Inflammatory Mechanisms of Cardiovascular Remodeling

Toshihisa Anzai, MD, PhD

Inflammation and fibrosis play an important role in the development and progression of cardiovascular diseases. Acute coronary syndrome (ACS) is caused by rupture of inflamed atherosclerotic plaque and subsequent atherothrombosis. Recent studies have shown that inflammatory markers such as C-reactive protein (CRP) can predict ACS development and have demonstrated the effectiveness of new therapeutic approaches targeting inflammation. Studies have also shown that an enhanced inflammatory response after myocardial infarction (MI) is associated with cardiac rupture, ventricular aneurysm formation, and exacerbation of left ventricular (LV) remodeling. Inflammation is a physiological reaction in which fibrosis is induced to facilitate the healing of tissue damage. However, when an excessive inflammatory response consisting mainly of monocytes/macrophages is induced by various factors, impaired reparative fibrosis and resulting pathological remodeling processes may occur. A similar phenomenon is observed in abdominal aortic aneurysm (AAA) expansion. In contrast, myocardial diseases such as inflammatory dilated cardiomyopathy (DCMI) and valvular diseases such as aortic valve stenosis (AS) are characterized by chronic inflammation mediated mainly by T lymphocytes and the associated enhancement of reactive fibrosis. Thus, inflammation can take 2 paths (the inhibition or promotion of fibrosis), depending on the phase of inflammation, inducing pathological cardiovascular remodeling. Elucidation of the regulatory mechanisms of inflammation and fibrosis will contribute to the development of new therapeutic approaches for cardiovascular diseases.

Key Words: Cardiomyopathy; Inflammation; Myocardial infarction; Remodeling; Valvular diseases

Although inflammation, degeneration, and tumorigenesis are the 3 main pathogenetic components of various diseases, the pathogenesis of cardiovascular diseases (CVD), which are considered benign, is mainly mediated by inflammation and degeneration. Inflammation in particular is considered an important target that can be therapeutically intervened. We reported in 1997 that a post-myocardial infarction (MI) increase in the serum C-reactive protein (CRP) level can predict subacute cardiac rupture and poor clinical outcome. Subsequently, high-sensitivity CRP (hsCRP) was identified as a biomarker for evaluating microinflammation, enabling the detection of pre-MI inflammation of coronary atherosclerotic plaques. This was followed by the demonstration of an important role of inflammation in vascular remodeling, a precursor condition of coronary atherosclerotic plaque rupture, leading to the establishment of the concept of acute coronary syndrome (ACS). Subsequent studies also revealed that, although inflammation following tissue damage is an essential physiological reaction in the healing process, an excessive inflammatory response inhibits reparative fibrosis and thereby affects not only post-MI left ventricular (LV) remodeling but also aortic remodeling, leading to the development/progression of abdominal aortic aneurysm (AAA). In contrast, in the chronic phase of inflammation, reactive fibrosis is promoted, leading to tissue fibrosis and resulting in pathological remodeling as well as progression of myocardial and vascular diseases. Inflammation, which not only plays an important role in the pathogenesis of this variety of diseases but is also involved in disease progression, may be an important therapeutic target for primary and secondary disease prevention. The aim of this article is to give a general overview of the pathophysiology of various forms of cardiovascular remodeling mediated by inflammation and discuss future treatment strategies.

Coronary Artery Remodeling and Inflammation

It was traditionally believed that atherosclerotic plaque gradually grows to cause angina pectoris and subsequent MI. However, in the 1990s, studies revealed that approximately half of patients with MI experienced sudden-onset MI without preceding angina and that the majority of patients who experienced angina before MI had new-onset unstable angina. Other studies showed that approximately 70% of patients with acute MI had coronary artery stenosis <50% before MI onset, suggesting that MI is caused by rupture of a plaque that has expanded towards the adventitia, the so-called positive vascular remodeling, with subsequent thrombus formation and abrupt coronary artery occlusion, leading to the establishment of the concept of ACS. Studies also revealed that thinning of the fibrous capsule of the plaque resulting from activated matrix metalloproteinases (MMPs) and decreased collagen fiber production is involved in the mechanism of plaque rupture, followed by the demonstration of the predictive value of the inflammatory marker hsCRP for ACS development.
by a number of studies.\textsuperscript{10,11} Ridker et al demonstrated that the hsCRP level is a stronger risk factor for coronary artery disease than conventional risk factors such as total and high-density lipoprotein cholesterol levels.\textsuperscript{12} A cohort study including 27,939 healthy adult women showed that hsCRP levels equal to or higher than the median were associated with an increased incidence of MI and other cardiovascular events regardless of the low-density lipoprotein cholesterol (LDL-C) level.\textsuperscript{13} In metabolic syndrome (MetS), an increasing number of diagnostic criteria are associated with an increasing level of hsCRP,\textsuperscript{14} suggesting that an altered adipocytokine level resulting from visceral fat accumulation induces inflammation and thereby increases the risk of ACS development. In fact, the cardiovascular event-free curve of patients with MetS almost overlapped the corresponding curve of patients with hsCRP levels $\geq 3$ mg/L.\textsuperscript{15} In patients with chronic kidney disease (CKD), another risk factor for ACS that is drawing increasing attention, increased hsCRP levels are observed in association with a reduced clearance of inflammatory mediators, oxidative stressors, and advanced glycation end products, as well as the activation of neurohumoral factors, and may play a role in the increased incidence of cardiovascular events (i.e., cardioenral syndrome) in CKD patients.\textsuperscript{16} Similarly, patients with chronic obstructive pulmonary disease have been shown to have increased hsCRP levels, reflecting chronic airway inflammation, which may contribute to the increased incidence of cardiovascular events in these patients.\textsuperscript{17} Drugs with proven efficacy for ACS prevention, such as statins\textsuperscript{18,19} and aspirin,\textsuperscript{20} reduce hsCRP levels, suggesting that they exert their effects through their action on lipid metabolism and platelet aggregation as well as via an anti-inflammatory action.

**Prognostic Significance of Serum hsCRP After ACS**

An increased serum hsCRP level is observed after ACS development, reflecting inflammation of the atherosclerotic plaque and post-MI inflammation. The FRISC (Fragmin during Instability in Coronary Artery Disease) study group measured the hsCRP and troponin T levels of 917 patients with unstable angina and found that those with a hsCRP level $\geq 10$ mg/L within 24 h after admission had poorer long-term outcomes.\textsuperscript{21} The hsCRP level, although higher in patients with higher troponin T levels, correlated with the long-term outcome independently of the troponin T level, suggesting that the former serves as a prognostic factor by reflecting atherosclerotic plaque inflammation. The hsCRP level has also been shown to be an independent prognostic factor for restenosis after coronary artery stenting and cardiovascular events.\textsuperscript{22} These findings suggest that, in cases of unstable angina with myocardial necrosis, increased inflammatory markers also reflect the inflammation of coronary artery atherosclerotic plaques and thus can predict coronary events.

![Pathophysiology of post-myocardial infarction (MI) left ventricular (LV) remodeling.](Image)

Figure 1. Pathophysiology of post-myocardial infarction (MI) left ventricular (LV) remodeling. After MI, infarct expansion may occur and trigger a progressive increase in LV volume and a reduction in the LV ejection fraction, eventually leading to chronic heart failure. Infarct expansion is the expansion of a necrotic and weakened myocardium caused by wall stress and influenced by 3 major determinants: infarct size, LV wall stress, and defective infarct healing because of excessive inflammatory response. Subsequently, non-infarct hypertrophy and dilatation occur in association with activation of the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS) and production of reactive oxygen species (ROS) and inflammatory cytokines.
in the risk of MACE, including non-fatal MI, non-fatal stroke, and cardiovascular death, in the ACZ885 150mg group compared with the placebo group. These results suggest the efficacy of treatment strategies targeting inflammation for the secondary prevention of ACS.

**Post-MI LV Remodeling and Inflammation**

After MI, infarct expansion may occur and trigger non-infarct hypertrophy and dilatation, thereby inducing a progressive increase in LV volume and a reduced LV ejection fraction (EF), eventually leading to chronic heart failure. This process is referred to as LV remodeling (Figure 1). Infarct expansion (the expansion of a necrotic and weakened myocardium caused by wall stress) is known to be influenced by 3 major determinants: infarct size, LV wall stress, and defective infarct healing.\(^\text{24}\) Infarct size can be reduced by primary percutaneous coronary intervention (PCI) and wall stress can be reduced using β-blockers and vasodilators, but no approach has been established to intervene in infarct healing. In necrotic myocardium, neutrophils and then monocytes/macrophages and other inflammatory cells infiltrate to phagocytize necrotic tissue and secrete MMPs to lyse unnecessary proteins (debris clearance). An excessive inflammatory response in this process may exacerbate weakening of the infarcted myocardium and interfere with healing processes such as angiogenesis and the replacement of collagen fibers.

Our group demonstrated that a post-MI increase in serum CRP predicts cardiac rupture and ventricular aneurysm during the subacute phase, as well as 1-year cardiac death.\(^\text{4}\) We also divided patients with a first anterior MI who underwent primary PCI according to the post-MI maximum CRP level into those who were lower or higher than the median and prospectively evaluated the course of LV remodeling. Although no significant intergroup difference was observed in LV volume on admission, the LV end-diastolic and LV end-systolic volume indices at 2 weeks and 6 months post-MI were significantly higher in the high CRP group than in the low CRP group, suggesting exacerbated LV remodeling in the former group.\(^\text{25}\) Moreover, patients treated with β-blockers within 24h post-MI showed a suppressed increase in CRP level and a lower incidence of cardiac rupture, while those aged ≥70 years or with concomitant CKD showed a greater increase in CRP level and exacerbated LV remodeling during the 2 weeks after MI.\(^\text{26-28}\) CRP is produced in the liver in response to proinflammatory cytokines that are mainly expressed by the monocytes/macrophages infiltrating the infarction site. We previously reported that an increased peripheral blood monocyte count is observed prior to monocyte/macrophage infiltration, peaking 2–3 days post-MI, and that a maximum monocyte count ≥2900/mm\(^3\) is associated with an increased long-term incidence of MACE, such as cardiac death, hospitalization for heart failure, and re-MI, leading to poor clinical outcomes.\(^\text{29}\) A subsequent study showed that the treatment of MI-model rats with granulocyte-macrophage colony stimulating factor (GM-CSF) resulted in an increased peripheral monocyte count, an increased expression of monocyte-chemoattractant protein (MCP)-1, and the subsequent increase in macrophage infiltration in the infarcted myocardium, which interfered with reparative fibrosis at the infarction site and induced exacerbated LV remodeling.\(^\text{30}\)

Although these findings demonstrate that an excessive inflammatory response mediated by monocytes/macrophages exacerbates post-MI LV remodeling, separate evidence demonstrates that post-MI treatment with steroids, which have an anti-inflammatory action, increases the incidence of cardiac rupture,\(^\text{31}\) suggesting that inflammation is a double-edged sword.\(^\text{32}\) Furthermore, high mobility group box-1 (HMGBI) protein, a nuclear DNA-binding protein and inflammatory mediator that is passively released from necrotic cells and actively secreted by inflammatory cells, has been shown to predict post-MI poor prognosis, although the treatment of MI-model rats with neutralizing antibody to HMGBI protein for 1 week after the model’s development resulted in reduced macrophage infiltration into the infarction site but simultaneously exacerbated LV remodeling 2 weeks post-MI.\(^\text{33}\) Thus, treatment strategies targeting post-MI inflammation must be designed to control excessive inflammatory response while promoting the normal healing process. We have demonstrated that dendritic cells (DCs), which regulate immune responses, play an important role in the control of the inflammatory response and its transition to healing.\(^\text{34,35}\)

**Regulatory Role of DCs in Post-MI Inflammation and Healing**

DCs have a strong antigen-presenting capacity and play a pivotal role in the induction of an immune response and tolerance in acquired immunity, as well as organ protection in innate immunity by controlling the excessive inflammatory response via the expression of the anti-inflammatory cytokine IL-10 in sterile inflammation, as demonstrated in a hepatic ischemia-reperfusion injury model.\(^\text{36}\) To determine the involvement of DCs in post-MI LV remodeling, our group developed a mouse model in which the animals were selectively depleted of bone marrow-derived DCs by transplanting bone marrow harvested from CD11c-DTR-GFP transgenic mice in which the human diphtheria toxin receptor (DTR) and green fluorescent protein (GFP) genes were introduced downstream of the promoter for CD11c, a DC marker, into wild-type mice and diphtheria toxin was administered. The introduction of MI in these model mice resulted in exacerbated LV remodeling compared with control MI-model mice.\(^\text{37}\)

The immuno histochemical staining of myocardial specimens collected 7 and 14 days post-MI showed increased infiltration of Mac-3-positive macrophages in the DC-ablated group. We then extracted the macrophages infiltrating the infarction site and analyzed their fractions by flow cytometry. The results showed an increased population of M1 macrophages, “classically activated” inflammatory macrophages, and a decreased population of M2 macrophages, “alternatively activated” anti-inflammatory macrophages involved in tissue repair, in the DC-ablated group.\(^\text{38}\) The development of MI is followed by the infiltration of proinflammatory Ly6C\(^\text{high}\) monocytes and M1 macrophages, which phagocytize necrotic tissue and lyse unnecessary proteins, and then the infiltration of anti-inflammatory Ly6C\(^\text{low}\) monocytes and M2 macrophages, which promote healing through tissue replacement by collagen fibers and angiogenesis.\(^\text{32}\) In this process, DCs are induced to differentiate from the same precursor cells as monocytes and macrophages and infiltrate the infarction site, which peaks at 7 days post-MI. These cells control the excessive inflammatory response by expressing the anti-inflammatory cytokine IL-10 and promote the mobilization of cells...
involved in healing as demonstrated by subsequent studies.\textsuperscript{38} To investigate whether this process actually occurs in the human MI, we performed the following study using human autopsy hearts.\textsuperscript{37} We compared the extent of reparative fibrosis at the infarction site as determined by Masson’s trichrome staining between patients who died of cardiac rupture during the subacute phase of MI and those who died of other causes during almost the same time frame and determined that the percent area fraction of fibrosis at the infarcted myocardium was significantly lower in the cardiac rupture group, suggesting the presence of suppressed reparative fibrosis in these patients. In contrast, a marked increase in the infiltration of CD68-positive macrophages was observed in the cardiac rupture group, although the infiltration of CD209-positive DCs into the same tissue section was significantly reduced in the cardiac rupture group. These observations suggested that the control of inflammation is lost under pathological conditions in which the infiltration or function of DCs is impaired, leading to consequences such as cardiac rupture, ventricular aneurysm, and LV remodeling.\textsuperscript{37} In fact, DCs induced from monocytes derived from CKD patients showed a reduced expression of DC maturation markers,\textsuperscript{38} suggesting an association between impaired DC function and severe inflammatory response and poor prognosis in MI patients with CKD or acute kidney injury.\textsuperscript{28,39}

Inflammation in response to tissue necrosis and infection is a physiological reaction that promotes tissue healing through fibrosis, but may become excessive in the presence of additional factors such as mechanical stress, autoimmunity, genetic background, activation of neurohumoral factors, oxidative stress, and aging, causing pathological remodeling through MMP activation and other mechanisms (Figure 2).

**Myocardial Inflammation in Cardiac Sarcoidosis (CS)**

CS is a condition in which inflammation and the immune response are major pathogenic factors. An excessive immune response to *Propionibacterium acnes* (*P. acnes*) has been suggested to be involved in the pathogenesis of sarcoidosis.\textsuperscript{40-42} We performed immunohistochemistry of surgical, autopsied, and biopsied myocardial specimens from patients with CS, myocarditis, and idiopathic cardiomyopathy using a *P. acnes*-specific monoclonal antibody and detected *P. acnes* in myocardial granulomas of 62\% of CS patients but not in patients with myocarditis or idiopathic cardiomyopathy.\textsuperscript{43} Focusing on DCs, which serve as antigen-presenting cells during the immune response, we analyzed autopsied and biopsied myocardial specimens from CS patients for DC infiltration and detected CD209-positive DCs infiltrating around the granulomas.\textsuperscript{44} The detection of non-necrotizing granulomas is a histological criterion for diagnosing CS, but has been detected in \( \leq 30\% \) of CS patients,\textsuperscript{45} suggesting the need for a more sensitive diagnostic marker. In the analysis of DC infiltration, we found increased infiltration of DCs and macrophages in the non-granulomatous area of specimens from patients who tested positive for granulomas and were later given a histologically confirmed diagnosis of CS compared with those with idiopathic cardiomyopathy. Further studies showed increased infiltration of DCs and a decreased proportion of M2 macrophages among the infiltrating macrophages in patients who tested negative for granulomas but were later given a clinically definitive diagnosis of CS based on the diagnostic criteria for designated intractable diseases established by the Japanese Ministry of Health and Welfare\textsuperscript{46} or the criteria proposed by the Heart Rhythm Society\textsuperscript{47} compared with control patients, suggesting that Th1-type inflammation was enhanced by antigen presentation by infiltrating DCs.\textsuperscript{44} Moreover, increased numbers of DC and decreased numbers of M2 among all macrophages in non-granuloma sections of myocardium showed high specificity for CS diagnosis, suggesting DC and macrophage phenotype as histopathological surrogates for the diagnosis of CS. These findings may also contribute to the future development of new therapeutic approaches to inducing immune tolerance of *P. acnes* using regulatory DCs.

**Dilated Cardiomyopathy (DCM) and Inflammation**

Recent evidence suggests involvement of inflammation related to viral infection and autoimmunity in the
pathogenesis of DCM. Sustained inflammatory cell infiltration is often observed in myocardial biopsy specimens collected from patients with clinical manifestations of DCM, suggesting an association between inflammation and impaired cardiac function.48,49 More specifically, it is proposed that myocardial infiltration by lymphocytes or macrophages at counts ≥14/mm² should be defined as inflammatory DCM (DCMI).50 We previously investigated the extent of myocardial infiltration by CD3-positive T lymphocytes and CD68-positive macrophages in 182 patients with DCM who underwent myocardial biopsy and found that 46% of all patients could be diagnosed with DCMI and had significantly poor long-term outcome in terms of cardiovascular death and heart transplantation.51 In chronic inflammation, as observed in DCMI, tissue-infiltrating inflammatory cells consist mainly of T lymphocytes and the cytokines and other factors expressed by these lymphocytes stimulate fibroblasts to produce extracellular matrix (ECM). Tenascin-C, a component of ECM, is considered to further promote inflammation and fibrosis, inducing pathological remodeling in the cardiovascular system.52 We then measured the expression of tenascin-C using myocardial biopsy specimens from DCMI patients and found that patients expressing higher tenascin-C levels had significantly less LVEF improvement after various treatments and poorer survival outcomes.53 Another interesting finding was that tenascin-C expression was significantly higher in DCM patients with concomitant diabetes mellitus than in those without it, suggesting that diabetes affects the development of myocardial fibrosis.53

**Chronic Inflammation and Tenascin-C Expression in Aortic Valve Stenosis (AS)**

We recently reported that chronic inflammation and fibrosis mediated by tenascin-C play a role in AS progression.54 Analysis of the surgical specimens of aortic valves collected from patients who underwent aortic valve replacement for atherosclerotic tricuspid AS, congenital bicuspid AS, and congenital bicuspid aortic valve regurgitation (AR) showed greater inflammatory cell infiltration comprised mainly of T lymphocytes in patients with atherosclerotic tricuspid AS or congenital bicuspid AS than in those with congenital bicuspid AR. Moreover, the expression of tenascin-C was particularly high in patients with congenital bicuspid AS, who also had the highest degree of valvular thickening caused by fibrosis. These findings suggest that, although atherosclerotic tricuspid AS is caused mainly by valvular calcification, congenital bicuspid AS is caused by valvular thickening resulting from enhanced chronic inflammation and fibrosis related to increased tenascin-C expression.54

**AAA and Inflammation**

The prevalence of AAA is increasing as society ages. Current treatment strategies for AAA consist mainly of surgical treatment for patients at a high risk of rupture, with no established effective medication therapy except antihypertensive agents.55 Pathologically, AAA is characterized by chronic inflammation, ECM destruction, and angio genesis in the arterial wall. Chronic inflammation of the arterial wall is believed to be induced by oxidative stress generated by smoking, hypertension, and underlying activation of neurohumoral factors.56–58 We have demonstrated by immunohistochemical staining increased macrophage and T lymphocyte infiltration, vasa vasorum proliferation of the media and adventitia, and increased vascular endothelial growth factor (VEGF)-A expression, as well as destruction of the wavy structure of elastin fibers as demonstrated by Elastica Van Gieson staining, in surgical specimens of human AAA compared with aortic specimens from patients who died of non-cardiovascular causes.4 We also reported similar pathological changes in CaCl₂-induced AAA model mice and that treatment with the soluble form of Flt-1 (sFlt-1), a molecule that inhibits VEGF-A activity, reduced inflammatory cell infiltration, neoangiogenesis, and MMP-2 and MMP-9 activity, and suppressed AAA formation. These observations suggest that proliferation of the vasa vasorum towards the adventitia results in the formation of a conduit for inflammatory cell transport, thereby
promoting inflammation and expansion of the AAA. In human AAA, increased expression of tumor necrosis factor (TNF-α) in both the circulation and the aortic wall of AAA has been demonstrated. We demonstrated increased expression of TNF-α converting enzyme (TACE), which cleaves the transmembrane precursor of TNF-α, in the aortic wall of human AAA and in a CaCl₂-induced AAA model. Temporal systemic deletion of TACE by the inducible Mx-1 Cre transgene attenuated TNF-α expression and ECM disruption. Increased activity of both MMP-9 and MMP-2, the numbers of Mac-2-positive macrophages, CD3-positive T lymphocytes and CD31-positive vessels in periaortic tissues, mRNA expression of CD68, MCP-1, TNF-α, VEGF-A, p47 and glutathione peroxidases in AAA were all attenuated by TACE deletion, suggesting its crucial role in AAA development.

We also demonstrated the involvement of the aforementioned HMGB1 in the expansion and rupture of AAA. In human AAA, HMGB1 was robustly expressed in inflammatory cells, smooth muscle cells, and endothelial cells in the aortic wall, and the AAA diameter, as determined by preoperative computed tomography, significantly and positively correlated with the expression of HMGB1 protein in the aneurysmal wall. Our results also revealed an increased expression of HMGB1 protein in both the circulation and the aortic wall of patients who experienced a ruptured AAA and underwent emergency surgery compared with those who underwent elective surgery.

In CaCl₂-induced AAA model mice, increased HMGB1 expression has also been demonstrated, and treatment with anti-HMGB1 antibody resulted in reduced infiltration of macrophages into the aortic wall, reduced expression of MMP-2 and MMP-9 at the protein level, and reduced AAA diameter. These results suggest the potential of HMGB1 as a new therapeutic target for preventing AAA expansion (Figure 3).

Conclusions

Inflammation and fibrosis are involved in the pathogenesis of various CVD. Inflammation of atherosclerotic plaque can lead to the development of ACS, and the efficacy of a new therapeutic approach targeting inflammation has been demonstrated in a recent large-scale clinical trial. The inhibition of reparative fibrosis caused by an excessive inflammatory response after tissue damage in the cardiovascular system appears to be involved in the development of post-MI cardiac rupture and ventricular aneurysm, as well as the expansion/rupture of AAA. In contrast, enhanced fibrosis in chronic inflammation may play a role in the progression of cardiomyopathy and valvular stenosis. The elucidation of the mechanism by which inflammation and fibrosis are regulated will substantially contribute to the development of new therapeutic approaches for these diseases.

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