REVIEW

Inflammation in acute coronary syndromes

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Extensive evidence supports a pathogenic role for both local and systemic inflammation in acute coronary syndromes. However, several important questions remain unanswered. Is the observed inflammatory process a precursor or a consequence of coronary plaque rupture? Is the inflammatory component of unstable coronary disease a potential therapeutic target? Finally, do infectious agents have a pathogenic role in coronary atherosclerosis and acute coronary syndromes?

Coronary plaque disruption, with consequent platelet aggregation and thrombosis, is the most important mechanism by which atherosclerosis leads to the acute ischaemic syndromes of unstable angina, acute myocardial infarction, and sudden cardiac death.[1] This insight has been obtained from clinicopathological studies over many years. These studies have identified a spectrum of alterations involving the plaque surface, including fissuring, erosion, ulceration, and rupture of the plaque surface.[2] All these changes involve disruption of the endothelium and the underlying connective tissue of the plaque capsule. Particular attention has been given to certain types of plaques that are prone to disruption. These "vulnerable plaques" are lipid rich atheromatous plaques that have a thin fibrous capsule.[3] However, the specific mechanisms responsible for plaque weakening and subsequent rupture have not been clearly elucidated. As a result, there has been considerable recent interest in further defining the factors that contribute to plaque instability and disruption.

There is substantial evidence implicating an inflammatory process in the pathogenesis of acute coronary syndromes. Local inflammatory cells can generate and release cytokines that have the potential to activate the endothelium, transforming its natural antiadhesive and anticoagulant properties. Furthermore, inflammatory cytokines may reduce matrix synthesis and increase its degradation, favouring plaque rupture. Finally, cytokines may enhance synthesis of endothelin in endothelial cells and macrophages, resulting in increased smooth muscle cell reactivity to local vasoconstrictors. The evidence supporting this hypothesis that inflammation is critical in the pathogenesis of acute coronary syndromes comes from a variety of sources.

EVIDENCE OF CORONARY ARTERY INFLAMMATION

Histological analysis of atherosclerotic coronary arteries taken from patients who died of acute coronary syndromes has shown that unstable or ruptured atherosclerotic plaques are characterised by the presence of foam cells, macrophages, lymphocytes, and mast cells.[4] Macrophages and to a lesser extent T lymphocytes were the dominant cell type at the site of plaque rupture or erosion. These inflammatory cells are activated, indicating ongoing inflammation at the site of plaque disruption.[5] Moreover, the shoulder regions of eccentric plaques are sites of predilection for active inflammation as is shown by the high number of activated mast cells, an inflammatory cell type that can induce matrix degeneration by release of both tryptase and chymase and can actively contribute to plaque destabilisation preceding rupture.[6] Furthermore, activated mast cells are abundant in the adventitial layer of infarct related coronary vessels and, interestingly, mast cell densities were twofold higher in normal segments of the infarct related artery, suggesting that the entire adventitial layer of the vessel is involved in an inflammatory process.[7] When activated, the mast cells can release histamine as well as other endogenous vasoconstrictors and the resultant coronary spasm can contribute to the clinical syndrome.

Van der Wal and colleagues[5] specifically examined the relation between thrombosis, plaque fissuring, and histological features in coronary atherosclerotic plaques from 20 patients who died of acute myocardial infarction. A deep intimal rupture extending into the lipid core was encountered in 12 cases, whereas in eight the erosions were only superficial. Ten atherosclerotic plaques had a distinctly attenuated fibrous capsule covering a large atheroma, whereas seven had a thick fibrocellular cap overlying a lipid pool, and three were fibrocellular lesions without a clear lipid core. In contrast to the variation in plaque morphology, the cellular composition at the lesion site was found to be more consistent. Macrophages and to a lesser extent T lymphocytes were the dominant cells at the immediate site of rupture or erosion in each case. Furthermore, these sites were always characterised by abundant expression of HLA-DR II antigens on both leucocytes and adjacent smooth muscle cells, suggesting an active inflammatory reaction. The authors concluded that, although both plaque architecture and cellular composition of the underlying atherosclerotic plaque were heterogeneous in complicated coronary artery lesions causing acute myocardial infarction, an active

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Abbreviations: CMV, cytomegalovirus; CRP, C reactive protein; ICAM-1, intercellular adhesion molecule-1; NF-κB, nuclear factor κB; VCAM-1, vascular cell adhesion molecule-1

inflammatory process always marked the site of plaque rupture or erosion. This suggests that inflammation plays a part in destabilising the fibrous cap tissue and thus in enhancing the risk of coronary thrombosis.

The results of postmortem studies of patients who died of acute coronary syndromes have been subsequently confirmed in vivo by studying atherectomy specimens.[8] Immunohistochemical analysis of atherectomy specimens taken from patients with both stable and unstable angina showed an increase in the percentage of interleukin 2 receptor (CD25) positive T lymphocytes in culprit lesions from patients with acute coronary syndromes. The number of atherectomy specimens containing interleukin 2 receptor positive T cells increased with the severity of the ischaemic syndrome: stable angina 52%, stabilised unstable angina 78%, refractory unstable angina 91%, acute myocardial infarction 90%. The percentage of activated T cells (CD25:CD3 ratio) was greater in lesions associated with refractory unstable angina (7.8%) and acute myocardial infarction (18.5%) than in lesions associated with either chronic stable angina (2.2%) or stabilised unstable angina (3.3%). These results indicated recent activation of the immune response within unstable plaques.

Interferon γ released from T cells plays an important part in destabilising atherosclerotic plaques by inhibiting the proliferation of smooth muscle cells and decreasing their synthesis of collagen.[9] Inflammatory cytokines can induce apoptosis of vascular smooth muscle cells in atherosclerotic plaques.[10] Macrophages within atherosclerotic plaques express matrix metalloproteinases, enzymes responsible for the degradation of collagen, and extracellular matrix, which thereby weakens the plaque structure and predisposes it to rupture.[11] Tumour necrosis factor α and interleukin 1 up regulate matrix metalloproteinases in macrophages and this may provide a link between activation of the immune system and plaque rupture.

Many of the genes involved in the inflammatory response that are pivotal in the atherogenic process are activated by the transcription factor nuclear factor κB (NF-κB). NF-κB is also necessary to transcriptionally activate monocyte chemoattractant protein 1 and it vigorously induces metalloproteinase genes.[12] Activated NF-κB has been found in the intima and media of atherosclerotic human vessels and the degree of NF-κB activation correlates with the extent of the atherosclerotic disease process. The role of NF-κB in acute coronary syndromes is further supported by the finding that NF-κB is selectively and highly activated in leucocytes taken from patients with unstable angina.[13] In a recent study 17 of 19 patients with unstable angina had pronounced NF-κB activation whereas only 2 of 83 patients with stable angina had evidence of NF-κB activation. Interestingly, both of these patient groups developed unstable angina within the next 24 hours, a finding that further supports the hypothesis that the inflammatory process is ongoing before the clinical syndrome evolves.

EVIDENCE OF SYSTEMIC INFLAMMATION IN ACUTE CORONARY SYNDROMES

Given the evidence for a localised inflammatory reaction at the site of plaque rupture, other researchers began to investigate the cellular components of the inflammatory response in the systemic circulation of patients presenting with acute coronary syndromes. Mazzone and colleagues[14] reported a transcardiac increase in the surface expression of the CD11b/CD18 integrin receptor in coronary sinus monocytes and granulocytes relative to control samples taken simultaneously from the aortic sinus. These activated leucocytes expressing increased numbers of the CD11b/CD18 integrin receptors interact with activated platelets and endothelial cells. There is also evidence that functional endothelial abnormalities may occur as a result of neutrophil interaction with the endothelium of large coronary arteries in ischaemic heart disease models.[15]

Further investigation into the role of activated leucocytes in acute coronary syndromes has shown that circulating neutrophils in patients with unstable angina and acute myocardial infarction have a low myeloperoxidase content.[16] Myeloperoxidase is the major constituent of primary azurophil granules in neutrophils and is promptly released after activation by various agonists. The resolution of unstable angina is associated with a return of neutrophil myeloperoxidase content to concentrations similar to those in patients with chronic stable angina and controls, suggesting that neutrophil activation was confined to the active phase of unstable angina. The magnitude of neutrophil degranulation was similar in unstable angina and myocardial infarction, suggesting that ischaemia–reperfusion is not the only stimulus responsible for neutrophil activation in acute coronary syndromes. Neutrophil activation may result from inflammatory mediators such as the complement system, aggregated immunoglobulins, or immune complexes or from inflammatory cytokines and fibrin degradation products.[17]

After in vitro exposure to immune or non-immune stimuli, monocytes express tissue factor on their surfaces and can specifically activate the coagulation cascade.[18] Circulating lymphocytes from patients with unstable angina, but not from control subjects or patients with stable angina, can induce tissue factor activity in cultured monocytes.[19] Lymphocyte induced expression of monocyte procoagulant activity appears to be a distinct feature of active unstable angina, as this feature was not detectable in the same patient group 8–12 weeks after the acute ischaemic event. During the acute phase, heparin administration reduced fibrinopeptide A concentrations (metabolite of thrombin) and monocyte procoagulant activity. A more recent study provides evidence that circulating lymphocytes from patients with unstable angina are activated directly and that an immunological reaction precedes the occurrence of unstable ischaemic symptoms.[20] In contrast to patients with stable angina, in patients with unstable angina expression of HLA-D is enhanced in both CD4+ and CD8+ lymphocytes, indicating that T lymphocytes are activated during the acute phase of unstable angina. The activation of T cells is further supported by the increased serum concentration of interleukin 2 receptor among patients with unstable angina.

INFLAMMATORY MARKERS IN ACUTE CORONARY SYNDROMES

The third area of research interest has been the investigation into the expression of inflammatory markers in acute coronary syndromes. Hirsh and colleagues[21] reported that local release of thromboxane B2 into the coronary circulation was associated with recent episodes of ischaemic chest pain in unstable angina. Increased urinary excretion of leukotrienes D4 and E4 was observed in patients with unstable angina. These findings are consistent with a systemically detectable inflammatory component in these patients. Increased concentrations of C reactive protein (CRP) have been reported in unstable angina[22] and in acute myocardial infarction.[23] More recently, Liuzzo and associates[24] have shown that concentrations of CRP and serum amyloid A in unstable angina increase independently of myocardial cell injury, as shown by normal concentrations of creatine kinase and troponin T. The authors also reported that higher concentrations of CRP at the time of hospital admission (> 3.0 mg/l) were predictive of a poor outcome in unstable angina.

The acute phase response of CRP and serum amyloid A is a non-specific phenomenon reflecting cytokine mediated hepatic production triggered by most forms of inflammation, infection, and tissue injury. The principle cytokines driving hepatic production of these acute phase reactants are interleukin 1 and interleukin 6. Concentrations of interleukin 6 are increased in patients with unstable angina and correlate

well with concentrations of CRP, and high concentrations are associated with an adverse prognosis.[25] Large scale epidemiological trials have shown that small rises in baseline CRP concentrations are associated with an increased risk of myocardial infarction and stroke in apparently healthy people.[26 27] Although the evidence is strong in support of CRP as an independent risk factor for ischaemic heart disease, the mechanisms underlying this association are unclear. CRP may simply reflect the ongoing inflammation critical to the development and progression of atherosclerosis. However, recent data suggest a direct pathogenic role for CRP in atherosclerosis. CRP can activate the classic pathway of complement activation by binding C1q and factor H.[28] Within atherosclerotic plaques CRP colocalises with the terminal complement complex.[29] CRP can also induce tissue factor expression by monocytes.[30]

Cell adhesion molecules

The endothelium is a dynamic organ system that maintains blood fluidity by inhibiting coagulation and resisting the adhesion of blood leucocytes through various mechanisms. However, in response to tissue injury, endothelial cells become activated and express cell adhesion molecules that mediate the binding of activated leucocytes and platelets to the endothelial surface. The process of leucocyte binding and extravasation is critically dependent on both selectin and immunoglobulin adhesion molecules on endothelial cells interacting with integrin receptors on leucocytes. Immunohistochemical analysis of human atherosclerotic coronary arteries shows expression of intercellular adhesion molecule-1 (ICAM-1) on endothelial cells, macrophages, and smooth muscle cells within the plaque.[31] Pasceri and colleagues[32] recently reported that CRP ($> 5\ \mu$g/ml) induces the expression of ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), and E selectin in both umbilical vein and coronary artery endothelial cells. This finding further supports the belief that CRP is not merely an inflammatory marker but may directly contribute to the development and evolution of atherosclerosis.

The extracellular portion of these molecules can be enzymatically cleaved and detected in serum and is referred to as soluble cell adhesion molecule. Shyu and colleagues[33] reported increased concentrations of soluble (s) ICAM-1 in patients with acute coronary syndromes. Ikeda and associates[34] found increased concentrations of sP selectin after an episode of chest pain in patients with unstable angina but not in those with stable angina or in control subjects. Concentrations of sICAM-1, sVCAM-1, and sP selectins remain increased throughout the first 72 hours after presentation in unstable angina and non-Q wave myocardial infarction, whereas the concentration of sE selectin falls during this time.[35] There is also evidence that an increased concentration of sVCAM-1 at presentation in unstable angina and non-Q wave myocardial infarction is associated with an adverse outcome. The increase in concentrations of sVCAM-1 provides prognostic information similar to increased concentration of CRP in these patients.[36] These data confirm the critical pathological role of inflammation in acute coronary syndromes and suggest that the intensity of the inflammatory reaction, as shown by increased concentrations of CRP or sVCAM-1, influences clinical outcome.

PERSISTENT INFLAMMATION FOLLOWING ACUTE CORONARY SYNDROMES

Acute coronary syndromes are characterised by persistent instability for weeks to months after the resolution of the clinical symptoms, resulting in recurrent episodes of unstable angina, myocardial infarction, or death.[37] Recent evidence suggests that the inflammatory process persists despite the resolution of clinical symptoms. Biasucci and colleagues[38] reported that serum CRP concentrations remain increased at the time of discharge and at three months' follow up in up to 50% of patients who presented with Braunwald class IIIB unstable angina. This finding of a persistent increase of CRP after an episode of unstable angina was associated with frequent hospital readmission for recurrent instability. Thus, there is a potential link between recurrent ischaemic episodes and persistent inflammatory stimuli. Ault and colleagues[39] have reported that there is evidence of continued activation of platelets in patients after an acute ischaemic coronary event. Platelet associated P selectin is a sensitive measure of platelet activation and platelet P selectin remained increased for up to one month after clinical stabilisation after unstable angina or acute myocardial infarction. This persistent platelet activation may be a consequence of sustained inflammatory stimuli. The authors also found a weak correlation between platelet activation parameters and concentrations of serum CRP. The concept of sustained vascular inflammation following an acute coronary syndrome is further supported by the finding of persistent increases of sICAM-1, sVCAM-1, sE selectin, and sP selectin for up to six months after unstable angina or non-Q wave myocardial infarction.[40]

INFECTION AND CORONARY ARTERY DISEASE

Finally, the potential pathogenic role of infectious agents in the initiation and progression of atherosclerosis remains controversial. The most commonly implicated pathogens are *Chlamydia pneumoniae* and cytomegalovirus (CMV). Original case–control studies found that patients undergoing cardiac surgery had higher titres of CMV antibodies than matched control patients.[41] CMV has also been implicated in restenosis following coronary intervention, with one study finding that 43% of seropositive and only 8% of seronegative patients developed angiographically definable restenosis.[42] The restenosis risk correlated with IgG antibody indicating the potential importance of prior rather than acute CMV infection. Also, cardiac transplant recipients who develop CMV infection more frequently develop graft atherosclerosis.[43] Similarly, small observational studies established a link between *C pneumoniae* seropositivity and acute myocardial infarction and chronic coronary artery disease.[44] It was recently reported that high concentrations of antibodies to human heat shock protein 60 and *C pneumoniae* are independent risk factors for coronary atherosclerosis and their simultaneous presence significantly increases the risk of disease.[45] Both *C pneumoniae* and CMV have been isolated from atherosclerotic lesions.[46 47] Despite abundant seroepidemiological data, the question that remains is whether infectious agents are a cause, a cofactor, or an incidental commensal of no pathological significance in coronary atherosclerosis and acute coronary syndromes.

CONCLUSION

There is extensive evidence to support a pathogenic role for both local and systemic inflammation in acute coronary syndromes. There is also evidence that increased concentrations of inflammatory markers at presentation can identify patients at high risk of future ischaemic events, suggesting that the intensity of the inflammatory response influences clinical outcome in acute coronary syndromes. More recent data suggest that the inflammatory process is sustained long after the clinical event has resolved and that this ongoing inflammation is associated with an increase in subsequent ischaemic events. However, some important questions relating to inflammation in acute coronary syndromes remain unanswered. Firstly, it remains to be elucidated whether the inflammatory process observed is a precursor or a consequence of coronary plaque rupture; the emerging data suggest that the inflammatory process is indeed a precursor of the clinical event. Secondly, is the inflammatory component of unstable coronary disease a potential therapeutic target? Finally, the pathogenic role, if

any, of infectious agents in coronary atherosclerosis and acute coronary syndromes needs to be established.

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