Mast cells in atherosclerosis

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Summary

The mast cell, a potent inflammatory cell type, is widely distributed over several tissues, but particularly prominent at the interface exposed to the environment to act in the first line of defense against pathogens. Upon activation mast cells release granules, which contain a large panel of mediators, including neutral proteases (e.g. chymase and tryptase), cathepsins, heparin, histamine and a variety of cytokines and growth factors. While mast cells have been demonstrated to be critically involved in a number of Th2 dominated diseases such as asthma and allergy, recent investigations have now also implicated mast cells in the pathogenesis of atherosclerosis and acute cardiovascular syndromes. In this review, we will discuss the contribution of mast cells to the initiation and progression of atherosclerosis and gauge the therapeutic opportunities of mast cell targeted intervention in acute cardiovascular syndromes.

Keywords

Atherosclerosis, mast cell, plaque stability, proteases, cytokines, acute cardiovascular syndromes

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Introduction

The mast cell is a highly potent inflammatory cell type; it was described first in 1876 by Paul Ehrlich who designated it the “Mastzelle”, referring to the prominent presence of basophilic storage granules, which he presumed to host tissue fertilisers (1–3). These granules in fact contain a whole array of mediators, such as the neutral proteases chymase and tryptase, the cathepsins, histamine, heparin, a large number of cytokines and chemokines such as tumour necrosis factor α (TNFα), interleukins (IL) and growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) (4). Mast cells participate in the first line of defense against pathogens such as bacteria and parasites, and release their granules after activation of dedicated mast cell receptors, among which the Toll-like receptors and immunoglobulin receptors. However, in some immune disorders such as allergies and asthma mast cells can display an exaggerated response to stimuli.

Mast cells derive from bone marrow cells and circulate in the peripheral blood as mast cell precursors to be recruited to specific tissues and organs such as lung and skin where they mature into mast cells. Mast cells also reside in the vessel wall, particularly in perivascular tissue, as well as in the heart, which may hint to a role of mast cells in pathogenesis that affect these tissues, such as atherosclerosis, the major underlying cause of acute cardiovascular syndromes (e.g. myocardial infarction and stroke). Atherosclerosis is currently viewed as a progressive lipid driven chronic inflammatory process. The inflammatory response involves monocytes, macrophages, T-lymphocytes, neutrophils and dendritic cells (5–7). As disease progresses, a deposit of lipid rich cell debris will form in the atherosclerotic lesion, overlaid by a fibrous smooth muscle cell rich cap, which may erode to become prone to rupture (8). In the final stage of the disease, upon rupture or erosion of the plaque, the highly thrombogenic contents of the necrotic lipid core will come in contact with the peripheral blood, thereby initiating a thrombo-coagulant response. Thrombosis and coagulation will conspire to the escalation of disease, which results in partial or entire ischaemic arterial occlusion, also referred to as acute cardiovascular syndromes (8). As mast cells are particularly abundant in diseased arteries (9–11) and have the capacity to release a wide variety of tissue remodelling and inflammatory mediators upon activation, these cells could well be part of the ongoing pro-inflammatory reaction that culminates in acute cardiovascular syndromes. This notion is based on a number of in vitro and human pathology studies, but direct experimental proof for a contributory role of mast cells in cardiovascular diseases has long been lacking. Recently, a series of in vivo studies by various groups have provided more compelling evidence for such a role. In this review, we will summarise current knowledge on the contribution of mast cells to atherosclerosis and acute cardiovascular syndromes as obtained from patient studies and in experimental models of disease.
Mast cells in atherosclerosis: pathology and patient studies

The first papers to describe a role for mast cells in cardiovascular diseases date back to the early 1950s (12, 13). Mast cells were identified in heart tissue of animals and in patients with clinical atherosclerosis. Myocardial tissue from these patients was seen to display reduced mast cell numbers as compared to non-diseased controls. Combined with the prominent presence of heparin in mast cell granules, this observation led the authors to attribute atheroprotective activity to mast cells. In fact, this is one of few papers proposing a protective role for mast cells in cardiovascular diseases. In the following years, paradigm gradually shifted towards a proatherogenic function of this cell type. For example, studies by the group of Kovanen et al. demonstrated in human coronary artery specimen that mast cell numbers, both in the intima and perivascular tissue (adventitia) of atherosclerotic plaques increased with disease progression (9–11, 14). These findings were corroborated for human aorta and coronary arteries by Atkinson et al. (15) as well as for carotid artery tissue by Jeziorska et al. (16). Two distinct mast cell phenotypes could be observed in the plaque intima: a tryptase positive and a tryptase/chymase-positive subset (10). In a subsequent study Kaartinen et al. could show that cytoplasmic secretory mast cell granules in the intima were associated with apoB-100-containing lipoproteins. Moreover, these granules were found to be ingested by foam cells and smooth muscle cells (17), suggesting a role of mast cells in foam cell formation and thus plaque expansion. Further support for a deleterious role came from studies showing co-localisation of mast cells, expressing the pro-angiogenic factor bFGF, with intraplaque neovessels (18–20). The studies showing co-localisation of mast cells, expressing the pro-angiogenic factor bFGF, with intraplaque neovessels (18–20). The authors hypothesised that by releasing the vasoactive agent histamine, mast cells can induce leakage of these microvessels, paving the way for intraplaque haemorrhage and subsequent plaque destabilisation.

Human coronary artery specimen analysis also revealed subendothelial mast cells in close proximity to microthrombi, suggesting that activated mast cells also could contribute to plaque erosion (21). According to the authors, mast cell proteases tryptase and chymase as well as cathepsin G had, at least in vitro, the capacity to induce endothelial damage. While providing a plausible mechanistic foundation for the adverse effects of plaque mast cells, the above pathology studies leave unaddressed what causes the progressive mast cell accumulation and activation at later stages of plaque progression. Interestingly, adventitial mast cells in advanced lesion increasingly co-localise with nerve fibers (22) and a direct correlation has thus been suggested between neuronal factors such as nerve growth factor (NGF), mast cells and atherosclerosis (23). From these studies it was proposed that neurogenic stimulation of mast cells in perivascular coronary artery tissue may lead to plaque destabilisation.

Not only human pathology but also epidemiology studies have linked mast cells to cardiovascular diseases. Levels of immunoglobulin E (IgE), one of the major mast cell activators, were elevated in patients with unstable angina pectoris (24), but also in dyslipidaemic men (25). Recently, blood lipid levels were correlated to basophils and mast cells by genomic profiling of whole blood (26). A gene cluster was identified, the so-called lipid leukocyte module, that showed co-regulation with blood lipid levels; in particular histidine decarboxylase and the alpha subunit from the Fc part of the IgE receptor were demonstrated to associate with ApoB, high-density lipoprotein (HDL) and triglyceride levels. This direct connection between mast cells and blood lipids may well represent a new therapeutic opportunity for cardiovascular diseases. In contrast, a recent study failed to detect a correlation between plasma IgE levels and low-density lipoprotein (LDL) oxidation in a Pakistan cohort of cardiovascular disease patients (27). Analysis of other, larger cohorts of patients and risk factor matched controls should shed more light on the importance of plasma lipids on IgE-mediated mast cell activation in cardiovascular diseases.

The correlation between mast cell presence and activity and cardiovascular diseases have also prompted searches for mast cell derived biomarkers for cardiovascular diseases and a number of mast cell mediators have already been screened in patient populations. For example, histamine levels from patients with stable coronary artery disease were elevated as compared to control individuals (28), suggesting that histamine and associated mast cell activation is a risk factor for atherosclerosis-related disorders. The mast cell protease tryptase has also been investigated as risk predictor or biomarker in a number of studies, albeit with largely negative outcome. In a study by van Haesl et al. no differences were observed in serum tryptase levels between non-ischaemic patients, patients with unstable angina pectoris and patients with myocardial infarction (29). The authors concluded that mast cell activation during acute cardiovascular events is a focal process and not reflected systemically, disqualifying mast cell derived products as risk predictors. These data have been confirmed by Kervinen et al., showing unaltered tryptase levels in patients with acute coronary syndrome versus controls (30). Contradictory, increased tryptase levels have been detected in acute coronary syndrome patients with ST-segment depression (31). Tryptase levels were later shown to be elevated in patients with significant coronary artery disease in general as well (32). In fact, Deliargyris et al. described that patients in the highest quartile of tryptase levels had a 4.3-fold increased risk for coronary artery disease, identifying tryptase as an independent prognosticator along with age (33). Concordant with this finding, in a Chinese patient cohort study serum tryptase levels were significantly higher in patients with substantial coronary artery disease (e.g. myocardial infarction and unstable angina pectoris) than in those without (34). Interestingly, a second important mast cell protease, chymase, tended to be elevated in this patient group as well. Thus the actual value of mast cell mediators as cardiovascular risk predictor remains subject to debate. Taken together, the above human pathology and epidemiology studies clearly demonstrate that mast cells are present in the intima and adventitia of human arteries at numbers that increase with disease progression. Although these studies have revealed a link between mast cells and derived factors such as tryptase, chymase, histamine and specific growth factors and cardiovascular events, they left unaddressed the issue of causality. In the next paragraph, we will review the current status on mast cells in experimental atherosclerosis.
Mast cells in experimental atherosclerosis

Interest in mast cells in experimental atherosclerosis was aroused already in 1953, when Constantinides proposed a protective role for mast cells in atherogenic responses in rats, which was tentatively assigned to granule associated heparin (12). Two decades later similar conclusions were drawn by Sue et al. (35). Concordant with the prevailing emphasis in atherosclerosis research on lipid driven processes, attention gradually turned to the potential impact of mast cells on lipid metabolism. Kokkonen et al. demonstrated in the 1980s that mast cell granules can degrade LDL (36, 37) and that mast cell-derived heparin can bind LDL particles, which can be taken up by macrophages in vitro, resulting in foam cell formation (38, 39). Mast cell degranulation was furthermore shown to induce uptake of LDL by macrophages in the peritoneal cavity of rats in vivo (40). These studies collectively point to a proatherogenic crosstalk between mast cells and macrophages. In contrast, studies by Lindstedt et al. showed a limited amount of data in favour of a protective effect: an inhibitory effect of mast cell activation on LDL oxidation by macrophages (41, 42). Further evidence confirms the pro-atherogenic effects of mast cell activation. For example, in vitro studies recently identified the mast cell protease tryptase as major actor in mast cell-mediated lipid uptake, as it enhanced foam cell formation in THP-1 macrophages by suppressing LXRα activation in a PAR-2 dependent manner (43). Furthermore, mast cell granules were shown to bind LDL particles, which implies that mast cell granules may also help to retain LDL within the vessel wall (44). Vice versa, infusion of oxidised LDL in rats was demonstrated to induce mast cell degranulation and leukocyte adhesion, which will further exacerbate the disease process (45). Oxidised LDL not only led to mast cell activation in vivo, but also to upregulation of mast cell IL-8 expression (46). After degranulation, mast cell tryptase can induce IL-8 and monocyte chemoattractant protein-1 (MCP-1) expression by endothelial cells, thereby providing a further mechanism by which mast cells may trigger leukocyte infiltration into the vessel wall (47). Immunoglobulin G/oxLDL immune complexes present in the atherosclerotic plaque were shown capable of activating mast cells, which in turn will release histamine, tryptase, TNFα, MCP-1 and IL-8, amongst others (48). Mast cells were not only shown to modify LDL, but also HDL function and in fact, mast cell activation by compound 48/80 led to dysfunctional HDL with reduced cholesterol efflux capacity, suggesting an inhibitory role for mast cells in the initial step of reverse cholesterol transport (49). Taken together, these findings identify mast cell-dependent lipoprotein modification, lipoprotein mediated mast cell activation and subsequent leukocyte recruitment as a plausible cascade by which mast cells contribute to the progression of atherosclerosis.

The first solid in vivo evidence for a causal role of mast cells in atherogenesis was provided in 2007, when systemic mast cell activation was shown to aggravate atherothrombotic lesion formation in the brachiocephalic artery in apoE-deficient mice (50). Interestingly, inhibition of mast cell activation by the mast cell stabiliser cromolyn prevented this effect. Importantly, focal mast cell activation in perivascular tissue of advanced atherosclerotic carotid artery plaques in apoE-/- mice led to a lesion morphology with features of instability as indicated by the presence of intraplaque haemorrhage, massive intraplaque apoptosis and leukocyte recruitment. In vitro studies showed that in particular macrophages were highly susceptible to mast cell-induced apoptosis and both histamine and the mast cell proteases were identified as the main factors involved in this process. This is in agreement with studies by other groups which show that mast cells and derived mast cell proteases can induce apoptosis of a variety of arterial wall cells, such as smooth muscle cells and endothelial cells (51–55). Furthermore, the mouse study cited above (50) corroborated previous pathology studies that mast cells indeed induce leakage of neovessels, which may be a major source of extravasated erythrocytes within the plaque (18–20). Histamine appeared to be causal in this adverse effect. Perivascular mast cell activation augmented leukocyte adhesion to the atherosclerotic plaque and, as already alluded to in previous in vitro studies, IL-8 and vascular cell adhesion molecule-1 (VCAM-1) were primarily responsible for this process. The mast cell stabiliser cromolyn prevented the mast cell-induced plaque instability, thereby rendering mast cell stabilisation a new therapeutic possibility in acute cardiovascular syndromes. In parallel, Sun et al. delivered further proof of a critical importance of mast cells in atherosclerosis using mast cell-deficient Kit(Wt/Wt) W-/- mice, backcrossed to LDL receptor-deficient mice (56). Mast cell-deficient mice had reduced atherogenesis in the aorta, and this anti-atherogenic phenotype could be rescued by repopulation with bone marrow-derived mast cells. Adoptive transfer of bone marrow derived mast cells from IL-6 or interferon (IFN)γ-deficient mice failed to rescue the phenotype, identifying mast cell derived IL-6 and IFNγ as major culprits. The same group recently showed that mast cell activation induces expression of adhesion molecules such as VCAM-1, intercellular adhesion molecule-1 (ICAM-1), P- and E-selectin by endothelial cells and to promote neutrophil adhesion in a TNFα and IL-6 dependent fashion (57). The Kit(Wt/Wt)/W-/- LDLr-/- mouse was also used in a study to determine the role of mast cells in lipoprotein metabolism. Similar to Sun et al. a reduction in lesion size was observed in mast cell-deficient mice, which was partly ascribed to lowered serum total cholesterol and triglyceride levels and partly to reduced vascular inflammation via ICAM-1 (58).

Mast cell activators and stabilisers have been exploited in a number of studies to dissect mast cell function in atherosclerosis. Tang et al. described that mast cell activation by intraperitoneal injection of compound 48/80, a general mast cell activator, resulted in enhanced plaque progression in carotid artery plaques (59). Conversely, in a hamster model of atherosclerosis, the mast cell stabiliser tranilast was shown to inhibit plaque destabilisation (60), and this was attributed to inhibition of mast cell chymases. It should, however, be noted that tranilast is known as a general mast cell stabilising and anti-inflammatory compound, which is not specific for mast cell chymases. In an attempt to address the potential involvement of mast cell chymases, apoE-deficient mice were treated with the specific chymase inhibitor ROS066852, resulting in reduced lesion progression in aorta and brachiocephalic artery. Of note, the number and size of intraplaque haemorrhages was sig-
nificantly reduced after focal perivascular mast cell activation in advanced carotid artery plaques upon treatment with this chymase inhibitor (61). These in vivo studies conclusively demonstrate that mast cells actively participate in the process of atherogenesis and that mast cell activation induces plaque destabilisation. Mast cell products such as tryptase, chymase, IL-6 and IFNγ appear to contribute significantly to the adverse effects of mast cell activation on plaque stability identifying chymase inhibition and mast cell stabilisation as a new therapeutic approach in the prevention of acute coronary syndromes.

### Triggers of mast cell activation in atherosclerosis

In general the consensus is now that mast cells act pro-atherogenic. Given the multitude of potent factors released by mast cells, intervention in mast cell factors per se will not always cover the whole spectrum of effectors. Moreover, mast cell releasate composition is highly dependent on the actual route of activation (4). Endogenous triggers of mast cell activation in cardiovascular disease may thus be of much greater therapeutic value, rendering their identification of great importance. Mast cells can be either activated by general mast cell activators such as IgE, but there may also be particular mast cell activators in cardiovascular diseases. As described above, oxidised LDL was demonstrated to induce mast cell activation, resulting in the release of a range of cytokines (45, 46). Another plausible candidate trigger may be microbes that reside in the plaque. As discussed before mast cells operate in the first line of host defense against pathogens such as parasites and bacteria. Various pathogens have been detected in human lesions, and of these in particular *Chlamydia pneumoniae* and *Aggregatibacter actinomycetemcomitans* can activate mast cells in vitro (62). Although both oxLDL and these microbes (63, 64) have been implicated in the pathogenesis of atherosclerosis, up to now there is no direct in vivo evidence that either of these factors are indeed major endogenous triggers of mast cell activation in acute cardiovascular syndromes. In search of other potential mast cell activators, attention turned to the complement system, which comprises approximately 30 proteins involved in the innate immune response (65, 66). During the development of atherosclerosis, several complement factors (67), and in particular C3a and C5a and their receptors, have been detected in advanced atherosclerotic plaques (68). Furthermore, activated complement has been detected in ruptured atherosclerotic plaques (69). Interestingly, mast cells express receptors for C3 and C5, by which mast cells can be activated (4). Again, up to now a direct role for complement-induced mast cell activation in atherogenesis has not yet been identified.

As mentioned earlier, perivascular mast cells in human lesions have been reported to co-localise with nerve fibres (22), while mast cells express receptors for a number of neuropeptides (4), fuelling the intriguing option of neuronal regulation of mast cell activation. In a recent study perivascular mast cell content was seen to correlate not only with disease progression but also with the number of nerve fibres in the perivascular tissue of human coron-
ary atherosclerotic plaque specimen (70). Furthermore, the authors showed that the focal treatment with neuropeptide substance P led to recruitment of mast cells to and activation in peri-vascular tissue, resulting in an increased incidence in intraplaque haemorrhage and thus plaque destabilisation. These adverse phenomena did not occur in mast cell deficient mice and could be inhibited by co-administration of spantide-I, an antagonist for the neurokinin-1 receptor for which substance P is a ligand. These data identify neuropeptides as potential mast cell activators in cardiovascular diseases and directly connect neuronal activation to vascular inflammation and acute cardiovascular syndromes.

Conclusions and therapeutic potential

Evidence is accumulating that mast cells affect atherosclerotic lesion formation, progression and destabilisation. Epidemiology, histopathology and experimental studies all point toward a pro-atherogenic role for mast cells, involving histamine, tryptase, chymase and cytokines such as TNFα, IFNγ, MCP-1, IL-6 and IL-8 (Table 1 and Fig. 1). Mast cell activation in atherosclerosis has been demonstrated to promote lipid uptake by macrophages, lipoprotein retention within the lesion, apoptosis of various vascular wall cells, leukocyte recruitment, vascular leakage and intraplaque haemorrhage, all resulting in plaque progression and destabilisation. Whether or not these deleterious phenomena represent acute effects that will be succeeded by a fibrotic, angiogenic healing response, remains to be determined. While the actual endogenous triggers for mast cell activation have not been elucidated yet, the picture may be rather complicated and variable from case to case as cytokines, IgE, modified lipoproteins, microbes, complement factors and neuropeptides within the plaque all could act in concert to mediate mast cell-induced acute cardiovascular syndromes. Intervention studies so far have lead to the identification of a number of promising therapeutic targets to inhibit pathogenic mast cell activity per se, such as mast cell stabilisers, or to intervene in dedicated mast cell constituents such as chymase, tryptase, histamine, IFNγ and IL-6. In theory, interruption of specific mast cell activation pathways, which are exclusively operational in cardiovascular diseases, may even be a more powerful strategy, as this may not interfere with regular mast cell function in host defense. Regardless of the route of intervention, the character of mast cell-mediated effects during acute stages of disease and the fact that many mast cell-targeted therapeutics are already widely prescribed to date, renders intervention in mast cell biology during critical episodes such as acute myocardial infarction, stroke and unstable angina pectoris a very attractive scenario. However, it needs to be established to what extent transient interference in mast cell function may perturb post-infarct healing responses, including collateral formation and angiogenesis. In conclusion, the apparent relevance of mast cells in atherosclerosis related disorders, and the impact of targeted intervention in mast cell function on disease progression clearly warrants further study, but opens the way to promising new therapies in acute cardiovascular syndromes.

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Conflict of interest

None declared.

References


