Brief Review

Mast Cells
Important Players in the Orchestrated Pathogenesis of Abdominal Aortic Aneurysms

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Abstract— Mast cells (MCs) regulate inflammation and immunity. Their granular content includes heparin, histamine, and several enzymes (tryptase, chymase, carboxypeptidase, and cathepsin G). In addition, activated MCs synthesize and release eicosanoids and a large number of cytokines and chemokines. Recent findings suggest a role of MCs in abdominal aortic aneurysms (AAAs) in humans, where they are found in the media and adventitia. Experimentally induced AAA in MC-deficient animals and animals treated with MC inhibitors demonstrate that MCs are involved in the pathogenesis of AAA via several different mechanisms. MC-dependent activation of metalloproteinases and the renin–angiotensin system, contribution to smooth muscle cell apoptosis, and release of proteolytic enzymes are some key examples. Human studies indicate that MCs are the main source of cathepsin G in AAAs and contribute to activation of the renin–angiotensin system via chymase and cathepsin G. Activated MCs also contribute to neovascularization, inflammation, and atherosclerosis, all hallmarks of AAA. Thus, we may envision that MC stabilizing agents, as well as leukotriene receptor antagonists and histamine receptor blockers already in clinical use for treatment of other diseases, could also be tested for their efficacy in preventing development and growth of AAA. (Arterioscler Thromb Vasc Biol. 2011;31:734-740.)

Key Words: aneurysms ■ angiogenesis ■ angiotensin II ■ apoptosis ■ immune system ■ macrophages ■ proteolytic enzymes ■ mast cells

The most common risk factors for abdominal aortic aneurysm (AAA) are male gender, age, smoking, and atherosclerosis. AAA is almost always associated with atherosclerosis, even though the pathogenesis for AAA is considered to be distinct from atherosclerotic occlusive disease (AOD). Activation of several types of inflammatory cells, for example, T cells, B cells, macrophages, and neutrophils, is important for the inflammatory response, an essential characteristic of both AAA and AOD. In AAA, the inflammation is mostly confined to the media and adventitia of the aorta, whereas in AOD, the inflammatory reaction is seen primarily in the intima, and to some extent also in the adventitia. As in AOD, mast cells (MCs) have been shown to be involved in the inflammatory response in AAA. Their role in AAA pathogenesis is the subject of this review.

The underlying major pathology in AAA formation is an imbalance in matrix synthesis and degradation, leading to weakening of the aortic wall and ultimately to progressive dilatation of the aorta. Important players in the scenario are various proteolytic enzymes synthesized and secreted by different proinflammatory and resident cells, notably macrophages, neutrophils, and smooth muscle cells. Accumulating evidence indicates that aortic MCs, which are a rich local source of neutral proteases and proinflammatory mediators, may enhance matrix degradation and regulation of inflammation at several levels. Accordingly, these cells could be of major importance in the pathogenesis of both aortic atherosclerosis and AAA.

Tissue MCs are the progeny of bone marrow–derived progenitor cells. Stem cell factor (SCF), a chemotactic factor for MCs, is secreted by various tissue cells. SCF facilitates homing of the circulating progenitors and also induces their terminal differentiation in tissues. In human aorta, 2 types of MCs can be distinguished, first shown by Kaartinen et al: MC_T cells, which expresses tryptase; and MC_TC cells, which also expresses chymase, carboxypeptidase A, and cathepsin G. MCs store these neutral proteases in cytoplasmic secretory granules. In response to an external stimulus, they become activated and release their granular contents. In addition to the proteases, all granules also contain histamine, and a variable fraction of them contain tumor necrosis factor (TNF)-α, transforming growth factor-β, vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and chemokines. Activation of MCs triggers prolonged synthesis...
of a large number of cytokines, chemokines, and eicosanoids, which is followed by their slow and sustained secretion. The release of all of the above listed substances contributes to inflammation, injury, remodeling, and angiogenesis in the vascular tissue in which the MCs have been activated.

Thus, MC granules contain mediators that may affect various molecular systems involved in the pathogenesis of AAA. Here, we evaluate the possible role of MCs regarding the following pathogenic mechanisms known to be operative in AAA: degradation of extracellular matrix, apoptosis of smooth muscle cells (SMC), activity of the renin–angiotensin system (RAS), and neovascularization.

In human AAA, the number of medial and adventitial MCs, particularly degranulated ones, is increased in atherosclerotic compared to non diseased aorta, and further increased in AAA, as illustrated by a positive correlation between the number of MCs and AAA diameter. In normal human aorta, MCs are found in the adventitia and the intima, but in specimens from human AAAs, they are located in the media, together with T cells and macrophages, and no MCs are identified in the intima.

Research concerning the pathogenesis of AAA is based on studies in experimental animals and humans, and the same is true for the role of MCs. Experimentally induced AAA is performed in normal or transgenic animals by various interventions, mainly intra-aortic elastase infusion, topical treatment of the aorta with CaCl2, or angiotensin infusion. Human studies are based mostly on aortic specimens obtained at open surgery, or in some cases plasma concentration of various markers. The advantage of animal studies is that they allow mechanistic studies to be conducted at various stages of the development of AAA. The disadvantage is, of course, that the results cannot be directly used to interpret development of AAA in humans. The disadvantage of analyses of human AAA tissue from patients undergoing surgery is that the surgical specimens represent the end stage of the disease.

**Experimental Evidence for the Role of MCs in AAA Formation**

Studies on the influence of MCs in experimentally induced AAA have used rats and mice with a genetic lack of expression of SCF or its receptor c-kit and, therefore, are MC-deficient. In addition, experiments have been performed in which degranulation of MCs has been prevented pharmacologically. Such experiments have provided compelling evidence that MCs play a role in the development of experimentally induced AAA.

Using a rodent aneurysm model based on elastase infusion into the aorta, Sun and coworkers reported absence of AAA development in MC deficient KitW-sh/KitW-sh mice. Interestingly, the MC-competent control mice developed AAAs associated with an increased number of MCs in the adventitia. Inhibition of AAA formation in the KitW-sh/KitW-sh mice was also seen after induction of AAA with CaCl2. The same authors also introduced bone marrow-derived MCs from wild-type mice and mice deficient in interleukin-6, interferon (IFN)–γ, or TNF-α to the MC deficient mice. If given MCs derived from wild-type bone marrow, they had a restored capacity for AAA formation and macrophage infiltration. AAA also developed after aortic infusion of elastase in the MC-deficient mice reconstituted with MCs derived from specifically TNF-α–deficient mice, but such cells from mice deficient in interleukin-6 or IFN-γ did not have this effect. This finding is somewhat unexpected, because TNF-α has been reported to be of importance in AAA development. The findings are, however, compatible with a stimulation of MC-induced AAA formation by IFN-γ secreted by the MCs, even if the secretion of TNF-α was knocked down. The same group also tested AAA formation after aortic elastase infusion in mice lacking mouse mast cell protease (mMCP)-4, a β-chymase that is the closest counterpart to human chymase. In contrast to wild-type mice, the mMCP-4–deficient mice did not develop AAA, and they also showed a diminished number of macrophages and T cells in the aortic wall. To make the story perfect, the authors demonstrated that, in contrast to mMCP-4–deficient mice reconstituted with bone marrow derived MCs from mMCP-4–deficient mice, those reconstituted with MCs derived from wild-type bone marrow developed AAA. Some of the differences, however, were not significant, indicating that mechanisms other than chymase are also important for AAA formation in this elastase-dependent mouse model of AAA.

Tsuruda et al, studying mutant rats deficient in MCs, found a significantly lower response to CaCl2-induced aortic widening compared to control rats. Neovascularization and elastin degradation were also reduced in the MC-deficient rats. Angiotensin II (Ang II)–induced AAA in apoE-deficient mice was reported to be attenuated by the MC stabilizer tranilast, an effect also seen after CaCl2-induced AAA formation in wild-type rats. Elastin degradation was also inhibited in tranilast-treated rats. Chymase inhibition has been demonstrated to prevent Ang II–induced AAA formation in apo-E deficient mice. Similar findings have been obtained for AAA formation in hamsters and dogs, in whom a specific chymase inhibitor prevented elastase-induced AAA formation.

The experimental studies cited above have all observed that AAA formation is associated with an increased number of MCs in the adventitial layer. This is understandable, because the intima in small rodents is almost a virtual layer. An important finding is that lack of MCs, or inhibition of their function, caused less inflammation, as judged by diminished accumulation of T cells and macrophages. Considering the anatomic distribution of MCs in the adventitia, it is conceivable that the activated MCs could induce influx of inflammatory cells via vasa vasora, that is, from the adventitial side.

The above finding of MCs being necessary for the accumulation of macrophages would suggest that MCs could regulate the inflammatory response upstream to the proteolytic activity induced by macrophages. One such regulatory link could be c-Jun N-terminal kinase (JNK). Both total and phosphorylated JNK are increased in human AAA compared to controls. In experimentally induced murine AAA, JNK has been shown to accelerate matrix degradation by proteases. JNK suppresses gene expression of lysyl hydroxylase, lysyl oxidase, and prolyl 4-hydrolase, which are crucial for biosynthesis and maturation of collagen and elastin.
c-Jun expression and JNK activity are upregulated by histamine,\textsuperscript{17} which is likely to be produced in AAAs mainly by MCs. JNK activity could provide a possible link between MC activation, histamine release, and AAA formation.

Based on findings from human AAA samples where an increased number of MCs are found in the media compared to controls and specimens with atherosclerosis, an important role of MCs can also be expected in human AAA.\textsuperscript{3,5} MC-derived chymase has been suggested to play a key role.\textsuperscript{18}

**Influence of MCs on Matrix-Degrading Activity in Human AAA**

Several proteolytic systems, for example, matrix metalloproteinases (MMPs),\textsuperscript{19} polymorphonuclear neutrophil-derived elastase,\textsuperscript{20} and cysteine proteinases,\textsuperscript{21} have been shown to contribute to the degradation of collagen and elastin in the aortic wall in human AAA. Neutrophils are abundant within the most luminal layer (blood interface layer) of the intraluminal thrombus (ILT),\textsuperscript{22} present in most AAAs of a clinically relevant size.\textsuperscript{23} Neutrophils are also present in the adventitia of AAA.\textsuperscript{22} It has been suggested that their content of elastase and cathepsin G contributes to degradation of collagen and elastin in the AAA wall. Accordingly, evidence indicates that the presence of ILT explains the increased elastin degradation in the AAA wall underlying the ILT, compared to wall segments without ILT.\textsuperscript{20}

Of the matrix-degrading proteolytic enzymes relevant in the pathogenesis of AAA, various members of the MMP family, particularly MMP-9 and MMP-2, have received most attention. These 2 gelatinases, found in both the ILT and the AAA wall, are considered to be important matrix-degrading substances.\textsuperscript{24} Unfortunately, many studies evaluating the role of MMPs have not differentiated between inactive proforms and the active forms of the enzymes. Such distinction is important, because the ratios between active and latent forms for both MMP-2 and MMP-9 are different in control and AAA. The serine proteinases chymase and trypsin released by human MCs activate proforms of both MMP-1 and MMP-3, and the latter in turn serves as an activator for the proform of MMP-2.\textsuperscript{25} Cathepsin G also activates the proforms of human MCs activate proforms of both MMP-1 and MMP-3, and the latter in turn serves as an activator for the proform of MMP-2.\textsuperscript{26} Cathepsin G also activates the proforms of both MMP-2 and -9 are different in control and AAA samples where an increased number of MCs are found in the media compared to controls and specimens with atherosclerosis, an important role of MCs can also be expected in human AAA.\textsuperscript{3,5} MC-derived chymase has been suggested to play a key role.\textsuperscript{18}

**Neovascularization and MCs**

Neovascularization is regularly found in human AAA, and the occurrence of neovessels is associated with the degree of elastin degradation and inflammation.\textsuperscript{37} The causes of neovascularization in AAA are not completely known. Vorp et al have suggested that the low oxygen tension in the AAA wall covered by an ILT is causally linked to neovascularization. Consequently, it is accentuated in the wall covered by a thick ILT,\textsuperscript{42} an area where inflammation and matrix degradation are more pronounced compared to wall segments not covered by the ILT.\textsuperscript{43}

The importance of neovascularization for growth and rupture of AAA in humans is not definitively proven. Choke et al investigated the presence of neovessels at sites of rupture in human AAAs and found them to be more prevalent at the edge of the rupture compared to nonruptured sites and specimens from nonruptured AAAs.\textsuperscript{44} The neovessels at the ruptured area were less mature than those found at other sites, rendering them prone to rupture. Increased mRNA for factors associated with neovascularization, such as \(\alpha_2\)-integrin, VEGF, vascular endothelial cadherin, and vimentin was also found to be increased at rupture sites.\textsuperscript{44} It should be mentioned that hypoxia-inducible factor (HIF)-1\(\alpha\) has also been identified close to rupture sites, indicating cellular hypoxia.\textsuperscript{45}

The number of MCs in human nonruptured AAAs was shown by us to be highest in areas that also show the most extensive neovascularization.\textsuperscript{37} In line with previous findings by Choke et al,\textsuperscript{44} we also found a strong positive correlation between markers for endothelial cells (CD31) and factors associated with neovascularization, (FMS-related tyrosine kinase-1, FLT-1, and VEGF). SCF, a growth factor and chemoattractant for MCs, was identified in endothelial cells in neovessels found in close proximity to MCs.\textsuperscript{10} The multiplicity of the potential mechanisms of MC-mediated angiogenesis has been reviewed previously.\textsuperscript{46}

In rats with AAAs induced by CaCl\(_2\), there is also evidence that the capillary density depends on the presence and function of MCs. Consequently, when treated with the MC-stabilizer tranilast, these rats had lower aortic capillary density compared to controls. In addition, the tranilast-treated mice had fewer MCs, T lymphocytes, and macrophages in their abdominal aortas. Similarly, treatment of MC-competent control rats with tranilast was found to significantly decrease capillary density in parallel with attenuated AAA formation.\textsuperscript{5} MC-deficient mice also showed a less pronounced neovascularization, as judged by the number of CD-31–positive microvessels.\textsuperscript{47} A causal role for neovascularization in the development of AAA is supported by
findings of significantly reduced elastase-induced AAA formation in rats, in which inhibition of Ets-1, an essential transcription factor for angiogenesis, was achieved by an Ets-1 decoy. In common with the finding in rats, Ets-1 seems to play a role also in rabbit AAA formation induced by elastase.

**RAS and MCs**

Many studies have shown that subcutaneous infusion of angiotensin II causes the development of AAA in experimental animals. These studies, usually performed in apoE-deficient or low-density lipoprotein receptor-deficient mice, cause AAA in the suprarenal abdominal aorta independent of blood pressure changes. Systemic activation of angiotensin leading to hypertension appears to be irrelevant regarding the blood pressure changes. Systemic activation of angiotensins, which converts angiotensinogen to Ang II and also angiotensinogen to Ang II, has also been reported in MCs. The role of chymase in AAA is of particular interest in hamsters, because chymase is more important than angiotensin-converting enzyme (ACE) for conversion of Ang I to Ang II in this animal species.

ACE inhibitors prevent AAA formation in rats subjected to elastase perfusion of the aorta, but angiotensin receptor blockers fail to do so. AAA formation in dogs, induced by local elastase infusion, is attenuated by a chymase inhibitor, an inhibition paralleled by decreased MMP-9– and Ang II–induced vascular injury, which is caused by local conversion of angiotensinogen to Ang I, and further to Ang II, followed by interaction with angiotensin receptors in the vascular tissue involved. MC-derived chymase converts Ang I to Ang II. In addition, cathepsin G, present in MCs and neutrophils, converts Ang I to Ang II and also angiotensinogen to Ang II, although more slowly. The presence of renin, which converts angiotensinogen to Ang I, and further to Ang II, is reported to be a requirement for AAA formation by Ang II. In this animal species, chymase is found in elastase-induced AAA in hamsters, and a specific inhibitor of chymase attenuates AAA formation in this animal model. ACE inhibitors prevent AAA formation in rats subjected to elastase perfusion of the aorta, but angiotensin receptor blockers fail to do so. AAA formation in dogs, induced by local elastase infusion, is attenuated by a chymase inhibitor, an inhibition paralleled by decreased MMP-9– and Ang II–induced activity. The ratio of ACE to chymase for angiotensinogen in the abdominal aortic wall varies between species; notably in humans, 70% is chymase-dependent. In human AAA, an increased local Ang II formation by both chymase and ACE has been reported.

In human AAA, the protein levels of angiotensinogen and angiotensinogen type I (AT₁) receptor, but not of AT₂ receptor, have been demonstrated by Kaschina et al to be increased compared to both normal and atherosclerotic aorta. The authors concluded that the presence of AT₂ receptor discriminates between atherosclerosis with and without AAA. The same authors also reported that components of RAS colocalize with neutrophils and MCs. The presence of AT₁ receptors is reported to be a requirement for AAA formation by Ang II also in experimental animals.

Accumulating evidence thus supports a role for renin, chymase, and cathepsin G released by activated MCs to contribute to Ang II formation. In this context, it should be emphasized that MCs seem to be of greater importance than neutrophils for cathepsin G–dependent formation of Ang II in human AAA. Ang II activates macrophages, leading to expression of monocyte chemoattractant protein-1 (MCP-1), which causes recruitment of more monocytes and macrophages in human atherosclerotic lesions.

A register-based study reported that patients treated with ACE inhibitors were less likely to be admitted for ruptured AAA compared to patients with other medications. The study, however, failed to show a similar effect of angiotensin receptor blockers. The authors suggested that angiotensin activation could be of importance for the risk of rupture. The finding, however, is at variance with a study based on a cohort from the UK Small Aneurysm Trial, which found that patients using ACE inhibitors had an increased growth rate of their AAs. The different conclusions from the 2 studies could be attributed to 1 study evaluating rupture risk, and the other, AAA growth. ACE inhibition increases bradykinin levels, and experimental studies indicate that decreased bradykinin activity may be associated with increased AAA formation. Deficiency of kinin B receptor aggravates AAA formation in apoE−/− mice. In addition, rats with a single point mutation resulting in kininogen deficiency have an increased tendency for AAA formation, which is associated with upregulation of cytokines, leading to AAA formation without influencing atherosclerosis. The final answer as to whether ACE inhibition prevents AAA growth in humans is, however, unresolved, but could be dependent on the balance between ACE and kinin/kininogen levels.

Taken together, there is evidence that angiotensin-induced AAA formation can be linked to the presence of MC-derived enzymes in the aortic wall. MCs are important for formation of Ang II, which leads to macrophage activation, and further macrophage recruitment, leading to MMP release. In this context, it should be noted that MC-derived tryptase, chymase, and cathepsin G are important for activation of MMPs.

**SMC Apoptosis and MCs**

Elastin and collagen are the principal load-bearing components in the human aortic wall, and in AAA, their contents decrease. Elastin, a macromolecule of tropoelastin, has an extremely long half-life and a limited period of synthesis, restricted to early life. Tissue repair in AAA, therefore, primarily depends on collagen synthesis by SMCs. Accordingly, the viability of SMCs in the aortic media is critical. In human AAA, the typically increased inflammatory response is associated with SMC apoptosis.

SMC apoptosis has been recorded also in experimentally induced AAA and is attenuated when MC function is absent, as demonstrated in MC-deficient animals and in normal animals treated with MC-stabilizing drugs. MCs may cause SMC death by the following pathways: TNF-α is released by activated MCs and binds to the “death receptors” on the surface of SMCs, which ultimately leads to caspase-mediated apoptosis of the target SMCs. SMC death may also be caused by anoikis, which implies that cells have lost their anchorage to the surrounding/underlying matrix. MC-derived chymase avidly degrades fibronectin secreted by SMCs, which leads to disruption of focal adhesion complexes and ensuing death of the SMCs by an anoikis pathway. Granzyme B, which was recently demonstrated to be present in human AAA, is also released by MCs, and can initiate SMC apoptosis. Finally, MC-derived chymase inhibits collagen synthesis by viable SMCs. Thus, even in the absence
of SMC death, MC activation may contribute to decreased collagen content in the inflamed aortic wall.

**Summary and Future Aspects**

A large number of studies regarding the pathogenesis of AAA have been published, but only during the last years have reports been published that describe molecular cascades suggesting specific roles for MCs in human and experimental AAA pathogenesis. Previous publications describing matrix degradation in the pathogenesis of AAA have mostly focused on MMPs, but the roles of cathepsins and neutrophil elastase have also been covered. In this review, we suggest a role for MCs in AAA development based on studies in humans and experimental animals. Contributing factors include MC-derived proteases, mainly chymase and tryptase, both for activation of other systems such as MMPs and for direct action on matrix components; for example, chymase induced activation of RAS and degradation of fibronectin. In addition, MCs influence SMC apoptosis and neovascularization, both considered to be important for the growth of AAA (see the Figure). Taken together, the findings of animal and human studies suggest that MC inhibition could be a promising mode of treatment to inhibit AAA growth.

Today, many AAAs are found unexpectedly in individuals undergoing abdominal examination by CT, MRI, or ultrasound. In addition, many countries have introduced screening for AAA. Based on 2 large trials, it has been shown that there is no significant reduction of the risk for rupture by prophylactic surgery for AAAs with a diameter 4.0 to 5.5 cm. Consequently, patients with AAA are recommended to undergo repair of their aneurysms, when the diameter exceeds 5.5 cm. Because the risk of rupture is related to the diameter, an important aim for AAA research is to find methods to attenuate growth of small AAAs. Randomized and cohort studies in humans, as well as animal studies, have been performed to find drugs that inhibit growth of small AAAs. Randomized trials in humans have only shown effects for roxithromycin and doxycycline, but with low levels of evidence.

Drugs targeting the pathways of MC-derived mediators may be of value in the future in the treatment of patients with AAA. Because MC inhibitors are used in the treatment of allergies, such drugs could be of value in the future for inhibiting growth of small AAAs without need for surgical repair.

It could be speculated that MCs are of importance also by mechanisms other than those reported in detail in this review. Because MCs are a major source of histamine, histamine-mediated mechanisms could play a role also in the pathogenesis of AAA. Interestingly, histamine H4 receptor blockers are potent inhibitors of MC-mediated activation of T cells and monocytes.

Activated MCs rapidly generate and release abundant quantities of eicosanoids, among them leukotrienes (LTs). Recently, it was shown that LT D4 induces MMP-2 activity in human AAA tissue in a cysteinyl LT receptor–dependent manner. Because LTs seem to regulate MMP expression and activity, LT receptor antagonists or inhibitors of selected key enzymes in the LT synthetic pathway might attenuate development of AAA and also the contribution of MCs in the pathogenesis of AAA.

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References


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