

## MAST CELLS AND MAST CELL GROWTH FACTOR: POSSIBLE ROLE IN AURICULAR THROMBOSIS

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### SUMMARY

• Although mast cells have been implicated in many vascular and inflammatory reactions, the mechanisms underlying such mast cell-dependent processes have not been fully clarified. Recently we have established an association between mast cells and local thrombus formation. The most useful model for studying molecular mechanisms of mast cell-thrombosis interactions appeared to be auricular thrombosis. In the present review we summarize our findings in patients with auricular thrombosis with regard to functionally important molecules that can either be expressed and released by cardiac mast cells or interact with mast cells via distinct cell surface molecules. The potential prothrombotic function of mast cells in atrial thrombosis is discussed.

### INTRODUCTION

• Mast cells (MC) are multifunctional immune cells in loose connective tissue. These cells are usually located in vicinity to small blood vessels (1-3). Human MC produce a number of vasoactive or thromboactive mediators including histamine, heparin, proteolytic enzymes or cytokines (4-7). Some of these compounds, such as heparin, tryptase and chymase, are almost exclusively expressed in MC. Human MC have been implicated in vascular and inflammatory reactions. However, the molecular mechanisms which are involved in MC dependent processes are not well understood.

It has been shown that differentiation of human MC from their progenitor cells *in vitro* is inducible by distinct stromal cells such as 3T3 fibroblasts (8). One factor involved has been identified and termed mast cell growth factor (MGF). This cytokine is also well-known as stem cell factor, steel factor or c-kit ligand (9-12). The receptor for MGF belongs to the tyrosine kinase family, subtype III, and is encoded by the c-kit proto-oncogene (13). Recently, this receptor has been clustered as CD 117 (14). Two molecular forms of human MGF have been described: a membrane bound form and a soluble one. These two forms are generated by tissue-specific splicing (11,12,15). Mice with mutations at the c-kit locus or at the MGF one typically exhibit anemia, absence of MC, white spotting of the skin, and sterility (1). Thus, MGF-c-kit receptor interactions are critical for normal hematopoiesis and development and survival of MC, cutaneous melanocytes and germ cells (13). Recent studies have demonstrated that multiple functions including growth, mediator production and secretion as well as chemotaxis (16,17) of MC are regulated by MGF (18,19).

MGF is expressed by stromal cells, including fibroblasts and vascular endothelial cells (13,20). *In vitro* studies have shown that thrombin, interleukin-1, and tumor necrosis factor (TNF- $\alpha$ ) can induce expression of MGF in endothelial cells (20). The soluble form of MGF is made abundantly and is present in human peripheral blood at a concentration of approximately 1-10 ng/ml (21) which is in the range that can promote mediator release from isolated human skin MC *in vitro* (22). How-

ever, very little is known about the tissue levels of membrane bound or soluble forms of MGF produced *in vivo*.

The c-kit receptor is expressed in immature bone marrow cells, oocytes, neural cells, and melanocytes. In addition, both immature MC progenitors and mature tissue MC express MGF binding sites.

### CARDIAC MAST CELLS

- Recently, the human cardiac MC have been characterized by our group (23). Phenotypical, functional and biochemical characteristics are closely related to MC of other body regions. Elevated number of MC were found in the atrial appendages of human hearts compared to the ventricular wall. The reason for this physiologic augmentation is unknown. We also investigated atrial appendages for changes in MC number, distribution and phenotype in disease states of the appendage, namely auricular thrombosis, in order to elucidate a possible role of MC and their mediators in thrombogenesis (24).

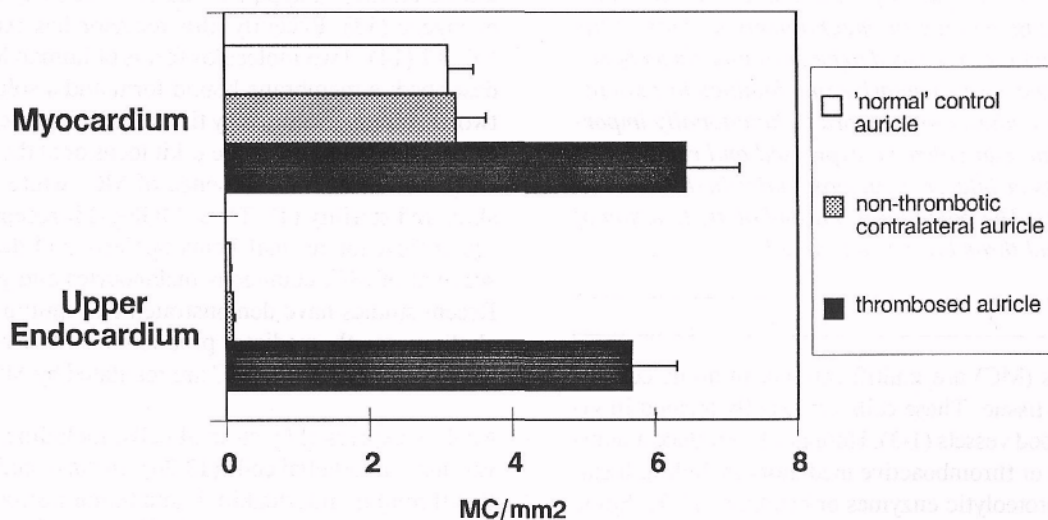
### AURICULAR THROMBOSIS

- The predilection site for thrombus formation in the human heart apart from cardiac infarction areas is the atrial appendage (25). The reason for the predilection of the auricle may be its distinct anatomy as well as the abnormalities in blood flow patterns. Patients with atrial fibrillation reveal a significantly reduced or absent blood flow with stasis (26-28). Other

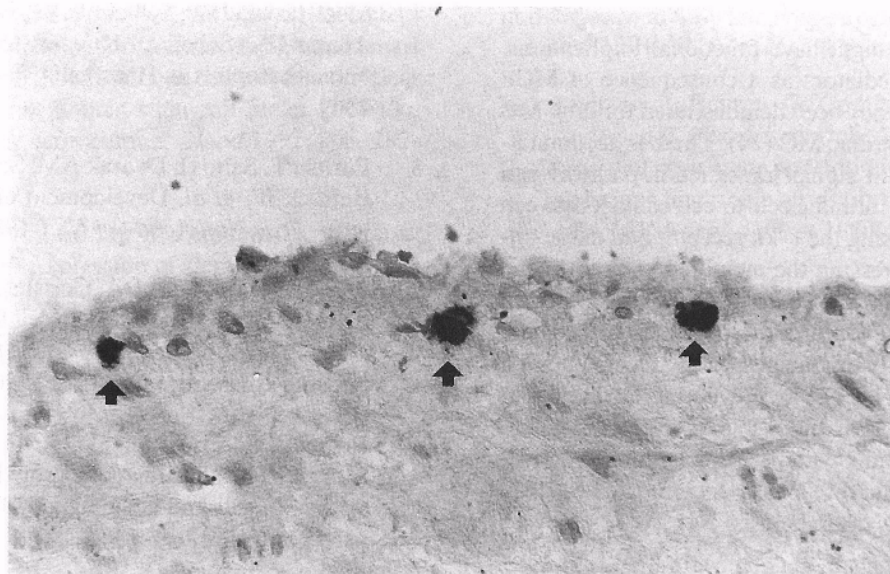
studies have shown that atrial appendage thrombus formation went along with auricular dilatation as well as decreased contraction (27,29). The increased incidence of thrombus formation in atrial fibrillation implies a role for hemodynamic factors. It has been demonstrated that atrial thrombus formation is associated with decreased atrial appendage contraction and atrial appendage dilatation (29). Anticoagulant therapy in atrial fibrillation lowers the risk of stroke indicating the involvement of humoral factors in thrombogenesis (30,31). Furthermore during anticoagulant therapy the thrombotic atrial mass may even decrease in size or dissolve (32-34). These observations point to endogenous prothrombotic mechanisms, since conventional anticoagulation exhibits no thrombolytic effect by itself. Endothelial and subendothelial cells may also contribute to local thrombus formation and/or thrombolysis due to their active role in the regulation of blood coagulation (35,36). So far, the exact molecular mechanisms and cell types involved in thrombosis and thrombolysis could not be defined.

### MAST CELLS AND MGF IN AURICULAR THROMBOSIS

- In recent studies (23,24), we could demonstrate a significant augmentation of atrial MC in patients with auricular thrombosis (Fig. 1) and a redistribution of augmented MC to the upper endocardium, close to the thrombus (Fig.2). These changes in the endocardium of thrombosed auricles were accompanied with an overexpression of the c-kit ligand MGF and downregulation of c-kit receptor on the redistributed MC. In addition, none of those phenomena were observed in fibril-



**Figure 1.** Number of mast cells (MC) per  $\text{mm}^2$  in thrombosed (black) and non-thrombotic contralateral auricles (grey) of patients suffering from auricular thrombosis and 'normal' control auricles (white). MC increase significantly in the myocardium and the endocardium of thrombosed atrial appendages but do not in the non-thrombotic auricles of the same patients. Moreover, MC redistribute to the upper endocardium in thrombosed auricles.



**Figure 2.** Three redistributed mast cells (MC) (arrows) in the upper endocardium in a patient with auricular thrombosis. MC were visualized by immunoperoxidase staining technique using mAbs to MC tryptase.

lating non-thrombotic contralateral appendages of the same patients or unaffected auricles of control patients suffering from atrial fibrillation (Fig. 1). Moreover, no significant correlation between MC number and atrial fibrillation onset could be found. Taken together these data, atrial fibrillation is not likely to trigger MC augmentation. The changes in thrombosed appendages were not due to an unspecific inflammatory process because neither macrophages, lymphocytes, nor polymorphonuclear leukocytes were significantly increased, neither in the endocardium, nor in the myocardium. This observation was farther supported by the fact that no upregulation of the leukocyte adhesion molecules ICAM-1, VCAM-1, ELAM-1, and PEC AM-1 could be substantiated in auricular thrombosis.

A possible link between MC and thrombosis has already been considered. In 1955, Sundberg reported on an increase of perivascular MC above average in certain thrombotic diseases (37). Three years later, Pomerance (38) observed an increase of MC in the adventitia of thrombosed coronary arteries. Studies by Kitamura *et al* (39) and Hatanaka *et al* (40) have shown that MC-deficient mice are hyperresponsive to thrombogenic stimuli. An augmentation of MC was also seen in coronary thrombosis in cocaine abusers and in early lesions of atherosclerosis (41,42).

The observed MC redistribution in auricular thrombosis is associated with an overexpression of MGF in the upper en-

docardium (24). This cytokine promotes the development of human MC from their progenitors present in bone marrow (43) and in peripheral blood (19). Additionally, MGF is a chemotactic factor increasing the directional motility of MC (16,17). Both effects of MGF could contribute to the MC augmentation in auricular thrombosis. MC accumulation in the endocardium of thrombosed auricles could be due to local differentiation from progenitor cells or due to chemoattractance of mature MC. The hypothesis of MGF as a key regulator of MC distribution is supported by several observations. Abnormal production of MGF was found in cutaneous lesions of patients with mastocytosis (44). Galli *et al* (45) administered human recombinant MGF to monkeys and observed an expansion of the MC population in many tissues and organs. It is reasonable to assume that locally overexpressed MGF plays an important role in the accumulation of MC either by local differentiation or by chemoattractance.

Redistributed MC in the upper endocardium of thrombosed auricles, where MGF, their natural ligand, is overexpressed, displayed weaker or absent reactivity with mAbs to c-kit when compared either to MC in the lower endocardium and myocardium of thrombosed appendages, or to MC in control auricles (24). One explanation for this phenomenon could be that receptors were covered by locally expressed MGF. On the other hand, MGF is known to cause downregulation of c-kit mRNA in MC, so that the loss of c-kit receptors from the cell

surface may be explained by ligand induced downregulation of c-kit protein production (46-48).

Our findings of MGF overexpression and c-kit downregulation in auricular thrombosis might have functional implications. MC can release their mediators as a consequence of MGF stimulation. This has already been demonstrated for lung MC (18) and confirmed for cardiac MC (24). There is accumulating evidence that the c-kit signal transduction pathway can also be activated through intimate cell-to-cell contact between proliferating cells expressing the c-kit receptor and tissue-anchored stromal cells expressing the membrane-bound ligand (11,49). Thus, it is tempting to speculate that the overexpressed c-kit ligand in the upper endocardium may cause or promote mediator release in locally augmented MC. Since MC are a unique source of heparin (1,50,51), preventing further apposition of thrombotic material, and a source of proteolytic enzymes, including prothrombolytic tissue plasminogen activator (52), one may speculate that locally accumulated MC can participate in endogenous thrombolysis.

## CONCLUSIONS

- The association between atrial appendage thrombosis, MC augmentation, MC redistribution and MGF overexpression may lead to the hypothesis that augmented and redistributed MC and their mediators play an important role in the pathophysiology of atrial thrombosis.

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