

Mast Cells and Atherosclerosis

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Key words: mast cells, atherosclerosis, inflammation, cytokines, lipoproteins,

IMAJ 2001;3:216-221

At the start of the new millenium, atherosclerosis and its complications such as myocardial infarction and stroke remain the major cause of morbidity and mortality [1]. The lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can be best described as an inflammatory process [2,3].

Recent studies have provided evidence that large numbers of mast cells are found in the various stages of atherosclerotic lesions [4]. It has been proposed that mast cells may play an important role in the development of atherosclerosis due to their ability to interact with lipoproteins, to deliver lipids to macrophages, and to release a large variety of cytokines that affect smooth muscle cells and T lymphocytes. Other mediators released by mast cells such as histamine, serotonin and heparin also have immunoregulatory and immunomodulatory effects relevant to the pathogenesis of atherosclerosis [reviewed in 5]. The role of the mast cell in atherosclerosis – from the early anatomical evidence of its localization in the plaque to its recently described active immunological roles – is discussed in this review.

Atherosclerosis — an inflammatory disease

Atherosclerosis is characterized by a focal, slow and progressive accumulation of cells, extracellular matrix and lipids in the intima of medium-sized and large arteries. The principal atherosclerotic lesions are fatty streaks, fibrous plaques and complicated lesions. The earliest recognizable lesion of atherosclerosis is the so-called fatty streak – an accumulation of lipid-rich macrophages (foam cells) and T lymphocytes within the intima. Fatty streaks develop into the more advanced and complex occlusive lesions, termed fibrous plaques. The cellular composition of the fibrous plaque includes macrophages, smooth muscle cells and activated T lymphocytes.

Data from several studies have shown that atherosclerosis is inflammatory in nature [2,3]. The discovery of mast cells, activated T lymphocytes and macrophages in atherosclerotic lesions, the detection of human leukocyte antigen class II expression, and the finding of local secretion of several cytokines all suggest the involvement of immune and inflammatory mechanisms in the pathogenesis of atherosclerosis [6,7].

According to the “response to injury” hypothesis of

atherosclerosis, endothelial dysfunction is the first step in atherosclerosis. Both endogenous and exogenous factors contribute to endothelial dysfunction. These include genetic predisposition, elevated serum low density lipoprotein levels, hypertension, diabetes mellitus, elevated plasma homocysteine concentration, free radicals caused by cigarette smoking, and infection caused by microorganisms such as herpes viruses or *Chlamydia pneumoniae* [2]. Endothelial dysfunction results in increased adhesiveness of the endothelium to leukocytes and platelets and increased permeability. The injury also induces the endothelium to have procoagulant instead of anticoagulant properties and to form vasoactive molecules, cytokines and growth factors [8]. In trying to remove or neutralize the offending agents, the ongoing inflammatory response stimulates migration and proliferation of smooth muscle cells that become intermixed in the area of inflammation to form an intermediate lesion. This response is mediated mainly by monocyte-derived macrophages and specific subtypes of T lymphocytes. Activation of these cells leads to the release of hydrolytic enzymes, cytokines, chemokines and growth factors that eventually lead to focal necrosis and plaque rupture. The cycle of accumulation and formation of fibrous tissue leads to fibrous cap, which overlies a core of lipid and necrotic tissue – the so-called advanced complicated lesion. At some point the artery can no longer dilate and the lesion may then intrude into the lumen and alter blood flow, leading to the well-known clinical signs and symptoms that result from ischemia to end organs such as heart and brain [1].

It has been established that atherosclerotic lesions contain a large number of T lymphocytes. While half of these cells express major histocompatibility complex class II antigen, some also express interleukin-2 receptors, indicating a state of activation [7]. Recent studies have shown that most of the T lymphocytes are memory CD45+ cells. These T cells are polyclonal in origin and bear the α/β T cell receptor [9]. Activated T cells may contribute to the development of the atherosclerotic lesion by inducing the chemotaxis and activation of different cell types such as mast cells, monocytes, macrophages and smooth muscle cells, as well as B lymphocytes.

Atherosclerotic lesions may contain immunoglobulin and complement component deposits not found in non-atherosclerotic arteries [10]. These include the C5b-9 complex – the end product of the complement cascade – indicating that

complement activation may occur within the atherosclerotic lesion [11]. It has been proposed that two types of antigens, namely oxidized low density lipoprotein and heat shock protein, induce an immune response in these lesions [12,13].

The cell

Mast cells are known to be essential resident effector cells in the elicitation of the allergic response. IgE-sensitized mast cells, on encountering specific antigen that is recognized by surface (FcεRI)-bound IgE, secrete and generate bioactive mediators that facilitate the development of allergic inflammation. Apart from activation by means of FcεRI, mast cells are also activated by cross-linking of surface FcγRIII molecules, anaphylotoxins (C3a, C5a), a range of small peptides including substance P and calcitonin gene-related peptide and β-chemokines, such as macrophage inflammatory protein-1α and monocyte chemoattractant peptide-1 [14]. In addition to classical IgE-mediated allergic responses, mast cells have been found to have a role in a range of other inflammatory reactions. For example, it has been shown that mast cells or their products are pivotal in mediating leukocyte recruitment *in vivo*, play a role in defense against bacterial infections, exert immunoregulatory roles by interacting directly with cells of the adaptive immune system, and present exogenous antigen to T lymphocytes *in vitro* [reviewed in 14]. These effects may be attributed to the wide range of cytokines, vasoactive amines, and other mediators released from mast cells, as well as to direct contact with other immune cells.

Although mast cells in different tissues share many characteristics, they are not a homogenous population. They exhibit histochemical heterogeneity based on the cytoplasmic granule protein content. This was observed in murine mast cells and later on in human mast cells, reflecting not only variation in staining properties of granules in mast cell populations at different anatomic sites but also variations in cell size, cytoplasmic granule ultrastructure, mediator content, sensitivity to stimulation by secretagogues or growth factors, and susceptibility to various pharmacological agents [14].

Mast cells reside at specific locations within the microenvironment, frequently poised strategically in tissues that interface with the external environment and often in proximity to blood vessels and nerves. These cells are capable of reacting, both within minutes and over hours, to a variety of physical, biological and chemical stimuli with either local or systemic effects. Mast cells also react in a coordinated and graded manner that appears to ensure an appropriate response. There is also a more sinister side to these well-engineered residents of the microenvironment – namely, that well-meaning effector mast cell functions may result in annoying to catastrophic allergic reactions and perhaps contribute to the pathology of auto-immune disease. The location of mast cells and the unique extent of their functional diversity no doubt account for the tendency of investigators to implicate mast cells in various

biological responses other than IgE-dependent allergic responses [reviewed in 14].

From LDL to foam cell – the granule carrier pathway

Elevated levels of blood LDL-cholesterol are a risk factor for atherosclerosis. LDL transports cholesterol from the liver to the peripheral cells via interaction with LDL receptors [15]. LDL can be oxidized or modified by endothelial and smooth muscle cells of the arterial wall and by mast cells to form ox-LDL [16]. Ox-LDL is the main source of lipids accumulated in macrophages to form foam cells in atherosclerotic lesions. Both the ox-LDL and other forms of modified LDL (glycation in diabetes, aggregation, association with proteoglycans, incorporation into immune complexes) are the major cause of injury to the endothelium and the underlying smooth muscle.

Modified LDLs are also chemotactic to other cells such as monocytes and granulocytes, and can also up-regulate the expression of genes for macrophage colony-stimulating factor and other chemotactic factors [17]. Mediators of inflammation, some of which are the products of mast cells such as tumor necrosis factor-α, IL-1 and M-CSF, increase the binding of LDL to endothelium and smooth muscle cells and increase the transcription of the LDL-receptor gene [17]. A vicious cycle of inflammation, modification of lipoproteins, and further inflammation can be maintained in the artery by the presence of these lipids.

In contrast to most mammalian cells, mast cells do not have LDL receptors on their surfaces but exert their effect on LDL metabolism by promoting phagocytosis of LDL by macrophages. It has been shown that mast cells can non-specifically bind to and degrade LDL without LDL internalization [18]. However, granule remnants derived from stimulated mast cells can bind a number of LDL particles. Interestingly, stimulated mast cells bind LDL remnants and subsequently internalize these particles. Because mast cells have no lipolytic ability the LDL remnants are not available for nutrition, rendering the cell metabolically inert. In response to this stimulus these lipid-laden mast cells exocytose their lipid-containing granules [1].

In contrast, the apolipoprotein B-100 of LDL can become proteolytically degraded by the enzymes of the mast cell granules. The granules' remnant-bound enzymes are chymase and carboxypeptidase A. Products of these degraded apoB-100, such as histidine, cysteine and tryptophan, have been shown to be capable of forming binding- and redox-inactive complexes with copper ions. The interaction of copper ions with LDL results in LDL oxidation, which can more easily be phagocytosed by macrophages in the arterial intima to form foam cells. The capacity of the exocytosed mast cell granules to carry LDL into macrophages is increased fivefold by the above mentioned

LDL = low density lipoprotein

ox-LDL = oxidized LDL

IL = interleukin

M-CSF = macrophage colony-stimulating factor

Ig = immunoglobulin

chymase activity. This is a result of the capability of heparin proteoglycan-bound chymase to proteolyze the LDL particles. On being proteolyzed, the LDL particles on the granules become unstable and fuse into larger lipid droplets, thus the capacity of each exocytosed granule to bind and carry LDL is increased [1]. It is well established that macrophages have scavenger receptors that mediate the uptake of ox-LDL or modified LDL better than unchanged LDL. Hence the LDL granule remnants released by mast cells can be phagocytosed by macrophages to form foam cells.

Activated mast cells can also metabolize high density lipoprotein by degrading apolipoproteins of HDL such as B48, apolipoprotein E and AI. When HDLs are degraded by mast cells *in situ* in the intima, their ability to induce the cholesterol efflux from macrophage foam cells is destroyed [19]. Thus, mast cells in the intima can influence both cholesterol influx into and efflux from macrophages. Disturbance of this balance may lead to fatty streak formation.

Mast cells and smooth muscle cells

Autopsies have shown that in patients with variant angina the number of mast cells in the adventitia was highest in the spastic coronary segment [20]. Also, the number of degranulated mast cells in the adventitia in affected segments of infarct-related coronary arteries was found to be significantly increased compared with non-affected segments [21]. In contrast to normal arteries where histamine is a potent vasodilator, in atherosclerotic arteries histamine has been found to be a powerful vasoconstrictor [22]. Indeed, after intracoronary injection of histamine, contraction of the coronary arteries was observed in atherosclerotic segments but not in the normal segments of these arteries [22]. Therefore, it may be hypothesized that in patients with myocardial infarction, coronary spasm at sites of plaque rupture is caused partly by local adventitial mast cell-derived histamine.

Histamine also has the ability to specifically bind to its receptors present on smooth muscle cells, resulting in cellular proliferation [23]. Part of the histamine released from the stimulated adventitial mast cells may diffuse directly into the media. The close proximity of mast cells to the vasa vasorum provides another possible route for released histamine to reach the medial layer. The possible functional relationship between mast cell-derived histamine and coronary spasm is also supported by the recent finding that in patients with variant angina the concentration of histamine in the coronary circulation was elevated shortly before coronary spasm [24]. In atherosclerotic coronary segments, smooth muscle contraction in response to histamine is likely to be vigorous, affecting the damaged endothelium that has lost its opposing vasodilatory capacity.

Most mast cells in the coronary adventitia contain not only tryptase but also chymase. Chymase is also known to convert

angiotensin I into angiotensin II. Angiotensin II receptors have been found on the medial smooth muscle cells of human coronary arteries. Thus, angiotensin II generated by chymase released from mast cells can act synergistically with histamine and augment the local constriction of the infarct-related coronary artery [25]. Activation of vascular chymase also plays a major role in myointimal hypertrophy after vascular injury by augmenting the production of angiotensin II. Transilat, a mast cell-stabilizing agent, was shown to down-regulate the chymase-dependent angiotensin II formation. Transilat was also found to suppress chymase gene expression, which was specifically activated in injured arterites and thereby prevented neointimal formation [26].

Smooth muscle accumulation in the arterial intima is a key event in the development of the atherosclerotic lesion, resulting from a combination of proliferation and direct migration of arterial smooth muscle cells from the media into the intima. Both proliferation and cell migration can be induced by cytokines and growth factors, such as fibroblast growth factor, platelet-activating factor and transforming growth factor- β , which are believed to be produced by mast cells within the arterial wall [27]. Thus, cytokines released by mast cells may be the molecular link between mast cells and smooth muscle cell proliferation in atherosclerosis. Mast cells may also contribute to the development of ischemic events by leading to micro-thrombi formation due to the effect of certain mediators such as PAF [28].

Rupture of the plaque

Rupture of a coronary plaque typically occurs at the site where inflammatory cells have accumulated [21]. Mast cells have recently been shown to accumulate at the site of coronary ruptured plaques associated with acute myocardial infarction [29]. In the normal coronary artery the majority of mast cells are found in the outer layer of the adventitia. However, mast cells appear in highest density in the shoulder regions of atheromas, which are prone to erosion and rupture, but are scarce in the fibrous cap of the lesion and in unaffected intima. The majority of mast cells in the shoulder regions of the atheroma contain tryptase and chymase whose enzymatic activity may contribute to matrix degradation leading to rupture of the plaque [21]. In addition, cytokines such as TNF- α , IL-5 and IL-8, which are produced by mast cells, appear also to exert an independent effect on collagen lysis [29].

Once the advanced stage of the atheroma has been reached, invasion of microvessels occurs. These microvessels appear to play a role in the supply of blood components to the plaque. It has been suggested that the microvessels tend to rupture, causing sudden hemorrhage inside the atheroma. This may be the final and often fatal event of atherogenesis. Mast cells may contribute to this process since they possess significant angiogenic properties [reviewed in 5]. Indeed, Kaartinen et al.

HDL = high density lipoprotein

PAF = platelet-activating factor

TNF- α = tumor necrosis factor-alpha

[30] demonstrated the selective localization of activated mast cells containing angiogenic factors around newly formed microvessels in human coronary atheromas, suggesting that these cells play a role in the neovascularization of these lesions. Moreover, by virtue of their neutral proteases, mast cells may also injure the microvessels and thereby produce intraplaque hemorrhage and, ultimately, unstable lesions [30]. Matrix-degrading metalloproteinases were found to be activated by mast cell proteases in atherosclerotic plaques [31]. However, since no tissue inhibitors of metalloproteinases were found in atherosclerotic plaque, it is believed that its activation may be an important mechanism in atherosclerotic plaque destabilization and rupture.

T cell–mast cell interaction

As mentioned previously, T cells may have an important role in the development of the atherosclerotic lesion. Mast cells have also been found to affect T cell function [14]. In addition to being major effector cells in the elicitation of allergic inflammation, mast cells are activated in various T cell-mediated inflammatory processes and reside in close physical proximity to T cells. Such observations and the wide spectrum of mediators produced and secreted by mast cells have led investigators to propose a functional relationship between these two cell populations. Indeed, mast cell activation has been reported to induce T cell migration either directly by the release of chemotactic factors such as lymphotactin or IL-16, or indirectly by the induction of adhesion molecule expression on endothelial cells. Mast cells are also able to present antigens to T cells, resulting in their activation in either a MHC class I- or class II-restricted and co-stimulatory molecule-dependent fashion. Adhesion molecule-dependent intercellular contact or MHC class II cognate interactions between T cells and mast cells results in the release of both granule-associated mediators and cytokines from the latter. Also, T cell-derived mediators such as β -chemokines directly induce mast cell degranulation. On the other hand, mast cell-derived cytokines such as IL-4 and IL-13 have been found to polarize T cells to preferentially differentiate into Th2 subset. Thus, T cell–mast cell interactions are bidirectional, fulfilling regulatory and/or modulatory roles that affect various aspects of the immune response [reviewed in 14].

The protective role

The hypothesis of a connection between mast cells and atherosclerosis was first put forward by Constantinides [32] who suggested that arterial mast cells, being a source of endogenous heparin, normally protect the arterial wall from atherosclerosis. Because mast cells disappear from very advanced lesions, this protective role is lost and atherogenesis is able to progress. In addition to heparin, mast cells are a source of tissue plasminogen activator with *in vitro* ability to induce

fibrinolysis [33]. The question that arises is whether mast cells have a role in endogenous thrombolysis, thus preventing ischemic events, or whether they play a key role in inducing atherosclerosis.

Convincing proof for the importance of mast cells in specific immunological or pathological responses *in vivo* has been difficult to obtain. There are several reasons for this. Many mast cell-associated mediators are produced by other cell types. As a result, evidence that one of these mediators has a critical role in a particular biological response is not in itself sufficient proof that mast cells are important in that response. A similar argument may be made about pharmacological experiments employing antagonists of specific mast cell-associated mediators or inhibitors of mast cell activation. As valuable as this approach is for evaluating the function of each individual mast cell mediator, resulting data may provide incomplete or, in some cases, misleading information on the role of the intact mast cell since other target cells as well as mast cells may be implicated. Further complicating the analysis of mast cell functions *in vivo* is that mast cell activation results in the elaboration of several mediators that may have diverse and sometimes opposing biological effects. It is therefore difficult to predict the net effect of mast cell degranulation. Finally, mast cell heterogeneity, which includes variations not only in morphology but also in mediator content and response to secretagogues and drugs, makes it difficult to offer succinct generalizations about mast cell function in biological responses [5].

It has been argued that atopic patients, who are prone to IgE-mediated mast cell activation, are protected against sudden cardiac death following myocardial infarction [34]. Their hemostatic balance is similar to that induced by aspirin. Asthmatic patients have prolonged bleeding time, depressed platelet aggregability, and delayed generation of thrombin *in vitro* [35]. It follows that the formation of thrombus in the coronary of these patients would be delayed. Interestingly however, autopsies of patients who died of acute asthmatic attack revealed increased circulating levels of heparin in the blood of atopic patients and lack of coronary atherosclerosis. It has been shown that heparin proteoglycans released from rat serosal mast cells inhibited proliferation of rat aortic smooth muscle cells in culture. Thus, by releasing heparin, mast cells may inhibit the proliferative and destructive role of smooth muscle cells in the atheroma [36].

Mast cell and myocardial injury

As early as 4 hours after myocardial infarction neutrophilic infiltrate can be found between myocardial fibers. In the coagulative necrosis stage, which takes place 2 to 3 days after myocardial infarction, neutrophils are the dominant cell type that plays a role in the inflammatory process. Neutrophils by virtue of their granules have a potent destructive ability. Both neutral and acid hydrolases, which degrade protein, can be found in the primary granules. The secondary granules store lysosomal enzymes and collagenase with the capacity to degrade

MHC = major histocompatibility complex

connective tissue. These destructive elements are used not only against bacteria and foreign particles, but also against self-injured cell and necrotic tissue such as myocytes in ischemic hearts.

It is reasonable to assume that C5a may activate cardiac mast cells in the context of active ischemia [37]. C5a is one of the most important chemoattractant factors produced during acute inflammation. In addition to attracting neutrophils to the site of complement activation, this peptide also enhances neutrophil adhesiveness to endothelial cells. C5a is also known to induce degranulation of cardiac mast cells and is present exclusively in the area surrounding the myocardial injury before initiation of reperfusion [38]. Alternatively, adenosine has been shown to induce mast cell degranulation through the A3 receptor and would be expected to increase in an ischemic area [39]. Reactive oxygen has also been shown to induce mast cell degranulation and might be an important factor in early reperfusion when production of reactive oxygen is highest [40].

A growing body of evidence supports a role for mast cells in neutrophil recruitment and neutrophil adhesiveness to myocytes associated with ischemia and reperfusion [41]. Neutrophil-induced cardiomyocyte injury requires the expression of myocyte intercellular adhesion molecule-1. This is an essential step necessary for neutrophils to migrate and to recognize the myocyte by the adhesion process [14]. IL-6 is the primary stimulus for myocyte ICAM-1 induction [42]. It has been demonstrated that IL-6 concentration is elevated in post-ischemic cardiac lymph. Also, IL-6 mRNA expression is induced early in reperfusion of the infarcted myocardium [42]. Cardiac mast cells degranulate after myocardial ischemia, releasing preformed mediators such as histamine and TNF- α or newly synthesized cytokines such as IL-6 [43]. Mast cell-derived TNF- α may be a crucial factor in up-regulating the expression and release of IL-6 by infiltrating neutrophils, thus initiating the cytokine cascade responsible for myocyte ICAM-1 induction and subsequent neutrophil-induced injury [44].

Mast cells have been found to induce apoptosis of cardiomyocytes via the activity of mast cell chymase 1 *in vitro* [45]. Apoptosis of myocytes may lead to cardiac failure and to the loss of muscle mass. Chymase concentration was found to be elevated as early as 6 hours after myocardial infarction, mainly in the healing scar region [46]. Chymase derived from mast cells has the ability to convert angiotensin I to angiotensin II in a non-converting enzyme-dependent fashion. The enhanced intracardiac angiotensin II formation leads to coronary constriction, impaired diastolic relaxation, myocyte enlargement and interstitial fibrosis, all of which aggravate cardiac function [47].

The clinical significance of many of these new findings is yet to be established. However, this work clearly supports a complex view of the pathogenesis of atherosclerosis, namely, that mast cells alongside other cells may have both effector and/or modulatory roles in this disorder.

ICAM = intercellular adhesion molecule

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