Mast cells and degradation of pericellular and extracellular matrices: potential contributions to erosion, rupture and intraplaque haemorrhage of atherosclerotic plaques

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Abstract

Mast cells are present in advanced human atherosclerotic plaques, where they are thought to exert multiple effects on their neighbouring cells and on the extracellular matrix of the plaque. Extensive efforts at delineating their role(s) in atherosclerotic plaques have unravelled mechanisms by which plaque mast cells may render advanced atherosclerotic plaques susceptible to erosion, rupture or intraplaque haemorrhage and so modulate their stability. In these mechanisms, the key effector molecules are mast-cell-derived neutral proteases and pro-inflammatory cytokines. These effector molecules are synthesized and stored in the cytoplasmic secretory granules of mast cells and, once the mast cells are activated to degranulate, are released into the microenvironment surrounding the activated mast cells. In the plaques, the key target cells are endothelial cells and smooth muscle cells and their pericellular matrices. In addition, the various components of the extracellular matrix of the plaques, notably collagen, are degraded when the released mast cell proteases activate matrix metalloproteinases in the plaques. By rendering the plaque susceptible to erosion, to rupture or to intraplaque haemorrhage, the mast cells may contribute to the onset of acute atherothrombotic complications of coronary atherosclerosis, such as myocardial infarction.

Introduction

Atherosclerosis is a disease of the inner layer of the arterial wall, the intima. When blood-derived lipoprotein lipids, notably cholesterol, start to accumulate in the intima, the long-lasting process of atherogenesis is initiated with the ultimate development of atherosclerotic plaques [1]. Atherosclerosis of epicardial coronary arteries and their major branches is the underlying cause of ischaemic heart disease. Acute coronary syndromes usually result from an occluding thrombus of the culprit coronary artery and clinically manifest themselves as unstable angina, acute myocardial infarction or sudden cardiac death. The most common cause of acute coronary thrombosis is plaque rupture (in approx. 75%), notably the rupture of a thin-cap fibroatheroma [2]. These plaques typically cause only minor stenosis of the coronary lumen and are morphologically characterized by a large lipid core which is covered by a thin fibrous cap. Such vulnerable plaques often contain a large network of neovascular sprouts located deep under the lipid core. The residual 25% of acute coronary thromboses are caused by superficial erosion of the coronary plaque [2].

The central role of inflammation in atherogenesis has gained wide acceptance and has forced us to re-evaluate our conception of all phases of this long-lasting disease [3]. Among the inflammatory cells in the plaques, i.e. macrophages, T-lymphocytes and mast cells, the spotlight has turned to mast cells much later than to the two other types of inflammatory cell [4]. Indeed, the plaque mast cells were rediscovered in the early 1990s and, by aid of the contemporary techniques of mast cell biology, these cells were assigned a role in the development of human atherosclerotic plaques and their clinical complications [5].

Mast cells: multipotent effector cells in inflammation

The mast cells are multipotent effector cells which originate in the bone marrow, circulate as progenitor cells and ultimately find their ways into the various tissues in the body [6]. A role for the chemokine eotaxin and its receptor, chemokine receptor 3, in the recruitment of mast cell progenitors from the circulation to human atherosclerotic plaques was postulated some years ago [7]. Like in other tissues, also in atherosclerotic plaques, the mast cell progenitors remain in the close vicinity of endothelial cells, where they slowly differentiate into mature mast cells filled with cytoplasmic secretory granules. The granules in all plaque mast cells contain histamine and the neutral serine protease tryptase, both bound to a heparin proteoglycan matrix. A variable
Interestingly, a fraction of TNF-α remains heparin-bound after secretion by activated mast cells. In the intimal extracellular fluid [13]. The strong affinity secreted in a fully active form and retain some of their activity is restricted. Importantly, the heparin-bound proteases are with histamine, granule proteases remain heparin-bound augmented and coronary spasm may ensue [12]. In contrast human coronary segment, the vascular tone in the segment is smooth muscle cells in the medial layer of an atherosclerotic lipoproteins becomes augmented [11] and, when it acts on acts on luminal endothelial cells, vascular permeability to and easily reaches the neighbouring cells. When histamine granule heparin and, as a small molecule, it diffuses away mast cells degranulate and exocytose the granule-associated effector substances into their microenvironment.

In the extracellular fluid, histamine is detached from granule heparin and, as a small molecule, it diffuses away and easily reaches the neighbouring cells. When histamine acts on luminal endothelial cells, vascular permeability to lipoproteins becomes augmented [11] and, when it acts on smooth muscle cells in the medial layer of an atherosclerotic human coronary segment, the vascular tone in the segment is augmented and coronary spasm may ensue [12]. In contrast with histamine, granule proteases remain heparin-bound after secretion. Thus their convection in the extracellular fluid is restricted. Importantly, the heparin-bound proteases are secreted in a fully active form and retain some of their activity in the intimal extracellular fluid [13]. The strong affinity of bFGF to heparin suggests that this growth factor also remains heparin-bound after secretion by activated mast cells. Interestingly, a fraction of TNF-α remains heparin-bound, while another fraction is released from heparin [14].

We have observed that the mast cells in the atherosclerotic plaques are surrounded by exocytosed granules (which we call granule remnants). Indeed, our method of analysing mast cell activation and degranulation in situ is based on this observation. Using this criterion of mast cell activation, we have observed that, in advanced atherosclerotic lesions, the fraction of degranulated mast cells is increased as a sign of active inflammation [15]. Classically, mast cell degranulation has been described in the setting of an acute anaphylactic reaction. In this type of mast cell activation, exogenous allergens bind to allergen-specific IgE molecules attached to IgE receptors on the surface of mast cells which are present in the outer surfaces of the body, i.e. the various mucosal tissues and the skin. Since it is unlikely that exogenous allergens ever reach atherosclerotic plaques, some other mechanisms of mast cell activation with ensuing degranulation must exist in coronary atherosclerotic plaques. Similarly to other sites of chronic inflammation (e.g. rheumatoid joints), plaque mast cells may become activated by neighbouring macrophages and T-lymphocytes. Moreover, atherosclerotic plaques contain various substances that may potentially activate mast cells, such as immune complexes and the complement-derived anaphylatoxin C5a. Since the mast cells in human coronary plaques express the receptor for C5a, it is likely that this activated complement is one of the mast-cell-activating systems in coronary atherosclerotic plaques [16,17]. Of particular interest is the observation that lipoproteins may also activate mast cells [18]. Indeed, since atherogenesis is a lipoprotein-dependent disease, this mode of mast cell activation, if present in human lesions, would be unique for atherosclerosis.

The simple immunohistochemical observation of mast cell activation, i.e. detection of extracellularly located granules in the vicinity of a mast cell, is unable to show the full potential of mast cells as effector cells in atherosclerotic plaques. Thus, in chronically inflamed tissues, such as atherosclerotic plaques, the mast cells are likely to undergo ‘piecemeal’ degranulation in which stored granule components are released in submicron vesicles [19] and are likely also to continuously release small quantities of de novo synthesized pro-inflammatory lipid mediators, growth factors, cytokines and chemokines [6].

On the basis of the above considerations, the influence of activated mast cells in human atherosclerotic plaques must be restricted to their immediate surroundings (Figure 1). Thus the location of mast cells in the plaques is of critical importance. As stated above, mast cells are located in the close vicinity of endothelial cells. In advanced atherosclerotic plaques, there are two regions in which mast cells may reside in the close vicinity of endothelial cells: (i) the superficial location next to the luminal endothelium; and (ii) the deep location next to the endothelial cells of newly formed microvessels. When activated, mast cells may contribute to the loss of endothelial integrity at either location.

**Potential mechanisms by which mast cells induce death or detachment of endothelial cells in atherosclerotic plaques**

The mechanisms leading to the loss of endothelial cells in human coronary plaques, i.e. to plaque erosion, remain enigmatic [20]. Apoptosis of endothelial cells has been suggested as one possible mechanism. Since endothelial cells are very resistant to apoptosis, specific pathological conditions are required in which subendothelially derived biochemical agents uniquely combine with extrinsic factors consisting of haemodynamic disturbances and various blood plasma-derived factors. In terms of the subendothelial factors, increased numbers of degranulated mast cells are present in the eroded (de-endothelialized) human coronary atheromas [8]. By secreting the strongly pro-apoptotic TNF-α, subendothelial mast cells may participate in the process of endothelial apoptosis [21]. In addition, proteolytic cleavage of the various components of the pericellular matrix of endothelial cells by mast-cell-derived proteases is likely to block the pericellular-matrix-mediated outside-in survival signalling and thus predisposes the endothelial cells to apoptotic death in concert with TNF-α. Indeed, we have observed recently that trypstatin, chymase and cathepsin G are capable of degrading vascular endothelial cadherin, a molecule involved in the outside-in survival signalling of endothelial cells [8]. Finally, proteolytic degradation of the endothelial basement membrane also loosens the attachment of endothelial cells to the wall of the atherosclerotic plaque and predisposes them to desquamation in areas of excessive shear stress and turbulent flow [8].

In plaque erosion, loss of the endothelial surface exposes pro-thrombogenic subendothelial matrix tissue to circulating blood, with ensuing local formation of a platelet-rich arterial thrombus. The ensuing wound-healing process leads to formation of a thicker and more stable fibrous cap when
**Figure 1** Schematic representation illustrating different protease-dependent actions proposed for mast cells in human atherosclerotic plaques

An activated mast cell is shown on the left in the subendothelial space of atherosclerotic arterial intima. The cytoplasm of the mast cell is filled with granules, which contain three neutral proteases, i.e. tryptase, chymase and cathepsin G (black dots) bound to the heparin proteoglycan matrix (dark blue) of the granules. The activated mast cell exocytoses some of its cytoplasmic secretory granules into the intimal fluid. In the intimal fluid, the granules lose their histamine, whereas the proteases remain heparin-bound. In the granule remnants formed, the heparin-bound proteases are resistant to inactivation and remain partially active. Four different mast cell protease-dependent actions are shown.

1. **Proteolytic degradation of fibronectin and vitronectin in the subendothelial basement membrane results in loss of outside-in survival signalling in the affected endothelial cells, which contributes to their apoptosis and detachment.** The resulting erosion of plaque exposes prothrombotic subendothelial surface and triggers local formation of an arterial platelet-rich thrombus (not shown). The growth factors released by activated platelets stimulate smooth muscle cells to grow and secrete collagen and thus contribute to stenotic thickening of the plaque.

2. **Proteolytic degradation of the fibronectin component of the pericellular matrix of subendothelial smooth muscle cells results in their apoptotic death.** The loss of plaque smooth muscle cells reduces local production of collagen and so contributes to thinning, weakening and ultimately to rupture of the plaque.

3. **Protease-dependent activation of the inactive proforms of matrix metalloproteinases (proMMPs).** The activated MMPs, again, degrade various extracellular components of the plaque, notably collagen and so contribute to plaque weakening.

4. **Proteolytic degradation of the thin basement membrane of a fragile neovessel present deeper in the plaque leads to endothelial apoptosis and rupture of the microvessel.** The resulting intraplaque microhaemorrhage tends to weaken the plaque and render it more susceptible to rupture.

Activated platelets stimulate the subendothelially located smooth muscle cells to proliferate [22]. Thus, by triggering endothelial loss, the mast cells actually tend to indirectly (i.e. via platelet activation) stabilize plaques, but at the price of inward plaque growth and increasing stenotic narrowing of the coronary lumen. This, again, leads to increased turbulence of flow and to a *circulus vitiosus* in which repetitive erosions may ultimately precipitate an occlusive thrombus.

The arterial intima is a peculiar tissue in that it lacks a capillary circulation. This is the major reason for the lack of mast cells in the deep intimal layers. In contrast with the intima, the outer layer of the arterial wall, the adventitial layer, is filled with tiny vessels, the *vasa vasorum*, which are surrounded by an abundance of mast cells [23]. In the advanced stage of atherosclerotic plaque development, invasion of adventitial microvessels frequently occurs in the deep hypoxic regions of the plaque [24], and these plaque microvessels are then accompanied by mast cells [25]. The mast cells located near the microvessels contain bFGF, a potent pro-angiogenic factor, and are likely to contribute to the growth of the neovascular sprouts [9]. Indeed, the mast cell is a pro-angiogenic cell par excellence. We also hypothesized that, by secreting neutral proteases with a potential to degrade the subendothelial matrix, the perivascularly located mast cells may injure the
fragile microvessels and play a role in microvascular leakage [25]. Such microvessel damage then produces intraplaque haemorrhages and ultimately contributes to the generation of unstable lesions [26].

Potential mechanisms by which mast cells induce loss of smooth muscle cells and of extracellular matrix in atherosclerotic plaques

The stability of advanced coronary plaques critically depends on the tensile strength of the fibrous cap covering the necrotic lipid core of the plaque [27]. The strength of the fibrous cap, again, depends on its thickness and collagen content. Since only smooth muscle cells are producing collagen in the cap, their survival and wellbeing is vital for plaque stability. Experiments with cultured smooth muscle cells have revealed that activated mast cells can induce apoptotic death of smooth muscle cells [28]. Notably, secreted chymase degrades the pericellularly located fibronectin which results in loss of outside-in signalling with ensuing apoptotic death of the smooth muscle cells [29]. Indeed, caps of ruptured human coronary plaques contain increased numbers of mast cells and reduced numbers of smooth muscle cells [30]. Moreover, chymase from exocytosed mast cell granules can also inhibit collagen synthesis in cultured smooth muscle cells by TGF-β (transforming growth factor β)-dependent and -independent mechanisms [31].

Mast cells can also contribute to the matrix degradation when their secreted neutral proteases activate MMPs (matrix metalloproteinases). Such activation is necessary because, in contrast with the mast-cell-derived neutral proteases, the MMPs are secreted as zymogens, i.e. as inactive pro-enzymes. One of these matrix-degrading enzymes is the interstitial collagenase MMP-1, which is also found in atherosclerotic lesions. We found that human chymase effectively activates pro-MMP-1 [32], and studies in other laboratories have shown that tryptase can activate prostromelysin (pro-MMP-1) [33], and studies in other laboratories have shown that tryptase can activate prostromelysin (pro-MMP-1) [32], and studies in other laboratories have shown that tryptase can activate prostromelysin (pro-MMP-1) [32].

As nicely illuminated by Johnson et al. [34], mast cell proteases seem to be able to activate MMPs in situ in human atherosclerotic plaques. These workers incubated small pieces prepared from freshly obtained carotid endarterectomy specimens with a synthetic mast-cell-degranulating agent and observed an increase in the activity of mast cell tryptase in the incubation medium and also an increase in MMP activity. This ex vivo study showed that degranulation of mast cells in an atherosclerotic plaque may activate the MMPs in the lesion.

In summary, the experiments have revealed several mechanisms by which activated mast cells could weaken the fibrous cap of an atherosclerotic plaque (Figure 1). They include (i) inhibition of collagen production by either down-regulating collagen synthesis in smooth muscle cells or by actually killing these collagen-producing cells, and (ii) degradation of extracellular collagen bundles. All of these effects would reduce the tensile strength of the cap and so increase the risk of plaque rupture.

Conclusion and future challenges

Most of the findings described in this summary are based on observations obtained in immunohistochemical studies using human tissues, and in experiments performed ex vivo, i.e. the experimentation has been performed using living tissue or cultured cells in an artificial environment outside the organism. Moreover, in the experiments, rodent (rat) mast cells, rather than human mast cells, were used. Nevertheless, the experiments have shed some light on possible mechanisms by which mast cells, by being multipotent effector cells, may intervene in the course of atherogenesis. Most importantly, the experiments have aided in adopting the mast cell as a member of the trio of pro-inflammatory cells in atherogenesis, the macrophage, T-lymphocyte and mast cell.

The various approaches described in this short summary are parts of a hypothesis-generating mast cell project. As stated very recently by Libby and Shi [4], “mast cell biology in atherosclerosis has now (this year) gone beyond the descriptive, observational phase.” This statement refers to the recent hypothesis-testing work, which has been performed in living animals. Thus Bot et al. [35] performed pharmacological experiments in mice that implicate mast cells in intraplaque haemorrhage, macrophage apoptosis, increased vascular permeability and recruitment of further leucocytes to atherosclerotic plaques. Another example of a rigorous in vivo testing of the potential role of mast cells in atherogenesis is the work by Sun et al. [36]. They adoptively transferred mast cells from mice deficient in various cytokines to reconstitute other mice genetically lacking in mast cells. Their observations established direct participation of mast cells and mast cell-derived IL-6 (interleukin 6) and IFN-γ (interferon γ) in mouse atherogenesis, providing new mechanistic insight into the pathogenesis of atherogenesis.

The experimental usefulness of the described mouse models is remarkable, and the testing is rigorous considering the many counter-regulatory protective and redundant mechanisms present in vivo, but absent ex vivo. Obviously, this phase of research is an obligatory step towards a better definition of the role of mast cells in atherogenesis. The suggested availability of murine models of plaque rupture offers a great opportunity to evaluate at least some well-defined aspects of mast cell contribution in the late events of atherosclerosis [37]. Yet, in our extrapolations to human disease, we need to be cautious. For example, whereas human mast cells express only one form of chymase, mouse mast cells express several chymases [38]. The multiplicity of these proteolytic mast cell effectors in the mouse may reflect a particular role for them in host defence against bacteria. Also, some of the rodent chymases, although phylogenetically closely related to human chymase, have acquired a catalytic domain mutation that fundamentally changes specificity: namely from chymotryptic to elastolytic. Indeed, a cautious word was given by Bischoff [39], who recently summarized data on mast
cells and emphasized that most mast cell data derive solely from experiments in mice or rats, two species that obviously never suffer from allergic or other mast-cell-associated human diseases. Regarding experimental atherogenesis, despite the many studies of murine atherosclerosis, we do not yet know the relevance of the natural history of this model to the final events precipitated by erosion or rupture of human atherosclerotic plaques [40].

Nevertheless, the increasing knowledge of the molecular pathways that operate in atherogenesis provides us novel therapeutic targets, the activated mast cell being one such target. The ability of the mast cell stabilizer cromolyn to prevent all the adverse phenomena elicited by mast cell activation in mouse atherosclerosis, provides a promising example [35]. We certainly agree with the authors of this paper when they propose mast cell stabilization as a promising new therapeutic modality in the prevention of acute coronary syndromes which are caused by erosion or rupture of a coronary atheroma.

References

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