The role of mast cells in atherosclerosis

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Mast cells, atherosclerosis, plaque instability, proteases, therapy

Summary
Rupture of an atherosclerotic plaque is the major underlying cause of adverse cardiovascular events such as myocardial infarction or stroke. Therapeutic interventions should therefore be directed towards inhibiting growth of atherosclerotic lesions as well as towards prevention of lesion destabilization. Interestingly, the presence of mast cells has been demonstrated in both murine and human plaques, and multiple interventional murine studies have pointed out a direct role for mast cells in early and late stages of atherosclerosis. Moreover, it has recently been described that activated lesional mast cells correlate with major cardiovascular events in patients suffering from cardiovascular disease.
This review focuses on the effect of different mast cell derived mediators in atherogenesis and in late stage plaque destabilization. Also, possible ligands for mast cell activation in the context of atherosclerosis are discussed. Finally, we will elaborate on the predictive value of mast cells, together with therapeutic implications, in cardiovascular disease.

Schlüsselwörter
Mastzellen, Atherosklerose, Plaqueinstabilität, Proteasen, Therapie

Zusammenfassung
Die Ruptur einer atherosklerotischen Plaque ist der wichtigste Auslöser unerwünschter kardiovaskulärer Ereignisse wie Myokardinfarkt oder Schlaganfall. Daher sollten therapeutische Interventionen auf die Inhibition des Wachstums atherosklerotischer Läsionen zielen und außerdem der Destabilisierung der Läsionen vorbeugen. Interessant ist, dass bei Nagern und auch beim Menschen Mastzellen in Plaques nachgewiesen wurden. Zahlreiche Interventionsstudien an Nagern wiesen auf eine direkte Rolle der Mastzellen im Früh- und Spätstadium der Atherosklerose hin. Darüber hinaus wurde kürzlich beschrieben, dass aktivierte läsionale Mastzellen bei Patienten mit Herz-Kreislauf-Krankheiten mit größeren kardiovaskulären Ereignissen in Zusammenhang stehen.
In dieser Übersicht liegt der Fokus auf dem Effekt der von Mastzellen freigesetzten Zytokine bei der Atherogenese und Destabilisierung der Plaques im Spätstadium. Daneben werden mögliche Liganden für die Mastzellaktivierung im Kontext der Atherosklerose diskutiert. Schließlich befassen wir uns noch mit der prognostischen Bedeutung der Mastzellen sowie den therapeutischen Konsequenzen bei Herz-Kreislauf-Krankheiten.

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Atherosclerosis is still among the leading causes of death worldwide, responsible for major cardiovascular events such as myocardial infarction, stroke and peripheral artery disease. Over the recent years, evidence has accumulated in which a detrimental role for mast cells in atherosclerosis is described.

> Mast cells are long-lived cells commonly present in tissues exposed to the outside environment, such as the skin, the gastrointestinal tract and the lungs, where they play a key role in innate immunity by functioning as sentinels.

At these strategic places mast cells reside in close proximity to blood vessels, nerves and lymphatics, allowing them to rapidly act upon an encounter with foreign threats (1). Mast cells are derived from progenitor cells and originate in the bone marrow. They home to tissues in response to locally produced stem cell factor (SCF) by fibroblasts, stromal cells and endothelial cells. The receptor for SCF is c-kit, a tyrosine kinase receptor expressed on the membrane of mast cells. SCF is required for the differentiation and maturation of mast cells, a necessity that becomes evident in mice carrying a mutation in their c-kit gene, which results in complete mast cell deficiency (2, 3).

After activation, mast cells exert their adverse effects through the excretion of pre-formed granules loaded with a vast array of mediators, including histamine, vascular endothelial growth factor (VEGF), various inflammatory cytokines (for example TNFα and IL-6), and the proteases tryptase, chymase and carboxypeptidase A3 (CPA3) (4). These stored granules give mast cells their typical morphologic appearance which originally led to their name 'Mastzellen', meaning well-fed cells. Mast

cell activation may be triggered by different ligands, including IgE, complement components, neuropeptides and TLR ligands. Although their presence is highly important to combat bacterial and parasitic infections, mast cells are actually commonly known for their contribution to diseases such as allergic asthma and IgE dependent allergic responses.

A role for mast cells in the setting of atherosclerosis has first been described over 60 years ago. It was discovered that heparin attenuates atherosclerotic lesion development in high-cholesterol fed rabbits, and heparin was postulated to be produced by mast cells in the connective tissue surrounding the blood vessels (5). However, as the important contribution of inflammation to the atherosclerotic process received increasing recognition, mast cells were later in fact discovered to aggravate atherosclerosis.

> Atherosclerosis is a chronic disease of the middle and large-sized vessels, often occurring at sites of low or oscillatory endothelial shear stress.

In early atherogenesis, monocytes enter the vessel wall and differentiate into macrophages, which can scavenge modified LDL and turn into foam cells. Continued inflammation can eventually result in the formation of advanced unstable lesions, consisting of a large necrotic core, a thin fibrous cap, luminal erosions, a high inflammatory cell count and multiple leaky neovessels. Rupture of the plaque may lead to acute thrombus formation with subsequent adverse cardiovascular events such as myocardial infarction or stroke (6), for which lesional mast cells have recently been shown to have a predictive value (7).

This review focuses on the role of the mast cell in both early and late stages of atherosclerosis, with particular emphasis on lesion destabilization with subsequent plaque rupture. Also, we will elaborate on the predictive value of mast cells, as well as on potential therapeutic implications, in cardiovascular disease.

Atherosclerotic lesion development

The presence of both resting and degranulated mast cells has been established in human coronary arteries and aortas without signs of atherosclerosis and interestingly, mast cell numbers, as well as their activation status, are increased at sites of foam cell accumulation in the vessel wall (8). The buildup of intimal foam cells is a key event in atherogenesis, caused by excessive uptake of modified LDL particles by macrophages combined with insufficient cholesterol efflux. Mast cells may influence the impaired cholesterol regulation via different pathways.

- It has been demonstrated in *in vitro* studies, that the mast cell releasate strongly enhances uptake of LDL by macrophages. Also, LDL can bind to granule remnants derived from the mast cell, fuse into larger particles, and subsequently be scavenged by macrophages and smooth muscle cells (9).
- The mast cell may promote foam cell formation by secreting tryptase, which is capable of degrading pre-β-high-density lipoprotein (HDL). As HDL particles have the ability to remove cholesterol from the macrophage and transport it back to the circulation, inhibition of the production of mature HDL via mast cell tryptase can thus impair reverse cholesterol transport (10) and may aggravate atherosclerotic lesion development.

Upon activation, mast cells release a variety of chemokines, amongst which are CXCL1, CXCL2, CXCL10, CCL2, CCL3 and CCL5 (▶Fig. 1, ▶Tab. 1) (4). CCL2 is one of the chemokines which has been suggested to play a role in leukocyte recruitment to the atherosclerotic lesion (11). Also, inhibition of CCR5, the prime receptor for CCL5, was recently shown to decrease atherosclerosis (12). CCL3, CXCL1 and CXCL2 all have the potential to attract neutrophils to the site of inflammation (13–16) and indeed, we have recently observed a profound influx of neutrophils to the atherosclerotic plaque after chronic mast cell activation (17). Thus, mast cell derived chemokines may exacerbate atherogenesis by attracting multiple inflammatory cells to the lesion.

Most *in vivo* studies investigating mast cell effects in cardiovascular diseases have been performed in mice. Mast cell deficient Kit(W/W^{-v}) mice were first described by Kitamura et al. and these mice have, besides a lack of mast cells, impaired melanogenesis, anemia, and sterility (3). Although it has been shown that following acute restraint stress, cardiac histamine release is impaired in Kit(W/W^{-v}) mice (18), there is no data available on the effects on atherosclerosis in these mice. More recently, a mast cell deficient $Kit^{W\text{-}sh/W\text{-}sh}$ mice has been reported (2), which has been backcrossed on either an LDLr or ApoE deficient background. These mice are also mast cell deficient, but lack the anaemia and sterility. It should be taken into account however, that these mice have increased numbers of neutrophils and platelets in the blood that may affect atherosclerosis as well.

Direct *in vivo* evidence for a detrimental role of the mast cell in atherosclerotic lesion development was provided in 2007, when it was established that lesion formation in the brachiocephalic arteries was markedly aggravated after systemic mast cell activation in apolipoprotein E (apoE) knockout mice on a high cholesterol diet. Moreover, treatment with the mast cell stabilizer cromolyn in this experimental setup significantly reduced lesion size back to control level. The observed effects on atherosclerosis were independent of cholesterol metabolism, since no effect on plasma cholesterol levels were reported (19). These findings were confirmed by Sun et al., who demonstrated that mice deficient for mast cells ($Kit^{W\text{-}sh/W\text{-}sh}$), crossbred on an $LDLr^{-/-}$ background, displayed reduced atherogenesis. Interestingly, the observed reduction in lesion size was again increased after the transfer of TNFα deficient mast cells, but not after that of IL-6 or IFNã deficient mast cells, indicating an important role for these inflammatory cytokines in atherosclerosis. Cholesterol levels were lower in IL-6 deficient mast cell reconstituted mice, but not in mice receiving wild type or IFNγ deficient mast cells (20). In a study performed by Heikkilä et al., mast cell function in atherosclerosis was

investigated in LDLr$^{-/-}$Kit$^{W\text{-}sh/W\text{-}sh}$ as well. In line with the previous reports, mast cell deficient mice developed significantly reduced atherosclerotic lesions in the aortic sinuses compared to control LDLr$^{-/-}$ mice (21). However, serum cholesterol and triglyceride levels were lower in mice lacking mast cells, which was accompanied by a decrease in pre-β-HDL. These data indicate that although mast cells clearly aggravate early atherosclerotic lesion development, the exact contribution of alterations in cholesterol metabolism versus inflammatory actions remains to be further elucidated.

Mast cell derived mediators, plaque destabilization

Advanced atherosclerotic plaques containing a thick fibrous cap and preserved lumen area may remain stable for years without apparent clinical manifestations (22). Therefore, it is more compelling to study the mechanisms behind plaque destabilization, in which mast cells are thought to be of importance as well (►Fig. 1, ►Tab. 1). A potential connection between the mast cell and unstable lesions was first proposed by Kovanen et al. who observed increased numbers of degranulated mast cells in ruptured coronary plaques and in plaques displaying erosions, compared to unaffected intimas, in autopsy material from patients who had died from myocardial infarction (23). A causal role for mast cells in plaque destabilization was later demonstrated after perivascular mast cell activation in apoE$^{-/-}$ mice with established atherosclerotic lesions in their carotid arteries. Focal mast cell activation at the lesion site profoundly increased the incidence of intraplaque haemorrhages, while also the percentage of apoptotic cells in the lesion was increased, both indicative of reduced plaque stability (19). Also, mast cell deficient LDLr$^{-/-}$ Kit$^{W\text{-}sh/W\text{-}sh}$ mice have been described to contain more stable lesions with decreased apoptosis and increased collagen content and fibrous cap thickness (20).

Various mast cell derived mediators are postulated to contribute to plaque destabilization, amongst which are the specific

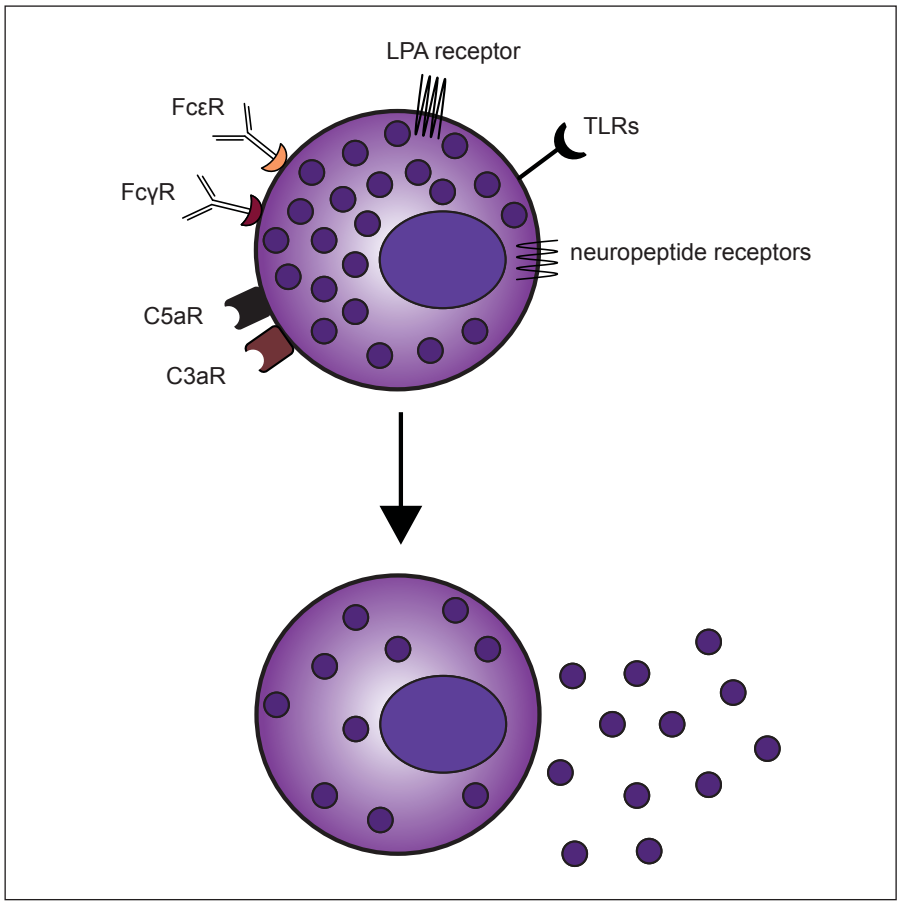

Fig. 1 In atherosclerosis, potential mast cell activators are: IgE, complement components (C5a, C3a), TLR ligands (e. g. EDA, fibrinogen and HMGB1), OxLDL-IgG immune complexes, lysophosphatidic acid (LPA), neuropeptides (substance P, NPY). Their effects are summarized in Table 1.

Tab. 1 Proposed effects of mast cell derived mediators on atherosclerosis

mast cell derived mediator	proposed effects on atherosclerosis	reference
granule remnants	enhanced uptake of LDL	9
tryptase	• degradation of pre-beta-HDL particles • chemotaxis • matrix degradation • increased intraplaque haemorrhage (in vivo)	10, 32
chymase	• conversion of pro-MMPs to MMPs • cleavage of extra-cellular matric proteins • generation of angiotensin II • proapoptotic effects on smooth muscle cells and endothelial cells • chemotaxis	26–30
histamine	• increase of vascular permeability • induction of vasospasms	19, 34
chemokines (incl. CXCL1, CXCL2, CXCL10, CCL 2, CCL3, CCL5)	attraction of lekocytes to the atherosclerotic lesion	4, 11–16
inflammatory cytokines (incl. IL6, TNF alpha, IFN gamma)	aggravation of the lesional inflammatory process	20
bFGF, VEGF	induction of plaque angiogenesis	33

neutral proteases chymase and tryptase. In humans, mast cells are divided into two subtypes according to their protease content, namely MC_T cells (expressing tryptase α and β) or MC_{TC} cells (expressing α and β tryptase, chymase and carboxypeptidase A), both of which have been identified in atherosclerotic plaques (24). Also, murine mast cells are classified as either connective tissue type mast cells (expressing chymase mMCP-4 and –5; and tryptase mMCP—6 and –7) and mucosal mast cells (expressing chymase mMCP-1 and –2) (25). In the perivascular tissue of murine atherosclerotic plaques, mostly the connective tissue type mast cells are present. Besides the differences in protease content between murine and human mast cells, increased levels of IL-4 and TNFα and a higher expression of the IL-3 receptor have been observed in murine mast cells, which is important to keep in mind when comparing human data to murine data. The potent protease chymase is stored and secreted as a fully active enzyme, involved in a number of pathways that may affect atherosclerotic lesion stability. For instance, chymase acts as an angiotensin converting enzyme generating the potent vasoconstrictor angiotensin II, a potent stimulus for the generation of reactive oxygen species (26). Furthermore, chymase facilitates the conversion of pro-matrix metalloproteinases (pro-MMPs) to the active enzymes MMP1 and MMP3, which in turn may activate MMP2 and MMP9, leading to matrix degradation (27). Also, chymase itself has the ability to cleave the extracellular matrix proteins vitronectin and procollagen, all possibly contributing to thinning of the fibrous cap.

A number of *in vitro* studies have demonstrated that chymase exerts pro-apoptotic effects on smooth muscle cells and endothelial cells via disruption of focal adhesion complexes necessary for cell survival (28, 29). While smooth muscle cell apoptosis leads to additional impairment of the integrity of the cap, apoptosis of endothelial cells may result in plaque erosions and leaky neovessels. Finally, it has been reported that chymase acts as a chemoattractant, attracting multiple immune cells such as neutrophils to the site of inflammation (30). A direct *in vivo* role for chymase was confirmed after inhibition of chymase in $apoE^{-/-}$ mice, which indeed significantly improved atherosclerotic plaque stability by increasing lesional collagen content, decreasing necrotic core size and reducing the incidence of intraplaque haemorrhage (31).

Similarly to the effector functions of chymase, tryptase released by the mast cell can also induce chemotaxis and matrix degradation. The importance of tryptase in atherosclerosis was demonstrated by Zhi et al., who showed that systemic lentiviral overexpression of tryptase in $apoE^{-/-}$ mice aggravated both plaque size as well as the frequency of intraplaque hemorrhages (32). The increase in incidence of hemorrhages observed in this study were suggested to be caused by enhanced lesional angiogenesis due to alterations in the expression of plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) following tryptase overexpression. However, it is unlikely that tryptase is the sole culprit actor in mast cell induced angiogenesis.

> In fact, the potent angiogenic mediator basic fibroblast growth factor (bFGF) is known to be stored and secreted by the mast cell as well, and a positive correlation has previously been found between numbers of bFGF positive mast cells and microvessel density in human plaques.

Also the percentage of bFGF positive mast cells was higher in advanced atherosclerotic lesions compared to the unaffected intima (33). Furthermore, mast cells secrete vascular endothelial growth factor (VEGF), which is well-known to induce neovascularization. Although a direct role for mast cell derived VEGF has not been demonstrated yet in atherosclerosis, it is likely that VEGF may also contribute to the formation of intraplaque neovessels. Increased leakage of these intraplaque neovessels may be induced by the mast cell product histamine. Histamine exerts its effects by binding to members of the histamine receptor family, which consists of histamine receptors H1, H2, H3 and H4. Binding of histamine to the H1 receptor on endothelial cells was shown to induce vascular permeability (34). Vascular leakage, but also macrophage apoptosis, induced by mast cell derived histamine was shown to be solely dependent on the H1 receptor (19). Smooth muscle cells also express the H1 receptor and are promoted to contract upon binding of histamine, which can generate vasospasms. Rozenberg et al. demonstrated a role for the H1 receptor by treating $apoE^{-/-}$ mice with an H1 receptor antagonist, which resulted in reduced lesion formation. Correspondingly, genetic deletion of the H1 receptor decreased lesion size, while no effects were observed after blocking or deleting the H2 receptor (35). Mice deficient for H1 receptor displayed reduced vascular permeability for LDL in the aortic wall, which was suggested to account for the observed effects on atherosclerosis.

Mast cell activators

Multiple mediators are capable of inducing mast cell activation (▶Fig. 1), the most famous of which is IgE mediated degranulation. Mast cells express the high affinity receptor FcεRI which can bind to IgE in a practically irreversible manner. Each cell carries a range of IgE molecules bound to the FcεRI with varying antigen specificity. Upon encountering the antigen, mast cells are promptly triggered to degranulate. Recently, mice deficient for the FcεRIα on an $apoE^{-/-}$ background were shown to display reduced lipid depositions in the aortic arch when fed a western type diet. Furthermore, lesions contained fewer macrophages, T cells and apoptotic cells, as well as lower levels of inflammatory cytokines. *In vitro*, IgE activation was seen to affect inflammatory signalling and apoptosis in macrophages, smooth muscle cells and endothelial cells, therefore in this study, mast cell specific effects remain to be identified (36).

In order to discover potential therapeutic leads for mast cell stabilization in atherosclerosis, it is crucial to further elucidate the underlying mechanisms of mast cell activation in the setting of atherosclerosis. Ligands present in the lesion or the underlying adventitial tissue, capable of inducing local mast cell activation, may thus be promising targets. For instance, the complement component C5a has been detected in human atherosclerotic plaques in lipid-rich inflammatory regions (37), and

mast cells are known to express the receptor for C5a. Interestingly, treatment with a C5a receptor antagonist decreased lesion size in a murine vein graft model, while perivascular application of C5a during disease initiation aggravated lesion formation. More importantly, C5a application combined with cromolyn treatment significantly reduced lesion size to control level, indicative of a mast cell dependent effect (38). In late stages of vein graft disease, C5a was seen to promote vein graft destabilization in a mast cell independent fashion (39). Although mast cells are also known to express the receptor for C3a via which they can be activated, the interactions between C3a, mast cells and atherosclerosis have not yet been elucidated.

Toll like receptors (TLRs) are pattern recognition receptors widely expressed by cells of the innate immune system. Murine mast cells express TLR 1 to TLR9, with the exception of TLR5, while human mast cells express TLR1 to TLR7, TLR9 and TLR10. Currently, the mechanism of mast cell activation via TLRs is still under debate, in particular the effects on degranulation versus mere cytokine release. While some groups have shown that activation of TLR2 on murine and human mast cells with peptidoglycan results in degranulation, others have been unable to reproduce these results (40). Also, mast cell degranulation following TLR4 activation with for example LPS has not often been observed; instead, this triggers the release of various pro-inflammatory cytokines and chemokines. With regard to atherosclerosis, TLR4 is of particular interest, for different endogenous ligands present in the plaque are proposed to be able to induce TLR4 signaling, including heat shock protein 60 (hsp60), extra domain A of fibronectin (EDA), oxidized LDL (oxLDL), fibrinogen and HMGB1 (41). However, it should be noted that endotoxin contamination may also induce TLR4 signalling, complicating this area of research and sometimes leading to the finding of 'false-positive' TLR4 ligands. Den Dekker et al. showed that mast cells promote plaque destabilization in a TLR4 dependent manner *in vivo* via chymase-induced smooth muscle cell apoptosis (42).

In contrast to previous reports, mast cell degranulation as measured by chymase release after TLR4 activation was observed. It was postulated that TLR4 signaling induces release of proinflammatory cytokines, including IL-6, which then in an autocrine manner trigger mast cell degranulation with chymase release, thereby causing smooth muscle cell apoptosis. Since TLR4 in that study was activated by *E. coli* lipopolysaccharide (LPS) and inhibited by a general TLR4 antagonist, it remains to be investigated which exact endogenous ligands contribute *in vivo* to mast cell activation in atherosclerosis.

In addition to serving as an endogenous ligand for TLR4, oxLDL may also form antigen-antibody complexes with autoantibodies directed against oxLDL, mainly of the IgG isotype. Immune complexes are present in the circulation of patients with cardiovascular disease (43), as well as in the atherosclerotic plaque itself. Mast cells express FcγRs that bind monomeric IgG with low affinity while they can bind to antigen-antibody complexes with a relatively high affinity. *In vitro* studies have shown that oxLDL-IgG immune complexes are capable of inducing mast cell activation, as indicated by increased secretion of tryptase, chymase, histamine, TNFα, IL-6 and CCL2 (44).

One of the major lipid parts of modified LDL is lysophosphatidic acid (LPA). This naturally occurring lysophospholipid has recently been described as a potential mast cell activator in atherosclerosis (45). With the use of mass spectrometry it was established that several LPA species are present in the plaque, especially in the necrotic core area. Exposure of mast cells to LPA *in vitro* resulted in exacerbated degranulation and cytokine release. Similarly, intraperitoneal injection of LPA increased tryptase levels in the peritoneal fluid, which was absent in mast cell deficient $Kit^{W\text{-}sh/W\text{-}sh}$ mice.

In vivo, perivascular application of LPA in established lesions increased the macrophage content and the frequency of intraplaque haemorrhages. These effects were suggested to be mast cells specific, since cromolyn was able to reduce both the number of haemorrhages and the percentage of lesional macrophages. Also, LPA administration in mast cell deficient mice did not significantly increase the percentage of macrophages nor the intraplaque haemorrhages.

Adventitial mast cells have been found in close proximity to sensory nerve fibers in human coronary arteries, and the number of mast cell to nerve contacts was seen to increase with lesion progression (46). These nerves stained positive for the neuropeptides substance P and calcitonin gene-related peptide (CGRP), both capable of stimulating mast cells. Furthermore, mast cell numbers were seen to correlate with the number of neurofilament$^+$ nerve fibers in coronary artery specimens (46). Taken together, these findings led to the hypothesis that neuronal activation may trigger adventitial mast cells in the setting of atherosclerosis. Indeed, it was established that local application of substance P in $apo^{-/-}$ mice increased both mast cell numbers as well as their activation status and moreover, the frequency of intraplaque haemorrhages was significantly enhanced. These findings were postulated to be mast cell specific, since substance P application in mast cell deficient $apoE^{-/-}Kit^{W\text{-}sh/W\text{-}sh}$ mice was ineffective (47). Recently, also neuropeptide Y (NPY) has been detected in human endarterectomy plaques. Its expression was twofold higher in unstable lesions compared to stable plaques and local lentiviral overexpression of NPY in the carotid artery of $apoE^{-/-}$ mice resulted in increased perivascular mast cell activation, as well as exacerbated atherosclerotic lesion formation (48). *In vitro* activation of murine mast cells with NPY was shown to induce IL-6 and tryptase release. Since long, it has been suggested that cardiovascular events may be elicited by (chronic) psychological stress. As stress is known to activate sensory nerves with subsequent release of, amongst others, different neuropeptides, it is thus compelling in light of the previous reports to further elucidate the effect of stress-mediated mast cell activation in atherosclerotic plaque destabilization.

Therapeutic implications, conclusions

In addition to the mast cell mediated effects demonstrated in murine experimental models, several case studies in humans reported on a link between acute mast cell

activation and cardiovascular events. These findings were first described as the occurrence of an allergic reaction acutely followed by typical angina pectoris, confirmed by clinical and laboratory parameters, now collectively referred to as the 'Kounis syndrome' (49). It is postulated that mast cell derived mediators such as histamine, proteases and cytokines excreted upon allergic reactions or anaphylactic insults may be responsible for the coronary events. Additional research is required to elucidate whether angina pectoris is caused by vasospasms induced by histamine or by actual plaque destabilization with subsequent embolization. Regardless, the Kounis syndrome clearly illustrates the major impact mast cell activation may have on coronary events.

A few studies have addressed the association between allergic diseases (atopy) and the occurrence of thromboembolism and cardiovascular disease. In a case-control study, the incidence of atopic sensitization and allergic rhinitis were found to be increased in patients with venous thromboembolism (50). Moreover, in both the Bruneck Study and the ARMY study, enhanced atherosclerosis was observed in patients with allergic diseases (51), suggested to be caused by either a systemic inflammatory response in allergy, or by shared effector pathways in both diseases, such as mast cell activation and leukotriene synthesis. In contrast, a recent study performed by Skaaby et al. failed to show a significant association between atopy and the incidence of ischaemic heart diseases or stroke (52). Thus, it remains to be elucidated whether an actual association between allergic diseases and cardiovascular disease exists.

Recently, the atherosclerotic plaques of 270 patients suffering from carotid artery stenosis have been evaluated for the presence of mast cells (7). In that study, the number of mast cells significantly increased with lesion progression and particularly in unstable lesions, mast cells were seen to accumulate in the rupture-prone shoulder regions. A correlation was observed between mast cell numbers and microvessel density, intraplaque haemorrhages, lesional macrophage and neutrophil content. Most importantly, during a three year follow-up period, adverse events were significantly associated with increased lesional mast cell numbers. Also, higher plasma tryptase levels were observed in patients experiencing a secondary event, indicative of a predictive value for mast cells in cardiovascular disease (7). However, conflicting evidence prevails in literature regarding the usefulness of tryptase as a biomarker for adverse events. For example, elevated tryptase levels have been observed in patients with significant coronary artery disease (53), while a separate study performed by van Haelst et al. failed to show differences in serum tryptase levels from patients with acute coronary syndromes as compared to controls (54). In addition to these studies, several other groups have reported either affirmative or negative findings for the predictive value of tryptase, often limited by a relatively small cohort size. Therefore, future studies are necessary to appoint a definite role for tryptase as a biomarker in cardiovascular disease.

Besides mast cell derived mediators, ligands for mast cell activation have also been investigated as potential biomarkers in cardiovascular disease. Increased levels of the immunoglobulins IgG, IgA and IgE have previously been shown to associate with myocardial infarction and cardiac death in men with dyslipidaemia (55). However, a recent study performed by Willems et al. did not show a correlation between plasma oxLDL-IgG, total IgG and total IgE levels and characteristics of a vulnerable plaque in patients which have undergone carotid endarterectomies (43). These inconsistencies may be caused by the difference in the location of the atherosclerotic plaques (coronary versus carotid arteries) between the studies. Also, other inflammatory diseases may raise circulating immunoglobulins as well, which in general is a major challenge in the search for predictive markers.

As basophils share some phenotypic similarities with mast cells, such as the FcεR1-expression via which both cell types can be activated, it is interesting to note that some studies have investigated a potential association between blood basophil numbers and the occurrence of cardiovascular disease. However, no correlation was found between the percentage of blood basophils and asymptomatic carotid atherosclerosis (56), the intima-media thickness of the common carotid artery (57) or the frequency of restenosis after stenting (58).

Mast cell stabilization in murine models for atherosclerosis have thus far shown beneficial effects, and taking into account the ample mechanisms via which the mast cell is capable of aggravating atherosclerosis, pharmaceutical mast cell stabilization in patients with cardiovascular disorders may thus yield promising results. As of yet, limited clinical trials in humans suffering from atherosclerosis have been performed using mast cell stabilizers. The antiallergic drug Tranilast has been tested in the PRESTO trial which enrolled over 10 000 patients undergoing percutaneous coronary intervention. No effects were observed in major adverse cardiovascular events or restenosis after a follow-up of nine months (59). It should be noted that Tranilast also exerts effects on other cell types, such as fibroblasts and endothelial cells, making it difficult to draw any conclusions regarding mast-cell specificity. Interestingly, a patent for the use of mast cell stabilizers in the treatment and prevention of cardiovascular disease has recently been published (US8445437 B2), which may direct further research towards investigating the therapeutic potential of mast cell inhibition in the clinic. However, considering the important role mast cells play as a first line of defense against pathogens, care must be taken with complete systemic mast cell inhibition, and unwanted side-effects such as infections should be tightly monitored.

Multiple interventional murine models have demonstrated a direct causal role for mast cell activation in both

- atherosclerotic lesion development and
- plaque destabilization.

Moreover, increased mast cell activation in humans has been associated with adverse cardiovascular events.
Therefore, mast cell stabilization in patients suffering from cardiovascular disease may be a promising therapeutic strategy in order to prevent major complications such as myocardial infarction or stroke.

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Conflict of interest

The authors declared that they have no conflict of interest.

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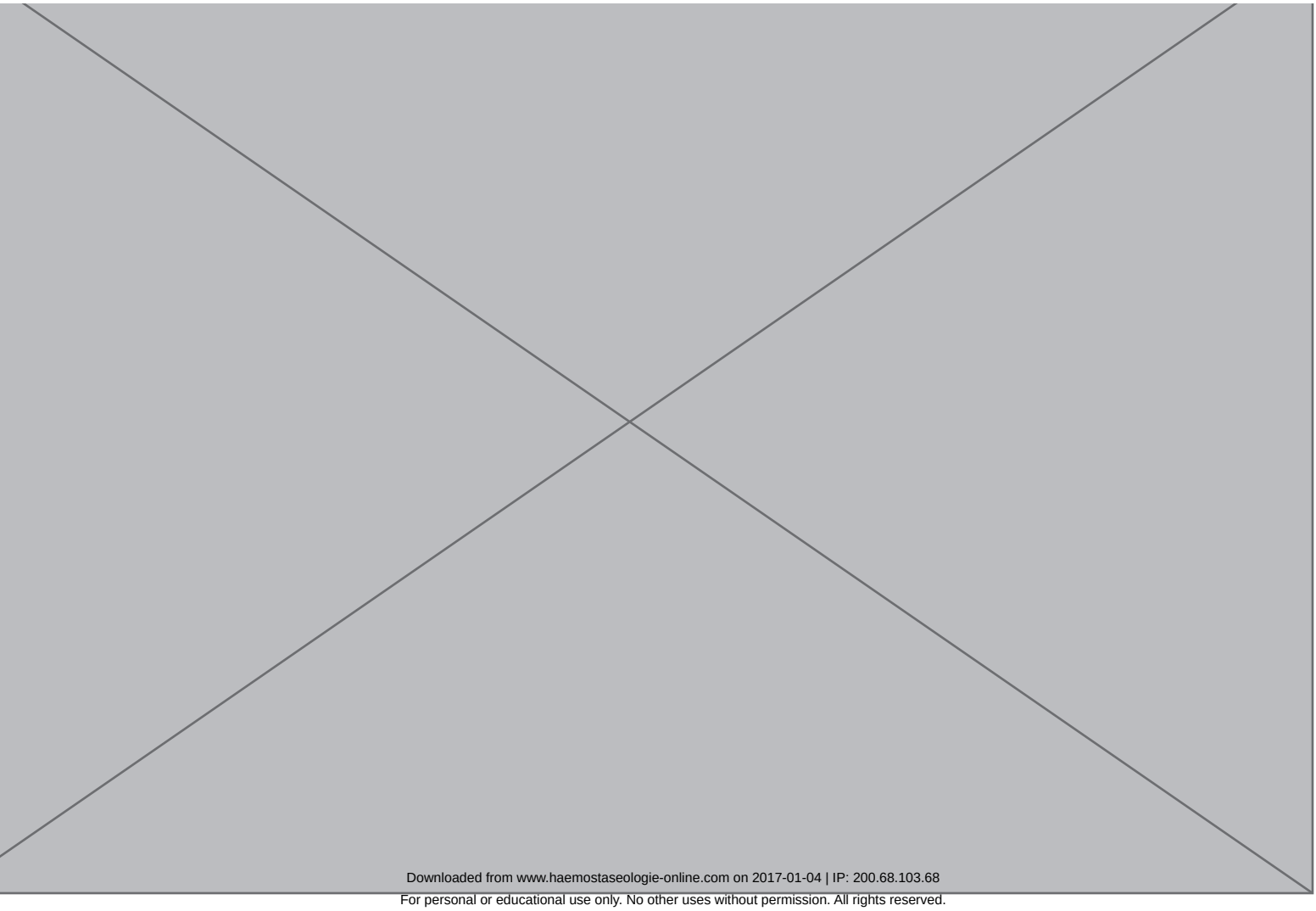

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