Review Article

Inflammatory Regulation of Valvular Remodeling: The Good(?), the Bad, and the Ugly

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Heart valve disease is unique in that it affects both the very young and very old, and does not discriminate by financial affluence, social stratus, or global location. Research over the past decade has transformed our understanding of heart valve cell biology, yet still more remains unclear regarding how these cells respond and adapt to their local microenvironment. Recent studies have identified inflammatory signaling at nearly every point in the life cycle of heart valves, yet its role at each stage is unclear. While the vast majority of evidence points to inflammation as mediating pathological valve remodeling and eventual destruction, some studies suggest inflammation may provide key signals guiding transient adaptive remodeling. Though the mechanisms are far from clear, inflammatory signaling may be a previously unrecognized ally in the quest for controlled rapid tissue remodeling, a key requirement for regenerative medicine approaches for heart valve disease. This paper summarizes the current state of knowledge regarding inflammatory mediation of heart valve remodeling and suggests key questions moving forward.

1. Introduction

The heart valves are the sole mediators of unidirectional flow through the cardiovascular system. These valves flex open and close 30 million times per year, subjecting the thin and flexible cusps or leaflets to demanding tissue strains and hemodynamic stresses. The fact that these tissues thrive can only be attributed to the remarkable stamina and remodeling capacity of the indigenous valve endothelial and interstitial cells that populate these valves. Over the past decade, many exciting discoveries have been made regarding the unique phenotypes of these cells, yet it has only framed the beginning of our understanding of valve function and dysfunction. Heart valve disease remains a serious and increasing clinical problem for which no solution exists save prosthetic replacement. These come in the form of mechanical or processed biological tissue valves. While providing over 20 years of function in elderly patients, these technologies perform dismally in children and young adults, with undesirable lifestyle restrictions and significant medical requirements. Tissue engineering has the potential to alleviate these limitations by providing a living valve conduit that can grow and remodel with the patient. Current results in animal trials are very promising, but human trials to date suggest that there is still much more to learn. Foremost among these needs is to understand how valve tissue remodels in the midst of the complex mechanical and biological signaling environment in which it resides. This includes natural tissue remodeling that occurs over embryonic development into adulthood, homeostatic and pathological adaptation over the course of life and disease, and with different living tissue replacement strategies. Being a biological structure that evolves and adapts over the entire lifespan, it seems likely that similar signaling mechanisms would be utilized across this continuum. While literally hundreds of regulatory genes have been identified in valve phenotypes and discussed in several reviews, this review will focus on the role of inflammation. While it is well appreciated that inflammation is a major driver of valve pathology, recent evidence suggests that inflammatory cytokines are present in embryonic development and in remodeling valves, which suggests its presence may not be singularly negative.

2. Inflammation and Wound Healing

The general healing response to tissue injury involves three phases: inflammation, tissue formation, and tissue remodeling [1]. The healing response begins when the tissue is injured, blood comes into contact with collage or other components of the extracellular matrix, and a blood clot forms. The blood clot platelets release chemotactic factors that recruit leukocytes to the injury site and initiate the inflammation phase [2]. These leukocytes then secrete chemokines and inflammatory cytokines to enhance the inflammatory response [2]. Next, neutrophils enter the wound site to remove foreign materials, bacteria, and damaged tissue; macrophages follow to continue the process of phagocytosis [3]. Fibroblasts deposit new extracellular matrix in the tissue formation phase [3]. In the remodeling phase, the newly deposited extracellular matrix is cross-linked and organized [3]. There are many cell signaling events required for this tightly controlled repair process to take place. The cytokine transforming growth factor-β (TGF-β) has been shown to play a part in all three phases of healing [4].

The inflammatory phase is initiated when TGFβ and other growth factors are released from platelets [5]. TGFβ has been shown to be chemotactic and mitogenic for neutrophils, lymphocytes, monocytes, macrophages, and fibroblasts [6]. During the tissue formation phase inflammatory cells migrate to the wound and secrete additional TGFβ, which at higher concentrations may induce the expression of other growth factors and increase cell proliferation at the wound site, stimulate angiogenesis, and promote collagen deposition [6–8]. During tissue remodeling TGFβ continues to promote extracellular matrix production and inhibit its breakdown, which has been implicated the cytokine in scar formation [6, 9]. Scars are fibrous tumors characterized by overabundant collagen deposition [10]. Treatment with TGFβ has been shown to increase endogenous TGFβ production, collagen deposition, and scar formation; while exposure to anti-TGFβ antibody decreases endogenous TGFβ production, collagen deposition, and scarring [9–11].

3. Inflammation and Valve Homeostasis

Each valve is comprised of thin, fibrous leaflets or cusps that are attached to a relatively rigid annulus or root [12]. The atrioventricular valves are further supported by tendinous chords that connect the leaflet free edge to the papillary muscles [12]. The leaflets/cusps are a multilayer composite of collagen, elastin, and glycosaminoglycans that assist in its efficient biomechanical function [12]. The surfaces of these tissues are lined with endothelial cells (VEC) while the underlying matrix is populated with interstitial cells (VIC)—a constellation of subphenotypes with incompletely understood individual roles [13]. In general, the endothelial cells are responsible for sensing and integrating biological and mechanical signals from the blood, and transmitting signals to the interstitial cells [14]. The VIC in turn proliferate and remodel the surrounding matrix [15]. Both cell types are also affected by other microenvironmental cues, such as mechanical strain, tissue stiffness, and the presence

of other cell types such as inflammatory macrophages and circulating cells [16]. Mitral valve cell biology is much less understood because the tissue is much more heterogeneous in structure and composition, potentially reflective of its more diverse mechanical environment [17]. The mitral valve is unsurprisingly susceptible to a much wider range of dysfunctional conditions (explained later). Therefore, this section will discuss results from aortic valves.

The inflow (atrialis/ventricularis) surface is exposed to a rapid, pulsatile, unidirectional shear stress with cycle averaged magnitude of 20 dynes/cm^2 [18]. The outflow (fibrosa) surface experiences a much lower magnitude, nearly oscillatory shear stress [19]. VEC align perpendicular to the direction of flow *in vitro* and *in vivo* whereas vascular EC align parallel [20]. Microarray comparisons between aortic valvular and vascular endothelium in static culture and under fluid flow identify hundreds of significantly different genes, suggesting that VEC are a distinct endothelial phenotype [21]. Steady shear stress was protective against pro-oxidation and proinflammation in both cell types, but VEC were inherently less inflammatory than arterial endothelial cells [21]. Simmons and colleagues probed side-specific differences in aortic VEC gene expression [22]. Aortic side endothelial cells showed significantly less expression of multiple inhibitors of cardiovascular calcification, enhanced antioxidative gene expression, and a lack of differential expression of proinflammatory molecules; suggesting that the aortic side endothelium may be primed to protect against inflammation and lesion initiation in the normal valve. In a follow-on study, Guerraty et al. investigated the side-specific aortic valve endothelial gene expression of hypercholesterolemic pigs [23]. They identified differential expression on the aortic side of caspase 3, peroxisome proliferator-activated receptor-γ, tumor necrosis factor-α (TNF-α), and nuclear factor-κB- (NF-κB-) related pathways that were consistent with a protective endothelial phenotype that persisted at 6 months. In contrast to these results, Sucosky et al. showed that high magnitude pulsatile shear stress applied to fibrosa-side VEC (an "altered" shear stress state) induced upregulation of inflammatory receptors and expression of BMP-4 [24]. Similar to shear stress, cyclic mechanical strain or pressure can induce and modulate an inflammatory phenotype in aortic VEC [25–27]. Cyclic tissue strain at 5% or 20% magnitude increased inflammatory cytokine expression in aortic valve endothelium, but decreased at 10–15% [25, 26].

VIC are a heterogeneous cell population with up to five different cell phenotypes (fibroblasts, smooth muscle cells, myofibroblasts) in adult aortic valves [13, 15, 28–31], but generally exhibiting fibroblastic phenotypic characteristics. Normal aortic valve interstitial cells secrete and turnover proteins and glycosaminoglycans at a dramatically increased rate in comparison to other cell types *in vivo*, with a significantly higher index of proliferation [32]. This suggests that VIC continually repair mechanically induced tissue microdamage to enable long-term durability. VIC freshly isolated from higher pressure left-sided valves (aortic, mitral) were significantly stiffer and had more collagen biosynthesis than cells isolated from right-sided valve (tricuspid,

pulmonary) [33]. Collagen synthesis by valve interstitial cells was shown to be dependent upon the degree and duration of stretch by Ku et al., as there was a significant increase in [³H]-proline incorporation into stretched valve cells at 10%, 14%, and 20% stretch [34]. These results indicate that VIC-mediated matrix remodeling is regulated in part by the magnitude of local mechanical signaling. VIC exposed to 15% "pathological" cyclic strain increased expression of α-smooth muscle actin (α-SMA), bone morphogenic protein (BMP)-2/4, matrix metalloproteases (MMP) and cathepsin activity, apoptosis, and osteoblastic protein expression, but not at 10% [27]. Hypertensive (170 mmHg) cyclic pressure also increased expression of VCAM-1 and downregulated osteopontin [35]. VIC-VEC cocultures within 3D type I collagen scaffolds suggest that VEC help maintain a quiescent VIC fibroblastic phenotype [36, 37]. The presence of endothelial cells stabilized VIC proliferation, promoted a quiescent VIC phenotype, increased VIC protein synthesis, and decreased glycosaminoglycan loss [37]. The addition of steady shear stress to the cocultures further enhanced the effects of the endothelial cells, including a further decrease in myofibroblastic markers and increase in protein synthesis [37].

4. Inflammation and Calcific Aortic Valve Disease

The cause of calcific aortic valve disease (CAVD) is not completely defined, but inflammation plays a lead role in the initiation and progression of CAVD. Adhesion molecules, such as ICAM-1, VCAM-1, PECAM-1, CD34, and E-selectin, promote the participation of endothelial cells (EC) in both physiological and pathological inflammatory responses through the recruitment of leukocytes [38]. EC become activated, which is a phenotypic change characterized by the production of a variety of biologically active products (cytokines, growth factors, proteolytic enzymes, adhesion molecules), an increase in adhesion molecule expression, endothelial-leukocyte interaction, and permeability, in response to stimuli including circulating inflammatory cytokines, lipopolysaccharides, activation of the renin-angiotensin system, hypercholesteremia, CD40/CD40 ligand interactions, ischemia-reperfusion, physical trauma, diabetes, and hemodynamic forces [38–41]. In diseased aortic valves, VEC upregulate ICAM-1, VCAM-1, and E-selectin, and this occurs preferentially on the fibrosa surface of the valve [24, 38, 40]. The fibrosa or aortic side of the valve is also exposed to disturbed, oscillatory flow, high bending stresses, and is where calcific degeneration initiates [19, 42–45]. The hemodynamics on the valve fibrosa may make the cells more susceptible to inflammatory cell infiltration. *In vitro*, disturbed flow has been shown to cause pro-inflammatory cytokine release (BMP-2/4) and a pro-oxidant phenotype (NADPH, ROS) in VEC [21, 24, 46]. Oxidative, inflammatory, and chondrogenic/osteogenic gene expression profiles are upregulated *in vitro* in VEC grown under static conditions, which mimic hemodynamic conditions on the fibrosa side, when compared with steady shear stress conditions, which recreate ventricularis side hemodynamics

[21, 47]. Evidence of leaflet stress promoting CAVD is the discrepancy in age at the time of presentation with tricuspid and bicuspid valves [48]. Patients with bicuspid valves, which are subjected to higher mechanical stresses, on average present with CAVD two decades younger than those with tricuspid valves [49, 50].

Atherosclerotic risk factors, such as increased low-density lipoprotein cholesterol, increased lipoprotein(a), male gender, cigarette smoking, hypertension, elevated body mass index, and diabetes, increase the incidence of aortic stenosis and likely contribute to valve endothelial dysfunction [44, 51, 52]. The dysfunctional, activated VEC in early valve disease have increased permeability and upregulated adhesion molecule expression. Monocytes attach to adhesion molecules, migrate into the subendothelial space of the valve, and differentiate into macrophages [38, 40, 53]. Macrophages and T-lymphocytes have been shown to be present in aortic valve lesions [54–56]. These invading inflammatory cells, and likely the resident activated endothelial cells, secrete a number of cytokines and/or chronic inflammation effector molecules (e.g., HLA-DR IL-1β/2/6, TNF-α, TGFβ-1, BMP-2/4/7) [43, 56–59]. In addition, circulating low density lipoproteins (LDL) are able to migrate through the permeable endothelial layer, and oxidized forms are capable of deep tissue penetration [60, 61]. Sub-endothelial LDL accumulation can recruit additional inflammatory cells by mechanisms including the induction of macrophage chemoattractants, adhesion molecules, and cytokines [62, 63]. Circulating LDL particles may also deliver angiotensin converting enzyme (ACE) to valve lesions [64]. Angiotensin II, which is generated from angiotensin I by ACE, can compound the inflammatory responses already present by stimulating inflammation and macrophage cholesterol accumulation, increasing oxidant stress, and impairing fibrinolysis [65].

The cytokines and inflammatory effector molecules secreted into the subendothelium and fibrosa by immune cells and activated endothelial cells contribute to a local biochemical environment that promotes VIC differentiation, matrix remodeling, neovascularization, fibrosis, and calcification. TGFβ-1, BMP-2/4/7, IL-1β, TNF-α, lipopolysaccharide, and peptidoglycan have been shown to induce myofibroblastic differentiation, the expression of proinflammatory mediators, or the upregulation of osteogenesis-associated factors in VIC when applied individually *in vitro* [43, 57, 59, 66–69]. Impaired anti-inflammation mechanisms may also contribute to VIC-mediated pathogenesis of aortic stenosis as interleukin-1 receptor antagonist, which is the antagonist of interleukin-1β, was shown to be abundant in nonstenotic aortic valve leaflets and nearly absent in leaflets from stenotic valves [70]. Myofibroblastic activation is characterized by an increase in myofibroblast markers such as vimentin, α-SMA, and embryonic nonmuscle myosin heavy chain (SMemb); and increased cell migration, proliferation, and contractility [13, 29, 31, 71–73]. Activated VIC and inflammatory cells secrete MMP, and cathepsins that progressively destroy the primarily collagen and elastin valve matrix ultrastructure [71, 74–79]. Enzymatic cleavage of ECM can release bound growth factors such as TGFβ-1, which further promotes VIC

myofibroblast differentiation and MMP expression [80–83]. MMP and cathepsin inhibitors, such as TIMPs and cystatin C, are also expressed by activated VIC, but their role in CAVD is not yet known [77, 84]. Following healthy ECM destruction, activated VIC deposit a remodeled, fibrotic matrix characterized by disorganized collagen bundle accumulation, proteoglycan degradation, and fragmentation and stratification of elastin fibers [84–86]. This ECM remodeling results in a stiff aortic valve that is prone to restricted movement, stenosis, and eventual calcification [85].

5. Myxomatous Degeneration of the Mitral Valve

Myxomatous degeneration of the mitral valve or mitral valve prolapse (MVP) is a condition diagnosed with echocardiographically and characterized by abnormally thickened, redundant, floppy leaflets that are displaced into the left atrium during systole [87]. MVP is estimated to affect 1–3% of the US population, and some serious complications of the condition include progressive heart failure, thromboembolism, infective endocarditis, and sudden death [71, 87–90]. The mechanisms for the changes within the valve leaflets are unknown, but myxomatous valvular degeneration is characterized by collagen degradation, proteoglycan accumulation, and elastin fragmentation [85, 87]. This valve matrix remodeling allows stretching of the leaflets, resulting in a floppy valve that is prone to prolapse and regurgitation. It has been hypothesized that the leaflet remodeling may be a response to repeated mechanical stress [91].

There are several connective tissue inherited disorders that are associated with mitral valve dysfunction including Marfan syndrome (fibrillin-1 mutations), Williams syndrome (elastin mutations), osteogenesis imperfecta (collagen 1A1 and 1A2 mutations), Ehlers-Danlos syndrome (mutations in collagen 1A1, 3A1, 1A2, 5A1, and tenascin-x), and Stickler syndrome (collagen 2A1 and collagen 11A1 mutations) [92–100]. MVP is generally sporadic, however, and it is unlikely that more than 1-2% of MVP cases are associated with a connective tissue disorder [87]. A Marfan syndrome mouse model has indicated that increased TGF-β signaling contributes to collagen dysregulation and loss of valve matrix integrity in Marfan syndrome-related and possibly other forms of MVP [101]. Fibrillin-1 interaction with latent TGF-β binding proteins (LTBP) regulates TGF-β activation [102, 103]. LTBP forms a bridge between matrix microfibrils and latency-associated peptide (LAP), which remains noncovalently linked to TGF-β and aids in matrix sequestration. Work by Ng et al. suggests that TGF-β sequestered in the TGF-β, LAP, and LTBP complex is stabilized and/or less prone to activation due to interaction with fibrillin-1 and potentially other components of the extracellular matrix [101]. Pharmacological inhibition of TGF-β signaling with losartan has been shown to reduce Marfan syndrome pathology in mice and humans [104, 105]. Increased TGF-β signaling also supports the interstitial cell activated myofibroblast phenotype and excessive proteolytic activity found in myxomatous valves, as TGF-β has been shown to activate valve interstitial cells and upregulate the expression of ECM-degrading enzymes [71, 82].

6. Rheumatic Heart Valve Disease and Infective Endocarditis

Rheumatic fever (RF) is an inflammatory complication that may develop after a untreated throat infection by the group A β-hemolytic streptococcal bacteria *Streptococcus pyogenes* in susceptible children and teenagers [106]. Carditis, which is one of the most serious RF complications, occurs about 20 days after the infection in 40–50% of patients and can lead to valvular heart disease, heart failure, or death [107–109]. The streptococcal cell structures include the cell wall, capsule, fimbriae, peptidoglycans, cytoplasmic membrane, group-specific carbohydrates, and the M, T, and R antigenic proteins [110]. The streptococcal M protein and hyaluronic acid capsule have been established as the most important virulence factors in human infections, as both confer antiphagocytic properties upon the streptococcal cell and patients with acute RF have a high level of antibodies to streptococcal M protein [111–115]. The M protein is attached to the bacterial cell wall and membrane and extends from the cell surface as an alpha-helical coiled-coil dimer [116, 117].

RF-related cardiac complications are the result of an autoimmune reaction induced by molecular mimicry of human tissues by streptococcal M proteins [108]. The alpha-helical coiled-coil dimer streptococcal M protein has been shown to be structurally and immunologically similar to cardiac myosin, a known mediator of inflammatory heart disease, and other alpha-helical coiled-coil molecules [118]. The antistreptococcal/antimyosin monoclonal antibody mAb 3B6 from rheumatic carditis has shown that the group A streptococcal M protein *N*-acetylglucosamine, which is the dominant group A carbohydrate epitope, and cardiac myosin in the myocardium are the cross-reactive antigens involved in antibody deposition on the valve [119]. Cardiac myosin is not part of the valve, but mAB 3B6 was also shown to recognize laminin, a valvular extracellular matrix protein with alpha-helical coiled-coil domains that are highly homologous with streptococcal M proteins and cardiac myosins [118, 119].

In acute rheumatic carditis host M-protein antibodies bind the valve surface endothelium and/or the valve basement membrane structure protein laminin, which upregulates the endothelial expression of inflammatory adhesion molecules such as VCAM-1 [120]. The inflamed valvular endothelium leads to T-cell recruitment and infiltration through the endothelial layer [118]. T-lymphocytes enter the valve interstitium and cause further inflammation, degeneration, and remodeling. The resulting valve pathologies include neovascularization, chronic inflammation, commissural fusion, thickening, calcification, and thickened and shortened chordae in the atrioventricular valves [108].

Infective endocarditis (IE) is inflammation of the endocardial surfaces of the heart, most commonly the heart valve, caused by the presence of bacteria in the bloodstream and bacterial vegetations forming on valve leaflets [121].

The bacterial strains that cause IE include staphylococci, streptococci, and enterococcus, and the complications from this type of infection include severe valvular dysfunction, congestive heart failure, and death [122]. If there is altered blood flow around the valves or the valves have been damaged, from mitral valve regurgitation and thickening due to rheumatic fever, for example, the risk of bacterial attachment increases [121].

The bacterial vegetations that form on the valve surface are composed of platelets, fibrin, microorganisms, and inflammatory cells and are the result of mechanical or inflammatory valve lesions [121, 123]. Mechanically denuded endothelial layer lesions promote microbial adherence to the endothelium when bacteria is present in the bloodstream [123]. Endothelial denudation results in direct contact between blood and proteins within the valve interstitium, which include extracellular matrix proteins, thromboplastin, and tissue factor, and causes blood coagulation [123]. The damaged endothelium is colonized when microorganisms bind to the fibrin and platelet-containing blood clots and initiate monocyte activation and production of cytokines and tissue factor activity (TFA) [124]. The vegetation grows when cytokines and TFA activate coagulation cascades, attract and activate blood platelets, and induce cytokine, integrin, and TFA production from nearby endothelial cells [123]. Inflammatory lesions are the result of endothelial cells responding to local inflammation by expressing $\beta 1$ integrins, including very late antigen (VLA) [125]. $\beta 1$ integrins bind fibronectin to the valve endothelial surface [123]. IE-associated pathogens have fibronectin-binding proteins on their surface, therefore $\beta 1$ integrins provide an adhesive surface for the circulating microorganisms [123]. Following pathogen adhesion, endothelial cells internalize the bacteria; which causes endothelial TFA and cytokines production, blood clotting, the extension of inflammation, and vegetation growth [123, 126]. Internalised bacteria eventually lyse endothelial cells by secreting membrane active proteins such as hemolysins [123]. The vegetation growth and tissue damage caused by mechanical or inflammatory lesions can result in abscess formation and septic emboli may circulate to other organs [127].

7. Serotonin Metabolism-Related Valve Disorders

Clinical studies have identified an increased incidence of fibrotic aortic valve disease in patients using several classes of drugs that are structurally similar to serotonin (5-hydroxytryptamine, 5-HT) and in patients with carcinoid syndrome, which can result in a high serotonin concentration on the right side of the heart [128–131]. External serotonin administration *in vitro* and *in vivo* has been shown to increase VIC proliferation and α-SMA, collagen, and TGFβ expression, which are all indicative of myofibroblastic activation and fibrotic matrix remodeling [132–135]. No studies have directly linked aortic valve calcification and elevated serotonin levels; however, serotonin may mediate intermediate stage valve fibrosis. Recent evidence supports serotonin synthesis by valve cells [136]. This may contribute

to valve calcification through increased TGFβ signaling by serotonin receptor antagonists or transporter agonists in mitral valves [136]. The effects of serotonin on VEC are less well understood, but endothelial function is required for serotonin-mediated valve relaxation [137, 138]. These results suggest that controlling serotonin metabolism may be a novel means of selectively modulating valve cell phenotype *in vivo*.

8. Bone Marrow Stem Cell Contribution to Valve Repair and Disease

Bone marrow-derived mesenchymal stem cells (BMSCs) are found in adult circulation at a low concentration and are thought to regulate the immune response in settings such as tissue injury, transplantation, and autoimmunity [139]. Mouse and human valve studies have shown that at least 10% of VIC are BMSCs, but the specific role of BMSCs in valve disease is not well understood [140, 141]. The progenitors appear to be recruited with other inflammatory cells into the valve interstitium, but whether they initiate repair or promote disease is unclear. Tanaka et al. identified bone marrow-derived cells expressing myofibroblast and osteoblast markers near calcific nodules in aged mice, which was supported in humans by Helske et al. [79]. Understanding the recruitment and function of circulating stem cells in valve homeostasis and pathogenesis could have clinical benefits, as autologous BMSCs may be treated to express antifibrotic and anticalcific proteins before their mobilization to valves [142].

9. Inflammation and Valve Tissue Engineering

Tissue engineered heart valves could provide a great clinical benefit to patients with valve disease, especially children who require valve growth and younger patients who cannot tolerate the side effects of nonliving prosthetics [143, 144]. Understanding the role of host inflammatory response is essential to the successful implantation of tissue engineered valves, however. Repopulation and regeneration are the two main heart valve tissue engineering approaches that have been attempted [145]. A repopulated heart valve is created when a patient's cells repopulate an implanted decellularized porcine aortic valve [145]. A heart valve is regenerated by implantation of a resorbable matrix that remodels *in vivo*, resulting in a functional valve composed of the patient's cells and connective tissue proteins [145]. Neither of these approaches have achieved clinical success, however.

Long-term sheep implantation studies with decellularized heart valves and acellular valves seeded with cells before implantation showed significant tissue overgrowth, infiltration with inflammatory cells, and dilatation [146, 147]. The SynerGraft valve by CryoLife is a decellularized pulmonary valve that has been implanted in humans. Adult clinical trials have been encouraging, but in children, rapid inflammatory reactions caused death in 3 out of 4 patients [148, 149]. All pediatric valves displayed severe inflammation, including fibrosis, encapsulation, perforation, and deterioration of the leaflet tissues [149]. Matrix P plus valves, which are decellularized pulmonary porcine

valves, displayed early obstruction following implantation in pediatric patients [150]. A foreign body-type reaction accompanied by severe fibrosis and massive neointima formation around the decellularized porcine valve wall was found. Examination of the explanted valves showed inflammatory infiltrates, composed of T cells, B cells, plasma cells, dendritic cells, macrophages, and mast cells, in the tissue surrounding the porcine matrix. *In vitro* studies have shown that the inflammatory mechanism of decellularized valves may involve porcine collagen type I, but not porcine elastin [151].

In one of the few long-term *in vivo* studies with polymeric tissue engineered heart valves, composite biodegradable polymer valves were seeded with mesenchymal stem cells, cultured for 4 weeks, and implanted into a sheep model for up to 20 weeks [152]. *In vivo* monitoring showed leaflet coaptation, and explanted valves showed near-native trilaminar matrix striation with both endothelial-like and interstitial-like cell phenotypes. Elevated transvalvular pressure gradients, however, suggest that the cusps are still too stiff. Interestingly, these valves exhibited a marked inflammatory activation during their early remodeling period, characterized by the presence of endothelial inflammatory receptors (VCAM-1, ICAM-1), matrix metalloproteases, and interstitial cell myofibroblastic activation. Early matrix structure was virtually absent and disorganized. After 2-week *in vitro* conditioning, and further enhanced with *in vivo* implantation, the progression to organized tissue striation correlated inversely with the degree of activated valve cell phenotypes. Collectively, these results demonstrate that the degree of inflammation has a significant role on the fate of engineered tissues, and suggests that progressively reduced inflammation can help develop and maintain quiescent tissue.

10. Inflammatory Regulation of Valve Formation

The morphogenesis of the atrioventricular and semilunar valves is a complex process that occurs along with the changing cardiac morphology and hemodynamics of the growing embryo. The early embryonic heart is a tube of endocardium surrounded by myocardium [153]. Soon after the linear heart tube begins to loop the myocardium secretes cardiac jelly, a hyaluronan and chondroitin sulfate rich gelatinous matrix, into the atrioventricular (AV) junction and outflow tract (OFT) lumen [154–157]. At chick stage HH14 (mouse E9.0, human day 20), valvulogenesis begins when a subset of endocardial cells receive growth factor signals from the myocardium that initiate a cascade of signal pathways resulting in an endocardial to mesenchymal transformation (EMT) [153, 158]. EMT is characterized by endocardial cell activation; three antigens expressed only by activated endocardial cells include ES130, JB3/fibrillin, and TGFβ3 [159–161]. Activated endocardial cells lose cell-cell contacts proteins (PECAM1, NCAM1, DS-CAM), gain cell-matrix adhesions (integrins), gain mesenchymal markers (α-smooth muscle actin, α-SMA), and the acquire cell migratory and invasive capacity (transformation) [153, 157, 158, 162,

163]. The transformed endocardial cells invade the cardiac jelly and form the endocardial cushions that will eventually develop into mature atrioventricular and semilunar valves [157, 162].

Few studies have been performed to determine the role of inflammation on valve development. A recent study showed that leptin, a member of the IL-6 superfamily, mediates embryonic EMT [164]. TNF-α serum levels were found to be high in children with congenital heart disease and TNF-α and IL-6 levels were elevated in the myocardium of infants with tetralogy of Fallot or ventricular septal defects [165, 166]. Neonates with hypoplastic left-heart syndrome (HLHS) have evidence of an activated inflammatory response, as IL-6 levels are significantly elevated when compared with controls [167]. Fetal aortic valve endothelial cells express ICAM-1 and VCAM-1, but whether this is due to inflammation is unknown [41]. VCAM-1 deficient mice survive to E11.5–12.5 and display severe heart abnormalities including a reduction of the compact layer of the ventricular myocardium and intraventricular septum, blood in the pericardial space, and an absent epicardium [168].

Abnormal inflammatory signaling may play a role in the development of congenital heart defects. Children born to mothers' with the connective tissue disease lupus erythematosus (LE) have an increased risk for congenital heart block (CHB), a type of arrhythmia, and valve regurgitation [169, 170]. CHB can result in early death from delayed pacemaker placement or hemodynamic compromise from associated congenital heart disease [171]. The fetal heart defects resulting from maternal LE are thought to be the result of transplacental passage of certain maternal autoantibodies, including immunoglobulin G (IgG) antibodies to SSA/Ro and SSB/La ribonucleoproteins [169, 172]. These autoantibodies damage the fetal AV conduction tissue by inflammation in the early stage and later by fibrosis [173]. Maternal inflammatory insult has also been shown to result in fetal cardiac dysfunction [174]. Administration of intra-amniotic lipopolysaccharide caused an increase in maternal TNF-α and IL-6 serum levels, and the placenta showed severe maternal vascular dilatation and congestion [175]. No inflammatory activation was found in the fetal tissues, and the amniotic fluid revealed no significant increase in cytokines; but ultrasonographic examination of the fetal hearts showed that 65% of the fetuses exhibited atrioventricular valve regurgitation [175]. The maternal inflammatory insult was shown to activate placental labyrinthine macrophages, which then leads to an acute increase in placental vascular resistance and fetal cardiac dysfunction [175].

Abnormal nuclear factor-Kappa B (NF-κB) activation may play a role in congenital heart defects. NF-κB is a transcription factor that coordinates inflammation and cellular proliferation. Upregulated NF-κB activation was found in the myocardium of children with congenital heart disease and in the myocardium of infants with tetralogy of Fallot or ventricular septal defects [165, 166]. The inhibition of NF-κB during chicken heart development led to impaired OFT development and resulted in interventricular communication, double outlet right ventricle, and valvular

Morphology	Inflammation	Cell response

EMT

Matrix remodeling

Fibroblast differentiation

Quiescence

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Myofibroblastic activation
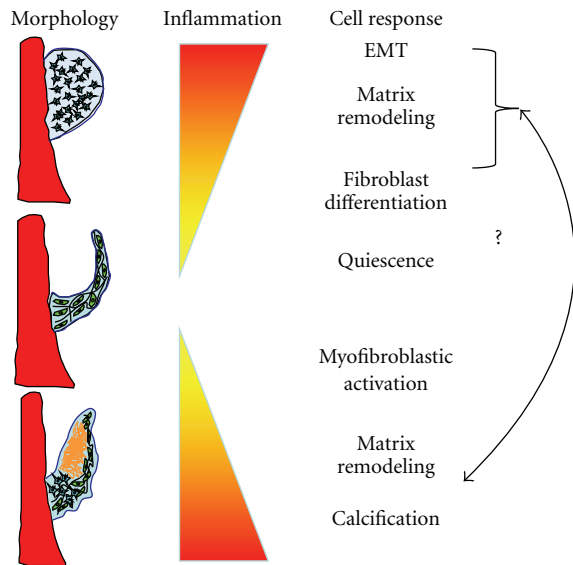
Matrix remodeling

Calcification

FIGURE 1: Inflammation and its signaling partners are involved in every stage of the heart valve life cycle, from embryonic morphogenesis (top), quiescent adult valve homeostasis (middle), and in valve pathology (bottom). Many structural and biological features of diseased valves resemble those from embryonic/fetal valves, suggesting similar signaling pathways are reactivated. Identifying and controlling these pathways represents an important and challenging task with potential for large payoff for heart valve disease diagnosis and treatment.

and great arteries stenosis [176]. Nuclear factor of activated T cells (NF-ATc) is a transcription factors that may be needed for cytokine gene expression in activated lymphocytes. Mice lacking NF-ATc died *in utero* from congestive heart failure at days 13.5–17.5 of gestation due to the absence pulmonary and aortic valve leaflets [177]. Adults with congenital heart disease were found to have elevated levels of endotoxin and of the inflammatory cytokines TNF-α and IL-6 when compared to controls, which indicates that adults with congenital heart disease could potentially benefit from novel anti-inflammatory therapies [178].

11. Conclusions

These studies highlight our evolving understanding of the complex role of inflammation in heart valve remodeling, and underscore how much more is left to be learned. While it is true that inflammation mediates the ugliest of pathological valve remodeling, the fact that it is also present during normal embryonic development suggests a potential benefit. Indeed, inflammation-mediated tissue formation, for example, during foreign body encapsulation, is among the most rapid processes available in our natural arsenal. Embryonic morphogenesis progresses on a significantly more rapid scale than wound healing, yet instead of fibrosis results in scar-free, mature tissue architectures. It is therefore time to reconsider the myopic view of inflammation as the harbinger of destruction, but rather as a universal agent of tissue remodeling, for good or ill. In this light, many more questions are revealed rather than answered (Figure 1).

How does inflammation promote positive remodeling, and what are the triggers for pathological change? Are there signaling paradigms in embryonic valve formation that are reactivated in pathological valve remodeling? If so, how are these controlled by inflammation? The answers will likely be heavily dependent on the microenvironmental context (patient age, gender, underlying inflammatory state, gene mutations, mechanical stress, etc.). However, this transformative notion suggests a new avenue to promote and control tissue remodeling in adaptive and regenerative heart valve applications. It is not yet known whether or how this may be achieved in tissue engineering applications, but strongly suggests that complete cessation of inflammatory signaling may not be the best approach. We hope with future research in this area these answers will lead to new clinically translatable therapies for patients with valve disease.

Acknowledgments

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