. By E18.5, the population of primitive macrophages becomes overwhelmingly diluted by cardiac-specific monocyte-derived macrophages²¹. Bone marrow-derived monocytes replace yolk sac and fetal macrophages soon after human birth in many systems²². There are some tissues that retain yolk-sac derived macrophage populations into adulthood. It has been shown that in some cardiac diseases such as myocardial infarction, tissue-resident macrophages are outcompeted by bone marrow-derived macrophages, which facilitate a loss of niche homeostatic function²³.

Postnatally, CCR2^{high}Ly6C⁺ inflammatory and CCR2^{low}Ly6C⁻ anti-inflammatory monocytes differentiate into classical, inflammatory, or M1 macrophages and non-classical, anti-inflammatory, or M2 macrophages from hematopoietic origins. Viewing M1/M2 phenotypes as a lineage dichotomy greatly oversimplifies actual macrophage phenotypic fluidity and context dependent functionality. For example, early studies have shown that macrophages can transdifferentiate between phenotypes²⁴. Further, it is not just M2 phenotypes that are beneficial for tissue healing and reparative remodeling, but combinations

of M1 and M2 act synergistically to improve tissue healing speed and ECM characteristics²⁵. Subpopulations within the M2 spectrum have shown to be valid in vitro targets or extremes with functional differences such as M2a (inflammatory resolution), M2b (immunoregulatory), and M2c (tissue remodeling). M1 inflammatory macrophages exposed to classic activation signals, such as IFN γ and LPS, express opsonic receptors, while M2 anti-inflammatory macrophages express more non-opsonic receptors. M1 polarization involves the synthesis of pro-inflammatory cytokines, such as tumor necrosis factor α (TNF α), interleukin 1- β (IL1 β), interleukin 6 (IL6), and expression of surface markers, such as C-C motif chemokine ligand 1 (CCL1)²⁶. M2 activation is dependent on activation signals, such as IL-4, IL-13, and IL-10, and expression CD163, tissue inhibitor of metalloproteinases 3 (TIMP3), C-C motif chemokine ligand 22 (CCL22), and matrix metalloproteinase 9 (MMP9)²⁶. It additionally involves the overexpression of scavenger receptors and the increase of IL-4, along with increased fibronectin expression, which may implicate involvement in cell adhesion and migration of cells during embryogenesis, wound healing, blood coagulation, and metastasis²⁷. In addition to biochemical stimulation of macrophages, mechanobiological activation such as integrin activation, cyclic stretch, and elongation has been shown to alter macrophage commitment^{28–30}.

Monocytes and Macrophages during inflammatory events

Monocytes either maintain their properties in tissues, depolarize to different monocyte subsets, or differentiate into macrophages³¹. There are several theories pertaining to the differentiation of monocytes to macrophages: monocytes are recruited to tissues as inflammatory monocytes, then anti-inflammatory, then to macrophages; anti-inflammatory monocytes are directly recruited from blood to supply monocyte-derived macrophages; some macrophages (particularly those in the heart, lung, brain, skin, and liver derived prenatally from the yolks-sac endothelium) are derived from embryonic sources and maintain themselves in tissues throughout adulthood^{32–34}. Inflammatory macrophages are non-adherent and highly mobile; however, when inflammatory cytokines are present, they adhere quickly and migrate through the endothelium³⁵. Anti-Inflammatory macrophages, in contrast, are derived from circulating monocytes and adhesive, which allows them to move along blood vessel walls when cytokines are not present³⁵. During inflammatory reactions, macrophages and monocytes adopt plasticity, in which blood monocytes are first recruited as a source of inflammatory macrophages, driven by chemotaxis of inflammatory signals, such as monocyte chemoattractant protein 1 (MCP1)³⁶. Ly6C⁺ monocytes are predecessors to early inflammatory macrophages, followed by Ly6C[–] macrophages that assist with wound healing and tissue regeneration through myofibroblast accumulation and collagen deposition³⁷. It is also worth noting that Ly6C^{+/–} populations extravasate differently with Ly6C⁺ populations showing a greater propensity, but there exists downstream population turnover between these groups³⁶. This remodeling from both monocytes and macrophages contributes to the synthesis of extracellular matrix and function³⁸. It is commonly accepted that dysregulation in mechanistic turnover between monocyte and macrophage subpopulations can facilitate chronic inflammation or unregulated fibrosis. Further studies need to be conducted to clarify whether there is direct differentiation between inflammatory

and anti-inflammatory components of the niche, or whether inflammatory predisposition is recruited at the progenitor level.

Monocytes/Macrophages in the Valve

The valve consists of 3 layers: (1) ventricularis in semilunar valves or atrialis in AV valves, which lies in the direction of blood flow, comprises of primarily radially oriented elastin and collagen, and provides elasticity, (2) fibrosa, which is located on the arterial side of semilunar valves and atrial side of AV valves, composed of circumferentially oriented, densely packed collagen, and able to withstand tension upon valve closure, and (3) spongiosa, which is primarily made of glycosaminoglycans and interposed between the ventricularis and fibrosa layers³⁹. While the valve certainly has endothelial cells (VEC) and some smooth muscle cells (SMC) at the root, the primary cells that are contained in the spongiosa of valve are referred to as valve interstitial cells (VIC). This is a relatively poor characterization scheme, since in reality VICs are a highly heterogeneous population of fibroblasts/fibroblast-like cells that previous literature categorized as quiescent (qViC), activated (aViC), and osteoblastic (obViC)⁴⁰. Fate mapping studies have shown active recruitment of CD45⁺ populations in the AV valve, which suggests their hematopoietic origins⁴¹. Anstine et al. better clarifies this by showing that these CD45⁺ cells recruited in the interstitium of the valve are monocytes and that this recruitment is age-dependent⁴². Postnatally, between P7-P30, there is increased CCR2⁺ F4/80⁺ macrophage populations present with variable phenotypic localization after P30 in the aortic valve correlating with differential flow pattern exposure⁴³. At some point during disease there is increased population turnover which results in aberrant macrophage presence⁵.

Valvulogenesis

Valvulogenic processes entail cellular migration, differentiation, and matrix remodeling. A few studies have characterized the roles of inflammatory factors during valvulogenesis. The remodeling phase of valvulogenesis is influenced by TNF α secreted by VECs and to a smaller extent VICs⁴⁴. This TNF α activity is regulated by NOTCH activity in VEC and in turn regulate apoptosis of VIC and therefore compaction⁴⁴. But whether or not macrophages contribute to TNF α or other cytokine levels during this stage and if macrophages regulate VEC activation and TNF α secretion also remains unknown.

Thrombospondin (TSP) 1-4, which can be produced by macrophages, functions as a secreted protein and regulates monocyte trafficking and adhesion as well as macrophage recruitment and activation, are readily detected in AV valves⁴⁵⁻⁴⁸. Moreover, TSP-1 can activate latent TGF-beta signaling⁴⁹, which is important for initiation of valvulogenic endothelial-to-mesenchymal transition (EMT) and myofibroblastic activation⁵⁰. Interestingly, mice deficient in Nedd4-2, a steady state suppressor of TSP-1, exhibit AV valve defects and embryonic lethality at mid gestation⁵¹. Whether endocardial derived cells that populate the cushion are also known to express TSPs that additionally alters niche monocyte/macrophage behavior have not been elucidated. Expression of intracellular adhesion molecule 1 (ICAM1) and vascular cellular adhesion molecule 1 (VCAM1) is high in fetal human valve endothelial cells (VECs) throughout valve development and significantly attenuated in

neonates and adults, suggesting that monocytes and other immune cells are recruited to the site of valvulogenesis⁵². Furthermore, TNF α and IL-6 can drive NFkB-induced EMT in quail embryonic valve endocardium as well as augmented expression of ICAM1 and VCAM1. TNF α , IL-6, and NFkB are upregulated in myocardium of patients with defective valve formation⁵³. Interestingly, VCAM-1-deficient mice are lethal at E12.5 and exhibit severe reduction of compact myocardium⁵⁴. Moreover, pharmacological inhibition of NFkB in chick results in several outflow tract abnormalities, including valvular hypertrophy and stenosis⁵⁵.

MMP2, another prominent macrophage-derived factor, has been implicated to participate in the early invasion of endocardial cells undergoing EMT, while VICs produce MMP1, 2, and 13 that play a role in matrix remodeling later on during valve development^{56,57}. Importantly, what is known is that during semilunar valve remodeling, presence of leukocytes that have engulfed VICs as well as apoptotic cells has been documented, suggesting a potential role in regulating VIC populations, but more work needs to be done to that end^{58,59}.

Role of Monocytes/Macrophages in Valvular Stenotic Processes

Aortic valve disease, spanning from early diagnosis tissue sclerosis to late stage stenosis/severe calcification, is denoted by the increasing prevalence and spatial localization of macrophages in zones of active remodeling as also seen in vessel stenotic processes^{60–62}. These populations play an important role in guiding tissue remodeling/structural repair and stimulating local stem/progenitor populations, and are also critical in both homeostatic and injury repair processes. It has been shown that active recruitment of circulating monocytes into the valve is an active age dependent process, yet mechanistic differentiation into macrophages that can drive disease has yet to be elucidated⁴². Thus, beyond this simple characterization of macrophage presence, the valve biology field has yet to determine how these sub-populations affect the microenvironment niche during the intermittent stages of the disease. However, we can garner a base understanding of their potential roles and homeostatic vs. aberrant immune response/tissue repair mechanisms from associated literature in myocardial and osteochondral development, regeneration, and repair. We find this appropriate to do so, because VICs undergo similar processes during disease events^{63–65}. Others in the field also similarly draw these sorts of comparisons⁶⁶.

In terms of cardiac macrophages (residing within myocardial-heart muscle) during the early embryonic to post-natal period, embryonically derived CX3CR1⁺ (CX3C chemokine receptor 1) populations further diversify into MHCII⁺ and MHCII⁻ cells. Coinciding age-related diversification, population density decreases and proliferation rates decline²⁰. Another study further examined the turnover and diversification of tissue resident macrophage populations using a combination of fate-mapping and parabiotic approaches. Circulating bone-marrow (BM) derived CCR2⁺ contribute to, and eventually overtake the cardiac macrophage pool, even in the absence of underlying inflammation⁶⁷. Furthermore, these infiltrating BM-derived populations preferentially give rise to MHCII⁺ populations, resulting in their relative increase over the CX3CR1⁺MHCII⁻ populations with age⁶⁷.

Study of monocytes/macrophages in an injured kidney fibrosis model recapitulates aspects of valve stenosis and denotes recruitment of Ly6C^{high} inflammatory-monocyte population, giving rise to 3 distinct macrophage subpopulations. Conditional ablation of the circulating monocyte populations was shown to prevent progressive fibrosis via pathologic collagen matrix deposition following ureteral obstruction. The group posits this effect was due to the disruption of paracrine interactions with resident fibroblasts, as the macrophage compartment interfering with their paracrine-stimulation of resident fibroblast⁶⁸. Marrow chimerism study further indicated while resident macrophages comprise 40% inflammatory population, they did not contribute significantly to fibrosis, and that remodeling activity is primarily driven by BM-derived, inflammation-recruited populations. From the bone biology/regeneration literature, macrophages are found to be involved in processes of fracture repair/bone regeneration and remodeling by promoting tissue vascularization and mesenchymal colony formation/condensation and proliferation⁶⁹.

Some groups have begun to probe mechanistic effects of the macrophage mediated inflammation in the form of cytokine production in promoting valve disease. Inflammatory macrophage derived microvesicles has been shown to promote VIC calcification via inflammatory cytokine production⁷⁰. From these readings, it can be inferred that these base repair mechanisms, while in certain contexts of other tissue may be beneficial, in this case serve to be pro-degenerative in disrupting intended tissue biomechanical function. The next round of studies from the valve disease field will need to focus on mechanistic studies of macrophage contributions to VIC behavior in 3D microenvironments.

Tissue Engineered Therapeutics

To date, the most widely used clinical valve therapies include mechanical and bioprosthetic valves. Both of these procedures have a limited timeline of 10-20 years, and require patients to take blood thinners and immunosuppressant drugs for life in an effort to ensure the long-term in vivo efficacy of these new valve(s). In these cases, modes of failure have been attributed primarily to macrophage infiltration of bioprosthetic valves, or fibrous tissue deposition onto mechanical valves, subsequent macrophage infiltration into the fibrous tissue, and sometimes calcification^{71,72}. This presents a need to provide more clinically translatable engineered valve therapeutics, and highlights tissue engineering as the most likely solution.

The valve tissue engineering field has congregated at two principal strategies to create biofunctional tissue engineered valve replacements: decellularized tissues and polymeric biomaterials that recruit autologous cells upon implantation⁷. While the potential of decellularized tissues is an attractive approach to valvular engineering, since it overcomes the engineering complexity of ECM structure and composition (growth factors, adhesive motifs, etc.), a commonly observed outcome of in vivo implantation of decellularized valves is rapid deterioration of the construct and an influx of CD31⁺CD8⁺ T-cells, which are known to drive macrophage recruitment, activation, and ECM degradation^{6,73}. Thus, immune-mediated deterioration of decellularized valves proves to be a strong burden to more clinically translatable therapies⁶. The biomaterials field has primarily focused on the development of non-immunogenic materials that can better facilitate integration in vivo.

Similar to failure with decellularized approaches, these materials also rapidly deteriorate due to chronic inflammation, or fail to reproduce native mechanical properties required for healthy function⁴⁰.

The true desired outcome for a tissue engineered valve is that the ECM mimetic must degrade at the rate of neo-tissue formation. One of the strongest candidates for neo-tissue formation is in situ tissue engineering, where a polymeric scaffold is engineered to co-opt the ability of macrophages to regulate fibrosis to deposit a valve-like fibrous tissue. This approach is extensively used in the vascular engineering field, but also is quite applicable for heart valves. Large mammal models for in situ engineering of vascular grafts using acellular ECM mimetics being used current have shown greater promise in terms of mechanical integrity, and function by some leading groups^{74,75}. But there still exist limitations towards translation with immunogenicity, and batch-to-batch variation. Most of these studies focus on end point leukocyte presence, which may not be appropriate since it is thought that if controlled, innate immune mediated tissue formation can be guided for desired engineering outcomes. To resolve some of these initial issues, some have shown promise with new materials, such as poly(glycerol sebacate), that can recruit specific denominations of macrophages (CD163⁺) to degrade scaffolds, form neo-vessels that retain mechanical properties, and have some preferred ECM deposition⁷⁶. While small diameter vascular grafts are a sensible starting point for heart valve engineering, complexity in valve structure and mechanical performance posits an even greater challenge for engineers. Today the immunology field is in agreement that M1/M2 delineation of macrophage phenotype and function is a semi-artificial assumption that becomes problematic when assessing in vivo engineering outcomes. What also should not be ignored is macrophages' ability to regulate fibrotic activity, more specifically to differentiate into fibrocytes and myofibroblasts^{2,77,78}. What is greatly needed is a better understanding of macrophage-mediated neo-tissue and more specifically ECM component production as a function of material properties to engineer tissues with desired heterogeneous spatial complexity. What may assist in resolving many of these outcomes is a revisit to and reinterpretation of the foreign body reaction studies of old.

Conclusions

While the field is slowly converging towards more detailed investigation of monocyte/macrophage regulation and contribution to valve development, homeostasis, disease and engineering approaches, there is much to still probe further. The next round of studies needs to better inform the field of the effects of the macrophage secretome in regulating valve developmental processes, the remodeling of VICs in engineered 3D platforms, and initiation mechanisms of local monocyte differentiation into macrophages. For better tissue engineering outcomes, more work understanding how material properties specifically guide macrophage mediated ECM remodeling is critical.

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Highlights

- We highlight potential mechanisms of myeloid contributions to valve development and disease
- We highlight monocytes and macrophages as therapeutic targets towards tissue engineered heart valves

Figure 1.
Graphical Abstract. Monocyte and macrophage regulation of valvular activity from valvulogenesis, homeostasis, disease mechanisms, and tissue engineering approaches.

Figure 2.

Valve Development. Macrophages localize themselves in the interstitial layer of the valve leaflet at the commissure and distal tip. During post-natal maturation, embryonically derived macrophages display CD206+ markers in early on and later MHCII+ populations emerge at the distal tip after the establishment of definitive hematopoiesis. Between P1 to P60 leukocyte presence in the valve increases from ~5% to ~12% in the aortic valve. CD206+ and MHCII+ macrophages are believed to have analogues M2 and M1 macrophages respectively, which may have functions in post-natal maturation processes or local leukocyte response to variable hemodynamic loading.

Figure 3.

Monocytes and macrophages driving valve disease. At steady state, circulating cells are actively recruited as a normal homeostatic process. Similarly, to vessel stenotic processes, disease initiation mechanisms are similar in valves as shown. Monocyte egress and differentiation into inflammatory macrophages is a commonly observed phenomenon in diseased valves, and it has been shown that inflammatory macrophage derived factors are able to drive disease mechanisms in VICs. How different phenotypes of macrophages can drive canonical and dystrophic means of osteochondral programming has yet to be clarified.