

Are mast cells the real culprit in atherosclerosis?

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This editorial refers to ‘Mast cells in human carotid atherosclerotic plaques are associated with intraplaque microvessel density and the occurrence of future cardiovascular events’[†], by S. Willems *et al.*, on page 3699–3706

Atherosclerosis in the presence of hyperlipidaemia is considered today as an inflammatory disease of the arterial wall. The dominant inflammatory cell is the macrophage; however, interaction with other inflammatory cells and red cells may be critical in the outcome of the plaque, i.e. whether plaques remain stable or become unstable. It has been repeatedly shown that macrophage interaction with T cells and dendritic cells is critical for the progression of plaques and for maintaining and sustaining inflammation. The role of mast cells in atherosclerosis has only been studied sporadically, and that too mostly in the aortic and coronary beds. Willems *et al.*¹ now show for the first time not only that severe carotid atherosclerotic disease is associated with a high number of mast cells but that the instability of the plaque may be influenced by the number of mast cells and their state of activation, which probably contribute to angiogenesis and plaque haemorrhage. Also, patients with high intraplaque mast cells had significantly greater cardiovascular events during a 3-year follow-up. However, the basic mechanisms involved will need further study in both animal models and man.

Paul Ehrlich’s doctoral thesis was a milestone in the study of mast cells, and he noted that they had similar staining characteristics to basophils, demonstrating metachromatic staining with aniline dyes.² Mast cell development depends on the expression of KIT ligand, also known as mast/stem cell growth factor (SCF), receptor from the pluripotent haematopoietic progenitor cells (HPCs) that express CD34⁺, CD117⁺, CD13⁺, FcεRI⁺, maturing in the target tissues, such as skin, mucosal surfaces, and probably also in the arterial wall, although other factors are necessary.³ The knowledge that anaphylaxis was linked to release of histamine, a component of mast cells, was the beginning of the understanding of the role of mast cells in immunological reactions especially in anaphylaxis and innate and adaptive immunity. Mast cells exert physiological functions when activated by stimuli such as IgE and IgG, FcR signalling by bacterial antigens, complement components, and endogenous inflammatory factors, which result in the immediate release of mast cell cytoplasmic

granules. The mast cells either produce or release proinflammatory cytokines [interleukin-6 (IL-6), interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), and others], chemokines [monocyte chemoattractant protein-1 (MCP-1), IL-8, RANTES (regulated upon activation, normal T-cell expressed and secreted), eotaxin, and leukotrienes], proteases [tryptase, chymase, angiotensin-converting enzyme (ACE), carboxypeptidase, and cathepsin G], growth factors [fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF)], histamine, heparin, and chondroitin sulfate proteoglycan. The proteases also exert their influence on LDL- and HDL-cholesterol. Mast cell tryptase and chymase promote macrophage LDL uptake and also result in functional changes in HDL₃ components, apolipoprotein (apo) E, apoA-I, and apoA-IV, that results in inability of foamy macrophages to promote cholesterol efflux.⁴ (Figure 1)

Constantinides in 1953/54 first noted the presence of mast cells in atherosclerotic lesions in rabbits and man.⁵ A higher prevalence of mast cells was shown predominantly in the adventitia of both aortic and coronary atherosclerotic lesions (raised lesions—fatty streak, fibrofatty plaque, and fibrous plaque) compared with those without any lesions in subjects 15–34 years of age dying of non-cardiac causes.⁶ In the aorta, the number of mast cells was significantly greater in the dorsal (lesion ‘prone’) as compared with the ventral (lesion ‘resistant’) half of the wall. Also, the number of mast cells was 10 times greater in the adventitia in atherosclerotic lesions as compared with the intima.⁶ By tryptase staining, mast cells have been demonstrated to be located in the endothelium and subendothelium, as well as in the shoulder regions of both early and more advancing plaques.⁵ Degranulated mast cells have been found at the site of rupture and those mast cells can activate matrix metalloproteinase (MMP)-1, -2, and -9 through release of proteases leading to weakening of the fibrous cap, which makes them a powerful participant in the conversion of a stable lesion into an unstable plaque even if their numbers are far fewer than macrophages.³ We have also shown that mast cell numbers were increased in a young man with transient vasospasm and minimal disease in the left anterior descending coronary artery.⁷ Histamine, a powerful vasoconstrictor, has been shown to be increased in the coronary arterial wall of patients

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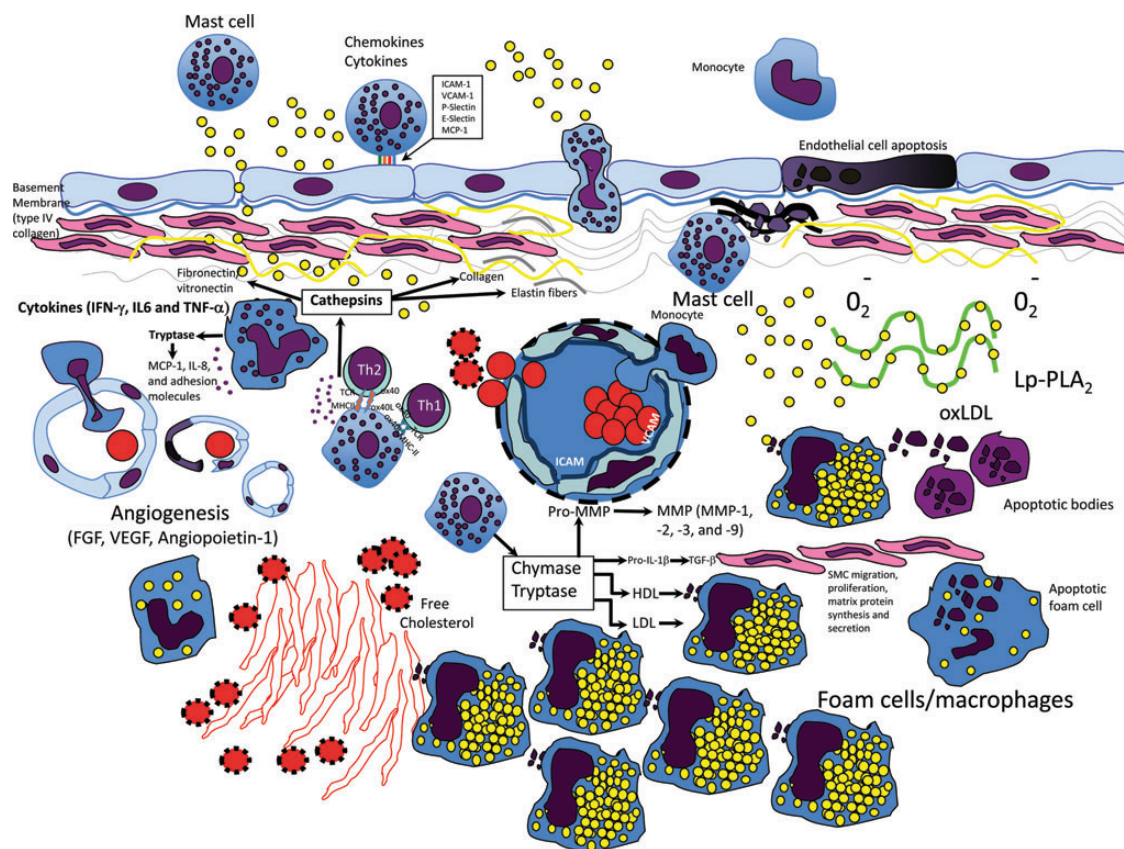


Figure 1 Mast cell function in atherosclerosis. Mast cells use integrins for interaction with intercellular adhesion molecule-1 (ICAM-1) from vascular endothelium. They reach the subendothelial space via the cell surface chemokine receptors and target tissue chemokines [monocyte chemoattractant protein-1 (MCP-1), eotaxin, and RANTES (regulated upon activation, normal T-cell expressed and secreted)]. Mast cells function by releasing various mediators such as histamine, heparin, proteoglycans, serotonin, cytokines [interferon- γ (IFN- γ), interleukin-6 (IL-6), and tumour necrosis factor- α (TNF- α)], chemotactic factors, and angiogenic factors such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and angiopoietin-1. Mast cell proteases, such as chymases and tryptases which are secreted as active compounds by the mast cell granules, convert pro-matrix metalloproteinases (pro-MMPs) to MMPs (especially MMP-1, -2, and -9) that degrade matrix molecules. Similarly, cathepsin G also degrades collagen, elastin, and fibronectin/vitronectin, which are essential for adhesion of smooth muscle cells (SMCs) and endothelial cells, and this lack of support and Akt dephosphorylation lead to their apoptosis. Likewise, the degraded matrix molecules also enhance leukocyte recruitment and liberate angiogenic factors. Chymases activate pro-IL-1 β and lead to production of transforming growth factor- β 1 (TGF- β 1) which induces SMC migration and proliferation. Proteases alter LDL such that there is enhanced macrophage LDL uptake and conversion to foam cells; at the same time, HDL₃ is degraded, resulting in destruction of its ability to efflux cholesterol from foam cells. Lp-PLA₂, lipoprotein-associated phospholipase A₂; oxLDL, oxidized LDL; TCR, T-cell receptor; VCAM, vascular cell adhesion molecule.

dying from coronary artery disease.⁸ The most compelling data of the involvement of mast cell in the generation of unstable lesions come from the laboratory of Biessen who showed in ApoE^{-/-} mice that activation of mast cells resulted in larger plaque area, an increase in macrophages, intraplaque haemorrhage, and iron deposition as compared with control non-stimulated mice.⁹

Willems *et al.*,¹ showed that intraplaque haemorrhage was significantly associated with the presence of mast cells. The authors also showed that as the number of mast cells increases in the carotid plaque, so does the microvascular density, and that the combined presence of macrophages and mast cells leads to an even greater increase in microvascular density, which is not surprising. However, what was surprising was the similar influence of neutrophils on microvessels when combined with macrophages. However, macrophages

alone also led to an increase in microvessel density, but neutrophils alone had no influence; thus suggesting that neutrophils and mast cells both increased microvessel density in the presence of macrophages and that the best correlation of microvascular density was observed with mast cells. Also, greater numbers of mast cells were observed in plaques with haemorrhage, and those subjects with haemorrhage were more likely to have future cardiovascular events at 3 years. In other words, the presence of mast cells correlates with microvascular density which is a predictor of haemorrhage, and mast cell numbers are significantly higher in symptomatic as compared with asymptomatic carotid disease. The only clinical marker that was elevated was plasma tryptase, and it too correlated with plaque mast cells. However, the information that is still lacking is how does all this happen and why. Are mast cells the real culprit

for plaque angiogenesis and haemorrhage? From the study of Willems *et al.*, we cannot draw these conclusions. All one can say is that there is an association. The significance of these findings would have been more compelling if the plaque protein levels of RANTES, eotaxin, TGF- β , and MCP-1 were elevated and also correlated with tissue mast cell number and extent of activation. The authors did show that higher plasma tryptase levels correlated with tissue mast cells, degranulating mast cells (per mm²), and microvascular density; however, plasma tryptase levels are not only elevated in those with carotid artery atherosclerosis and haemorrhage but also in patients with allergic reaction, gastrointestinal disorders, mastocytosis, infections, hypereosinophilic syndrome, and others. We are told very little about the general state of health of the 264 patients included in the analysis. Therefore, it may be a hard sell that increased plasma levels of tryptase occur in all patients due to carotid disease.

There is ample evidence that mast cells are extremely active cells and indeed play a central role in anaphylaxis and immunological reactions. We are beginning to understand with greater clarity the interaction between mast cells and T-cell subtypes (Th1, Th2, Treg, and CD8 cells), B cells, macrophages, and dendritic cells, not only in immunology but also pertaining to development and progression of atherosclerosis, as Willems *et al.* have now illustrated. However, the jury is still out!

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