Mast cells as immune regulators in atherosclerosis

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Chapter 2

The impact of mast cells on cardiovascular diseases

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Abstract

Mast cells comprise an innate immune cell population, which accumulates in tissues proximal to the outside environment and, upon activation, augments the progression of immunological reactions through the release and diffusion of either pre-formed or newly generated mediators. The released products of mast cells include histamine, proteases, as well as a variety of cytokines, chemokines and growth factors, which act on the surrounding microenvironment thereby shaping the immune responses triggered in various diseased states. Mast cells have also been detected in the vessel wall and are implicated in the onset and progression of numerous cardiovascular diseases. Notably, modulation of distinct mast cell actions using genetic and pharmacological approaches highlights the crucial role of this cell type in cardiovascular syndromes. The acquired evidence renders mast cells and their mediators potential prognostic markers and therapeutic targets in a broad spectrum of pathophysiological conditions related to cardiovascular diseases.

1. Introduction

Mast cells are innate immune cells characterized by a monolobular nucleus and numerous cytoplasmic granules[1], morphological features which distinguish them from a variety of cell types that comprise the immune system. Originating from hematopoietic stem cells within the bone marrow[2], mast cell progenitors are released into the circulation and, upon the influence of cytokine and chemokine signals, home in tissues and further differentiate into mature mast cells[3]. The place of maturation for mast cells is either mucosal surfaces or connective tissue, therefore granting them a wide distribution throughout the body. Tissue resident mature mast cells exert their effector functions after activation triggered by cytokines, antibodies and proteins specific for receptors present on their surface. The most widely studied mast cell activation pathway is the antigen-specific activation by Immunoglobulin-E (IgE) antibodies, which bind to the high affinity membrane receptor Fcepsilon Receptor-1 (FcεR1)[4]. However, during the last decades, additional agents triggering mast cell activation have been discovered, such as the Immunoglobulin-G (IgG) antibodies[5], complement system components[6], but also pathogenic Toll-like receptor peptides[7], as well as endogenous ligands like substance P (SP)[8] and endothelin-1 (ET-1)[9].

The mast cell secretory granules contain a variety of molecules such as proteoglycans (e.g. heparin and chondroitin sulfate), histamine, cysteinyl cathepsins, proteases (e.g. chymase and tryptase) and a broad spectrum of pro-inflammatory as well as anti-inflammatory cytokines[10]. Through their mediator release, mast cells are able to act on the adjacent cells and shape the local microenvironment.

In addition, mast cells residing in different tissues exhibit a diverse protease content, and can consequently be separated into various subpopulations. However, despite the differences in the granule protease composition of each mature mast cell subtype, their content can be actively shaped depending on signals received from the surrounding microenvironment[11,12]. Finally, the ultimate protease phenotype in a tissue may switch from one to another in a reversible way. Such *de novo* formation of mast cell mediators, which extends itself beyond the granule proteases, depends not only on the intrinsic characteristics of each tissue but also on local pathologic conditions at any given point, granting remarkable plasticity to mast cells regarding their effector functions[13,14].

Within the cardiovascular system, mast cells reside in close proximity to blood vessels, as well as in the heart of both humans and rodents[15], where they participate in physiological functions such as angiogenesis and local generation of the vasoconstrictive hormone Angiotensin II (Ang II)[16]. The majority of mast cells populating the human heart and vessels consist mainly of tryptase[17], while in mice they are recognized as connectice

tissue mast cells and contain granules filled with heparin, chymase and tryptase[18,19]. However, particularly in humans, the mast cell protease content in the vessels shows remarkable variation among subjects[20], attributing each protease a differential role in the pathophysiology of cardiovascular diseases. Yet, aside from proteases, there is a plethora of additional mast cell mediators which participate in the pathological events observed in cardiovascular syndromes (**Figure 1**).

Mast cells in the vasculature are mainly distinguished for their adverse effects in syndromes such as abdominal aortic aneurysm[21], myocardial infarction[22], and atherosclerosis[23], displaying thus a crucial role in the leading cause of death worldwide[24]. It is therefore intriguing to pinpoint the overall importance of mast cells in a wide variety of such conditions.

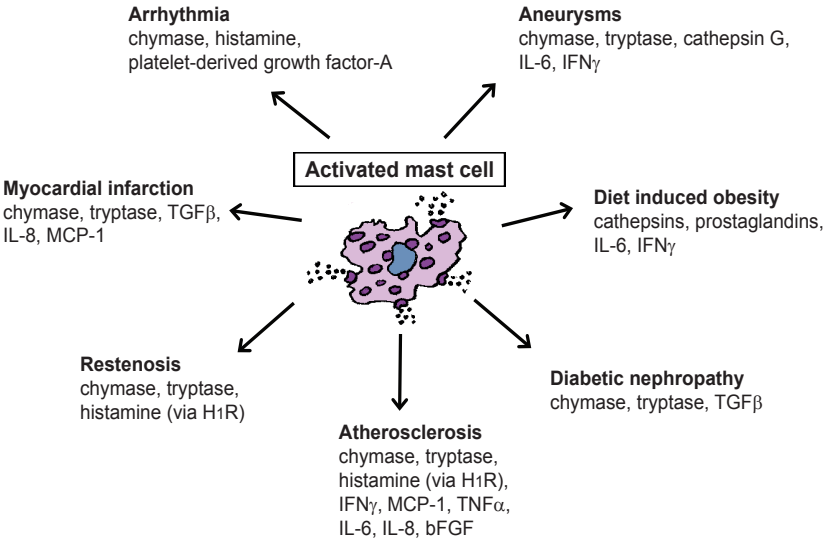

Figure 1: Mast cells in cardiovascular diseases. This figure depicts major cardiovascular diseases in which mast cells have been implicated. Specific mast cell mediators, summarized here, have been reported in each of these syndromes. *Abbreviations: IL-6: Interleukin-6, IL-8: Interleukin-8, TNFα: tumor necrosis factor-alpha, IFNγ: Interferon-gamma, TGF-β: transforming growth factor-beta, MCP-1: monocyte chemoattractant protein-1, bFGF: basic fibroblast growth factor.*

2. Diet-induced obesity

The incidence of obesity, due to high fat diet, has become a Western society epidemic and is closely linked to type 2 diabetes, as well as other metabolic and cardiac disorders[25]. Increased chronic inflammation at a low degree is observed in white adipose tissue (WAT) of obese humans and mice, with local infiltration of macrophages[26] and T cells[27]. Mast cells have also been detected in obese adipose tissue, located next

to microvessels and are directly associated with the pathology of this disorder. More specifically, Liu et al. reported in 2009 an increased number of mast cells in WAT of obese humans and mice, accompanied by elevated serum tryptase levels as well as local and systemic levels of inflammatory cytokines, chemokines and proteases, compared to lean subjects. Here, mast cells contribute to WAT apoptosis and angiogenesis. This effect is exerted *via* Interleukin-6 (IL-6) and Interferon-gamma (IFNγ) cytokines, which in turn increase cysteinyl cathepsin expression, thus promoting diet-induced obesity. Importantly, in the cited study, mast cells are observed to infiltrate obese WAT prior to macrophages. Likewise, mast cells are shown to co-localize with $CD8^+$ T cells in mouse WAT[28], suggesting a role in adipose tissue inflammation. An additional study reported that mast cell deficient, $Kit^{W\text{-}sh/W\text{-}sh}$, mice transplanted with hematopoietic-prostaglandin synthase deficient mast cells are not able to gain weight similarly as mice transplanted with wild-type mast cells, pinpointing the importance of mast cell derived prostaglandins in adipose tissue function[29]. As adipocytes themselves are also an important source of cytokines[30], this adds up to the local inflammatory burden. Interestingly, adipocytokines have been linked to mast cells in the context of allergic inflammation and asthma[31], introducing a possible crosstalk between the resident cells of adipose tissue and the infiltrating mast cells.

2.1. Type II-diabetes mellitus

Notably, obesity comprises an essential risk factor for the development of type 2-diabetes mellitus[32]. Non-insulin dependent diabetes is a metabolic disease defined by hyperglycemia and insulin-resistance, which is greatly influenced by obesity. Aside from their role in obesity, mast cells have been directly linked to type 2-diabetes. Mice fed a high-fat diet while lacking mast cells show higher glucose tolerance, compared to the wild type strain[33]. Since tumor necrosis factor-alpha (TNFα) was found to be overexpressed in obese mice[34], this cytokine has been considered a key mediator in the induction of insulin resistance[35]. However in experiments using TNFα deficient mast cells[33], TNFα did not contribute to the effect of mast cells in obesity, indicating that the metabolic changes induced by this cytokine may have been due to TNFα derived from other inflammatory cells than mast cells.

2.2. Diabetic nephropathy

Diabetes mellitus can often lead to serious renal complications, such as diabetic nephropathy, a condition showing increasing mortality rates over the years[36]. Diabetic nephropathy is marked by glomerular and tubular basement membrane thickening and hypertrophic mesangial matrix, while it can lead to tubulointerstitial fibrosis and

glomerulosclerosis[37]. In the past, human mast cells have been reported to infiltrate the diseased kidney at increasing numbers as diabetic nephropathy progresses[38], while participating in the initial mechanisms of tubulointerstitial injury[39]. Furthermore, mast cell infiltration in the kidneys of rodents was clearly established in renal diseases, the mast cell mediators chymase, tryptase and transforming growth factor-beta$_1$ (TGF-β_1) showing increased levels in injured rat kidney tissue[40]. Importantly, TGF-β_1 has already been reported to be a key cytokine responsible for the aggravation of renal fibrosis[41], *via* collagen overexpression by fibroblasts[42]. Chymase has been connected to various mechanisms that shape renal diseases[43], and regarding diabetes and diabetic nephropathy, chymase was suggested to participate in local Ang II formation[44]. In addition, tryptase was reported to enhance renal fibrosis while additionally being implicated in collagen synthesis, which illustrates a potential regulatory mechanism of mast cells on fibroblasts[45]. In fact, diabetic rats treated with the mast cell stabilizer tranilast displayed diminished chymase-positive mast cell infiltration in mesenteric vessels, diminished mesenteric vascular collagen deposition, and ultimately amelioration of the diabetes-induced vessel fibrosis[46], which was considered to result from inhibition of chymase and subsequent reduction in the generation of Ang II.

Seemingly, mast cells in both obesity and diabetes function either by establishing cell interactions with local and infiltrating cell types, or *via* their degranulation products acting *in situ*. Through these routes, mast cells may contribute to various life-threatening cardiovascular syndromes. For instance, in an experimental diabetes model, mice showed increased cardiac mast cell activation and defective heart function, which led to the development of cardiomyopathy[47]. However, upon treatment with the mast cell stabilizer nedocromyl, the cardiac mast cell numbers were decreased, followed by decreased collagen deposition and normalized cytokine levels. Furthermore, in a recent patient study examining obese subjects for subclinical atherosclerosis, serum tryptase positively correlated with metabolic risk factors, such as body-mass index[48], as well as atherosclerosis markers, suggesting that mast cell activation composes a tight link between obesity and cardiometabolic diseases.

3. Atherosclerosis

Atherosclerosis is the primary underlying cause of acute cardiovascular syndromes, such as stroke and myocardial infarction, principal causes of death in Western society[49]. This vascular disease is a chronic inflammatory condition, characterized by thickening of the arterial wall after lipid accumulation, which results in the formation of an atheromatous plaque[50]. Elevated levels of cholesterol-carrying low density lipoprotein (LDL), and its subsequent penetration through the vascular endothelial cell layer where it gets oxidized (oxLDL) are an initial feature of plaque

development. Circulating monocytes begin to accumulate in the area where they differentiate into macrophages and ingest oxLDL[51]. This gives rise to foam cells, the innermost cell type of an atherosclerotic plaque[52]. A progressed plaque consists of high numbers of inflammatory cells, enhanced extracellular matrix (ECM) degradation, excessive levels of cell apoptosis, and may eventually rupture, leading to thrombus formation and possible vessel occlusion.

3.1. Mast cells in atherosclerosis

The first to illustrate the importance of mast cells in atherosclerosis was Paris Constantinides in 1953, when he proposed a protective role for mast cells in rats, assigning this role to mast cell heparin[53]. Thereafter, a long silence in the field of mast cells and potential atherogenic mechanisms prevailed, with some frustration about the strong ability of mast cells to degrade and inactivate enzymes involved in lipoprotein metabolism. A renewed interest in the potential connection between mast cells and atherosclerosis was sparked when it was discovered that exocytosed granules of activated rat peritoneal mast cells were able to avidly degrade the apolipoprotein B-100 of LDL[54]. This initial observation led to the finding of activated mast cells being able to convert macrophages into foam cells, the hallmark of atherosclerosis[55]. Mast cells exert this effect *via* the "granule carrier pathway" in which the heparin and chymase components of the exocytosed mast cell granules act in concert. Thus, heparin first binds LDL particles, granule chymase then proteolyzes the bound particles and renders them unstable to fuse and form lipid droplets on granule surface, and ultimately macrophages phagocytoze the LDL-containing granules. This renewed interest in the mechanisms explaining the potential role of mast cells in atherosclerosis led to a search of mast cell proteases in the various layers of the healthy human arterial wall and in the various regions of atherosclerotic plaques. Indeed, it could be demonstrated that the mast cells in the human aortic wall are of two types, those containing only tryptase and those containing both tryptase and chymase[20]. Ever since numerous laboratories profoundly linked mast cells to the development of atherosclerosis and acute cardiovascular syndromes, such as atherothrombosis. In the human arterial wall, mast cells are located in the inner and outer layer (intima and adventitia) of a healthy aortic wall, first at a low density and then, during the progression of atherosclerosis, with a propensity to increased density in the vulnerable shoulder region of the developing plaque and also in the adventitia[20,56]. Most importantly, activated mast cells accumulate in the shoulder region of human coronary plaques, which is the site vulnerable to rupture[57]. In the atherosclerotic aortic wall of atherosclerosis-prone mice, mast cells are located mainly in the adventitia with only sporadic occurrence in the intima[58], while in humans they gather in both areas[56,59]. Moreover, mast cells in

the advanced atherosclerotic carotid and coronary arteries have been reported to be in close proximity to microvessel sprouting[59,60] and to be connected with intraplaque hemorrhage, *i.e.* with a key histological sign pointing to vulnerability of a plaque to rupture[61]. Notably, a recent publication on human carotid atherosclerosis described mast cells as the only immune cell type directly associated with future cardiovascular events[62]. Acquired evidence heretofore, recently reviewed by our group, appoints mast cells a pro-atherogenic role, as they have been reported to enhance leukocyte influx and local apoptosis, augment matrix degradation and induce hemorrhagic events, affecting plaque initiation, progression and destabilization[63].

3.2. Mast cell infiltration mechanisms

In the course of plaque development, mast cells infiltrate the vessels and are found in the intima and adventitia of human coronary arteries[64,65]. One mechanism of mast cell infiltration is *via* chemokine signals between eotaxin and its receptor C-C chemokine receptor-3[66]. However, mast cells carry additional chemokine receptors[67], which are also known to participate in transendothelial migration[68] during atherosclerosis development, indicating that there may be supplementary signals that attract mast cells to enter the arterial intima. Furthermore, activated mast cells can produce chemokines themselves[69], which may amplify the attraction signals and positively regulate their presence; possibly explaining the direct correlation between mast cell numbers and plaque progression. In addition, it has recently been reported that vascular endothelial cells (ECs) can induce immature mast cell attraction *via* the adhesion molecules E-selectin, P-selectin, vascular cell adhesion molecule-1 and platelet endothelial cell adhesion molecule-1[70]. Conversely, pro-inflammatory cytokines released by mast cells can induce upregulation of main adhesion molecules (e.g. P-selectin) in endothelial cells[71].

3.3. Mast cell activation mechanisms

It has been observed that apart from the numerical increase, mast cells appear in a degranulated state inside ruptured plaques of human coronary arteries[72], proposing therefore that mast cells exert their local effects through mediator release. Following that, we confirmed, using the atherosclerotic apoE-deficient ($apoE^{-/-}$) mouse model, that mast cells through their activation are responsible for disease progression, after observing an increase in plaque size upon systemic mast cell activation[73]. Concomitantly, Sun et al., (2007a) reported that double mutant LDL-receptor ($LDLr^{-/-}$)$Kit^{W\text{-}sh\backslash W\text{-}sh}$ mice show decreased plaque formation, lipid deposition and immune infiltration compared to the $LDLr^{-/-}$ strain, confirming a direct connection between mast cells and atherosclerosis.

Consequently, scientific research focused on possible target molecules that may activate mast cells inside the vascular microenvironment. Evidently, IgE was one of the primary candidate molecules to be investigated, with the first studies reporting that IgE serum levels were higher in patients suffering from angina pectoris[74] or dyslipidemia[75]. Moreover, IgE blood levels were detected to be significantly higher immediately after myocardial infarction[76]. Furthermore, in a recently published experimental study targeting immune activation in atherosclerosis, $LDLr^{-/-}$ mice treated with an antibody against OX40-ligand showed plaque regression which was partially appointed to reduced plasma IgE and lower mast cell infiltration[77]. Additionally, a few years ago it was stated that serum IgE could be detected within atherosclerotic s where it promoted cell apoptosis and cytokine expression in $apoE^{-/-}$ mice as well as in humans through FcεR1 in synchrony with Toll-like receptor-4 (TLR4)[78]. With respect to TLR4, despite the fact that the above effects were mainly described on macrophages, this receptor has also been found on the surface of mast cells where it was able to trigger membrane bound-mast cell activation[79], thus supporting a possible mast cell-TLR4 specific role in atherosclerosis. Moreover, antibodies of the IgG-class were found to activate human mast cells *in vitro*, after formation of immune complexes with oxLDL[80], indicating that various components of an immune reaction can contribute to intraplaque mast cell activation. Interestingly, oxLDL itself is one of the endogenous molecules that can initiate an immune response[81]. In relation to mast cells, oxLDL can directly activate human mast cells *in vitro*, partly through TLR-4 signaling[82]. In addition, oxLDL constituents, such as lysophosphatidic acid have lately been described to concentrate in the plaque and locally activate mast cells, contributing to plaque destabilization[83], and consequently illustrating a strong link between mast cell action and excessive lipid accumulation. Furthermore, components of the complement system, such as the anaphylatoxin C5a, are able to activate mast cells in the context of atherosclerosis through their specific C5a-receptor. Examination of human coronary plaques revealed that mast cells were positive for C5a-receptor[84]. A recent study directly linked C5a-specific mast cell activation in mice to plaque progression, showing that mast cell stabilization, using cromolyn, attenuated C5a-induced progression of vein graft disease, while mast cell activation using dinitrophenyl hapten augmented disease and increased plaque development[85]. Yet, apart from C5a, other proteins of the complement system may also act on mast cells inside the plaque microenvironment, since it has already been mentioned that certain mast cell subsets can get potently regulated by additional complement factors[86]. However, it is also the interaction with adjacent cell types inside the plaque that affects mast cell activation. For instance, ET-1, an endothelial vasoconstricting peptide, displays elevated levels in atherosclerosis patients[87] and induces cardiac mast cell activation in rats[88]. Furthermore, activated mast cells are able to enhance endothelial ET-1 expression *in vitro*[89]. Additionally, several bioactive molecules, such as reactive oxygen species,

present in the inflamed plaque at increased levels, may also function as local mast cell activators[90]. Lastly, an intriguing observation revealed that mast cells inside human coronary artery specimens of different atherosclerosis phases, reside proximal to nerve fibers[91], implicating the central nervous system in mast cell action. One link may be SP, a neuropeptide regulating inflammation[92] and stress-related cardiac syndromes[93]. Since mast cells were found to express the SP receptor, neurokinin-1[94], an experimental atherosclerosis mouse study was designed to identify the direct link between SP and these cells inside the plaque, revealing that mast cell presence and activation were enhanced upon intensified SP treatment, followed by plaque destabilization, while neurokinin-1 blockade prevented these changes[65]. Moreover, a very recent publication reported that an additional neurotransmitter, neuropeptide Y, can promote mast cell activation while also affecting plaque progression in apoE$^{-/-}$ mice[95]. Admittedly, there are probably other unidentified mast cell activators that elicit mediator release inside the plaque and further research needs to be conducted in order to identify the precise triggers of mast cell activation in atherosclerosis.

3.4 Mast cell mediated atherosclerosis progression

A vast amount of research has approached the role of mast cells in atherosclerosis by testing the effects of general mast cell inhibition achieved by pharmacological or genetic approaches. Among the evidence collected so far, it has been demonstrated that mast cells stabilized *in vitro*, using anti-allergic drugs, were able to inhibit foam cell formation, suggesting a direct role in plaque development[96]. In accordance, *in vivo* research, using the apoE$^{-/-}$Kit$^{W\text{-}sh/W\text{-}sh}$ mouse model, reports a reduction in aortic atherosclerosis progression, as well as leukocyte influx and inflammation levels[97]. Adding up to the above, a recent study using LDLr$^{-/-}$ mice, showed that mast cell stabilization with cromolyn not only reduced inflammation and atherosclerosis progression but also improved the lipid profile of these mice[98]. It is therefore evident, that mast cells, through their activation, can directly affect atherosclerosis progression.

However, as it may be expected, distinct mast cell activation pathways result in the release of diverse molecules, which individually affect the complex mechanisms of atherosclerosis in different aspects. Mast cell proteases are thought to be important in atherosclerosis (**Table 1**). For instance, chymase accounts for the disruption of vascular tissue homeostasis by indirect degradation of ECM *via* activation of pro-collagenase[99] and pro-matrix-metalloproteinase-9 (pro-MMP9)[100]. This serine protease can also directly degrade ECM by hydrolyzing its main components, such as fibronectin[101]. Furthermore, the direct degradation of fibronectin by chymase leads to vascular smooth muscle cell (VSMC) apoptosis[102]. Chymase can also induce VSMC apoptosis by disturbing the nuclear factor-kappa B (NF-κB) anti-apoptotic signaling pathway[103]. This

regulatory effect of mast cells, *via* chymase, on the VSMCs was recently found to be, at least partly, mediated through TLR-4 activation, in an *in vivo* atherosclerosis model[104]. Nevertheless, together with the induction of apoptosis, chymase can inhibit collagen production and hinder VSMC proliferation[105]. A combination of the above mechanisms can induce thinning of the fibrous cap of a plaque, and can lead to rupture. Moreover, this protease can also act on the vascular endothelium. Thus, chymase avidly degrades various components of the pericellular matrix of ECs, which may result in detachment of endothelial cells in turbulent areas of the arterial tree[106] and, in combination with TNFα, enhance EC apoptosis[107]. Chymase can also release latent TGF-β_1 from the pericellular matrix of ECs, and so render it susceptible for activation *via* interaction with binding sites on EC surface[108]. The activated TGF-β_1, again, can affect a multitude of signaling machineries involved in tissue remodeling, and it may also regulate the activity of infiltrating immune cells. Moreover, chymase is known to induce apoptosis of macrophages[73], while also stimulating immune effector cytokines and chemokines[109]. In addition, chymase has been demonstrated to degrade various apolipoproteins of high-density lipoprotein and thereby dampen the efflux of cholesterol from macrophage foam cells[110,111,112]. Also, experiments in the mouse have demonstrated that mast cell activation in the peritoneal cavity locally degrades apolipoprotein AI, and *via* this action impairs the entire macrophage reverse cholesterol transport pathway, *i.e.* cholesterol transport from the peritoneal macrophage foam cells to feces[111]. A number of studies report that chymase is responsible for the local production of Ang II[113,114]. More specifically, chymase-induced Ang II was able to induce reactive oxygen species production *via* binding to its receptor and activating NAD(P)H oxidase, aggravating hence the local inflammatory burden[115]. Subsequently, chymase expression, as well as Ang II levels, were found elevated in human atherosclerotic aortas[116], while, in a hamster model, Ang II levels were observed reduced after treatment with tranilast[117]. Ang II is also leading to VSMC apoptosis[118] and leukocyte infiltration[119]. A critical role for chymase, as a mediator for atherosclerosis progression, was demonstrated in apoE$^{-/-}$ mice in which this protease was inhibited, using compound RO5066852. These mice showed reduced plaque progression, increased plaque stability, through enhanced collagen deposition, and lower intraplaque hemorrhage levels[120].

Moreover, both chymase and tryptase were shown to be positively correlated with the degree of atherosclerosis in human aortas[121]. Tryptase is known to degrade ECM by activating pro-MMPs[122] while also breaking down fibronectin[106]. In addition, this protease has been suggested to actively participate in foam cell formation *in vitro*, through protease activated receptor-2 (PAR-2)[123], while enhancing MCP-1 and IL-8 levels on cultured endothelial cells[124]. Along that line, tryptase augments neutrophil migration by acting as an inducer of chemokine IL-8 secretion from endothelial cells[125]. Furthermore, tryptase acts on macrophages, by increasing lipid accumulation while

degrading high-density lipoprotein[126] and thus blocking the cholesterol efflux pathway. Yet, tryptase can affect various chemokines[127]. Additional evidence regarding the role of tryptase *per se* was gathered from apoE$^{-/-}$ mice that had recently undergone tryptase inhibition using lentiviral constructs. These mice presented increased angiogenesis and intraplaque hemorrhages[128].

Alongside with the protease secretion, inflammatory cytokines secreted by mast cells affect plaque phenotype. Human mast cells incubated with oxLDL-immune complexes *in vitro* were found to upregulate and secrete TNFα, Interleukin-8 and macrophage chemoattractant protein-1[80]. Moreover, TNFα, together with IL-6 and IFNγ, secreted by mast cells were able to regulate *in vitro* the expression of endothelial cell adhesion molecules[71]. Interestingly, Sun et al. (2007a) reported that mast cell specific IL-6 and IFNγ, but not TNFα, cytokine secretion can promote atherosclerosis *in vivo* upon induction of cathepsin activation and ECM degradation[58]. Furthermore, mast cells inside human coronary artery plaques were also capable of producing basic fibroblast growth factor, intensifying the link with intraplaque angiogenesis[61]. Finally, additional mast cell specific mediators such as histamine and heparin have been also reported to participate in mast cell mediated atherosclerosis progression; by inducing macrophage apoptosis[73] or enhancing foam cell formation[55] respectively.

Thereupon, the effects of mast cell activation are induced through the differential action of their degranulated mediators. which act in the surrounding microenvironment and alter the plaque phenotype either by increasing plaque size or by diminishing plaque stability. For that reason, a considerable amount of research focuses on the inhibition of specific mast cell activation pathways in an attempt to hamper atherosclerosis progression while still retaining beneficial mast cell effector functions, for example as components of the host-immune response.

Chymase	Tryptase
Breakdown of ECM components	Breakdown of ECM components
Vascular SMC apoptosis	PAR-2 activation
Collagen degradation	Increased lipid accumulation
Disruption of vascular EC layer	Disruption of vascular EC layer
Vascular EC apoptosis	Macrophage apoptosis
Induction of leukocyte infiltration in the subendothelial space	Induction of leukocyte infiltration in the subendothelial space
HDL and LDLproteolysis	HDL proteolysis
Cytokine/chemokine regulation	Cytokine/chemokine regulation
Macrophage apoptosis	Angiogenesis
Vascular Ang II production	Immunoglobulin synthesis

Table 1: Chymase and tryptase effects in atherosclerosis. *ECM: extracellular matrix, SMC: smooth muscle cells, EC: endothelial cells, Ang II: angiotensin II, ROS: reactive oxygen species, PAR-2: protease activated receptor-2, PAF: platelet activating factor, HDL: high-density lipoprotein; LDL: low-density lipoprotein.*

4. Restenosis

The available surgical intervention methods, used so far, to treat an atherosclerotic artery are bypass surgery, balloon angioplasty and stent placement; however, this often leads to restenosis[129]. The major differences between a restenotic and an atherosclerotic plaque are primarily the acute rate of inflammation and neointima formation induced by endothelial damage, as a consequence of the therapeutic intervention, as well as increased fibrosis caused by enhanced VSMC proliferation[130].

Seemingly, since mast cells have already infiltrated the vessels upon primary atherosclerosis development, restenotic plaques contain an increased amount of mast cells ready to degranulate upon trigger[131], with their mediators aggravating the restenotic burden. In particular, chymase-generated Ang II and TGF-β_1 are linked to fibroblast proliferation and neointimal formation[132]. Therefore, a general Ang II receptor inhibitor used in an experimental model of dog angioplasty showed reduced neointima development[133], an effect partly caused by blockade of chymase-induced Ang II. Moreover, tranilast used on dog carotid arteries after balloon injury was able to suppress chymase expression and hinder neointimal formation[131]. Tryptase is also implicated in restenosis, mainly through PAR-2 activation, affecting transendothelial leukocyte infiltration. More specifically, PAR-2 deficient mice showed reduced leukocyte adhesion in the endothelial wall which contributed to reduced neointima size[134]. In the same manner as Ang II, PAR-2 effects are partly linked to tryptase-induced activation.

Another mast cell mediator which has attracted attention through the years in restenosis is histamine, with prior *in vitro* data suggesting that it plays a role in EC and SMC proliferation and migration[135]. An *in vivo* study in which the histamine receptor H1 was inhibited in mice suffering endothelial damage after coronary angioplasty, reported diminished neointima size and reduced cell proliferation inside the plaque[133]. In line with that, *in vivo* data from pigs that underwent stent placement revealed that local histamine concentration is increasing over time[136]. In contrast to the above mentioned *in vitro* study the authors did not observe any histamine-triggered proliferation in cultured SMCs hetherto. Nonetheless, it is important to note that histamine effects should not be entirely ascribed to mast cells since macrophages[137] and platelets[138] can show inducible histamine production as well.

4.1 Drug Eluting Stents

In order to avoid the excessive VSMC proliferation that follows stent or angioplasty-generated trauma, Drug Eluting Stents (DES), containing specific anti-proliferative drugs, have been developed[139]. With regard to the role of mast cells in DES,

a very recent study proposed nitric oxide and ROS-scavenger coated stents as beneficial agents in mast cell stabilization inside the implanted artery[140].

Interestingly, a striking effect that highlights the importance of mast cells in acute coronary events is described under the name Kounis syndrome[141], in which hypersensitivity reactions, such as anaphylaxis, can occur upon DES placement and develop in a synchronized manner with acute coronary events. Recent studies have revealed that DES coating elements such as metal, latex and polymer, as well as the eluted drugs, can cause hypersensitivity reactions[142]. It is therefore suggested that mast cell activation may lead to increased inflammation, and possible thrombosis, in an acute fashion inside a restenotic environment, underlining the influential effect of these cells in yet another cardiovascular syndrome.

5. Myocardial infarction

The clinical outcome of a ruptured atherosclerotic plaque is mostly induced by the formation of a thrombus, which can destabilize upon high blood flow and relocate in smaller vessels, obstructing the circulation. Progressed stenotic plaques inside a coronary artery often lead to the occlusion of vessels that supply the heart muscle with blood, giving rise to an acute myocardial infarction (MI)[143].

Mast cells have been directly linked to plaque rupture, since they accumulate in the shoulder region of ruptured atherosclerotic plaques of patients that have suffered an MI[57]. Similarly, analysis of human coronary specimens after MI, displayed increased numbers of degranulated mast cells in the adventitia of ruptured plaques[64]. These cells were mainly positive for chymase, histamine and tryptase.

Consequently, attention turned to mast cell specific proteins in MI, with the first data coming from infarcted cardiac tissues in a hamster model. This study in hamsters presented increased chymase activity in the ischemic area, from day 1 and until day 56 post-MI, while chymase positive mast cell numbers were found elevated, particularly in the initial phase of the MI[144]. Moreover, chymase-mediated Ang II production was studied. Specifically, systemic Ang II production was blocked using an ACE blocker and the subsequent effects were compared to the overall Ang II effects obtained upon using an Ang II receptor inhibitor. This experiment showed a decrease in the mortality rate only upon Ang II blockade, suggesting indirectly that chymase-induced Ang II production is responsible for the detrimental effects in the ischemic tissue following an MI. A follow-up study from the same group, in which chymase was blocked in hamsters after mechanical MI induction, reported improved cardiac function and decreased mortality rates, highlighting the importance of this protease in infarcted tissues[145]. Therefore, from a therapeutic view, it has been proposed that chymase inhibitors

can significantly improve cardiac function upon combination with general Ang II inhibitors[146]. Nevertheless, the effects of chymase appear to be caused not only *via* Ang II but also partly *via* TGF-β. Chymase inhibition in rats showed reduced TGF-β expression in combination with reduced myocardial fibrosis levels and cardiac dysfunction[147]. Lastly, aside from TGF-β, chymase may additionally exert its effects through activation of MMP-9, since its inhibition in pigs resulted in lower levels of activated MMP-9 which was accompanied by a lower infarct size, as well as reduced inflammation and apoptosis levels[148].

Along this line, experiments using the mast cell inhibitor relaxin in a swine MI model have confirmed a reduction in the plasma histamine, together with a decrease in mast cell degranulation[149]; but also lower cardiac tissue injury. This points towards a role for histamine in MI, while highlighting the importance of mast cells in the pathophysiology of the ischemic tissue. In addition, upon examination of inflammatory levels in human serum after an acute MI event, MCP-1 was found significantly increased and positively correlated to IL-8[150], which may illustrate a partial implication of mast cells, since they can secrete both cytokines.

Interestingly, research on late-phase infarcted heart tissues in dogs suggested that tryptase contributes to the upregulation of MCP-1 and IL-8 in the cardiac endothelium, thereby leading to increased angiogenesis and promotion of the healing process in the ischemic myocardium[151]. Similarly, a recent study in infarcted rat hearts locally treated with mast cell granules isolated from rat peritoneal cells, demonstrated reduced cardiomyocyte apoptosis, increased angiogenesis levels and improved cardiac function[152], providing evidence in favor of a cardioprotective role exerted by mast cell granule contents. Thereupon, mast cells through their diverse mediator effects seem to participate not only in the generation of an MI, but also in the cardiac tissue recovery following it.

6. Arrhythmia

Atherosclerosis and MI are both linked to certain types of cardiac arrhythmia[153], a condition during which the myocardial rhythm appears disrupted as a result of irregular electrical activity. One of the classification methods for distinct types of arrhythmia is based on the location where the dysfunction occurs, with characteristic examples atrial and ventricular arrhythmias[154]. Atrial fibrillation, the most common type of arrhythmia, is tightly connected to pre-established atherosclerosis, with the latest study directly associating human carotid intima/media thickness with the occurrence of this type of tachycardia[155]. On the other hand, ventricular arrhythmias are frequently described as a result of an MI incident[156] upon over-excitation of cardiac nerve fibers.

Clearly, since mast cells are associated with both atherosclerosis and MI, attention was also drawn to their possible implication in cardiac arrhythmias. The primary indications in favor of such a relationship were collected in a 1986 review which scrutinized the arrhythmogenic effects of histamine in pivotal sectors of the heart muscle[157]. The role of histamine was explored in detail after induction of MI, in rodent and canine models, demonstrating that the presence of histamine is directly associated with post-MI generation of ventricular arrhythmias[158]; and moreover, that its concentration increases proportionally to the severity of the ischemic incident[159]. The mast cell inhibitor relaxin was able to lower histamine content in the heart and reduce ventricular tachycardia, expanding thus the beneficial outcome of mast cell stabilization in arrhythmias[149]. Histamine exerts its function through a series of G-coupled protein receptors (H_1-H_4) which may exhibit differential downstream actions. For instance, while H_2 receptor is reported to show an arrhythmogenic function[160], H_3 receptor is considered as benefactor for the alleviation of post MI-tachyarrhythmic events[161]. To make matters even more complex, it has recently been suggested that mast cells are not the only source of cardiac histamine, and therefore not the only cell type responsible for the ventricular arrhythmias observed after an MI incident[162]. Nonetheless, the majority of evidence so far highlights the detrimental role of mast cells in cardiac arrhythmias, which appears to be mediated through release of their mediators. For example, after blockade of chymase in an infarcted dog model, ventricular arrhythmias as well as serum Ang II levels were reduced[163], implicating thus chymase release and local Ang II formation in the generation of post-MI tachycardia. Furthermore, SP was reported to trigger renin release by cardiac mast cells in *ex vivo* ischemia experiments, with the latter further enhancing arrhythmogenicity[164,165].

Finally, recently obtained data connect mast cells not only to arrhythmias following pre-established cardiac disorders, but also to newly generated atrial fibrillation events which can subsequently lead to acute cardiovascular syndromes. Specifically, in a mouse study of atrial fibrillation, less fibrosis in the atrium was observed after cromolyn stabilization or genetic reconstitution with mast cell deficient $Kit^{W/W\text{-}v}$ bone marrow. This was appointed to a cross-talk mechanism between cardiac mast cells and myocytes or fibroblasts, through platelet-derived growth factor-A[166]. Based on the above, it is evident that the impact of mast cells in this cardiac disease is not entirely understood, since it has begun to attract full attention only recently.

7. Aneurysm

The development of an aneurysm is the consequence of vascular wall thinning, following accelerated smooth muscle cell death and increased ECM breakdown, accompanied by increased levels of inflammation, occurring for example in the abdominal

aorta (abdominal aortic aneurysm or AAA) or in the brain (cerebral aneurysm)[167]. Following these events, a vessel can rupture, leading to excessive hemorrhage and strongly increased risk of death[168].

7.1 Cerebral aneurysm

Among research linking mast cells to aneurysm formation, a distinctive study, on humans who suffered a cerebral aneurysm rupture, reported mast cells at increased numbers near ruptured vessels[169]. In another study, treatment of rats with mast cell inhibitors reduced macrophage infiltration and vessel thickening, and mast cells were proposed to induce the expression and activation of pro-MMPs in adjacent SMCs[170]. Furthermore, a report on human tissue specimens confirmed that mast cells, together with inflammatory macrophages, are abundant in ruptured aneurysms[171]. Lastly, another study demonstrated that mast cells which reside within the thin wall of a remodeled and eroded human intracranial aneurysm, were located in close proximity to newly formed microvessels and associated with micro-hemorrhages[172]. These observations suggested that mast cells contribute to the wall remodeling and degeneration in the small intracranial arteries which develop saccular aneurysms and may ultimately rupture.

7.2 Abdominal aortic aneurysm

The strongest evidence on the role of mast cells in aneurysms is obtained from abdominal aortic aneurysms[21]. Regarding the recruitment of mast cells inside the aneurysmal area, a study revealed that transplantation of mast cells from $apoE^{-/-}$ mice with a defect in the MCP-1 receptor, CCR2, in $apoE^{-/-}Kit^{W\text{-}sh/W\text{-}sh}$ mice reduced aortic aneurysm formation, in comparison to the $apoE^{-/-}$ strain, as a result of decreased mast cell presence; proposing a possible CCR2-mediated signaling pathway for the migration of mast cells inside the diseased vessel wall[173].

In terms of mast cell action in aneurysms, the impact of IL-6 and IFNγ has also been established in aneurysm formation, and reconstitution experiments in the $Kit^{W\text{-}sh/W\text{-}sh}$ mice have proven that these cytokines play a fundamental role in the induction of aneurysms[174]. In addition, mast cell deficient mice were protected from aneurysms, presenting decreased leukocyte infiltration in the injured area, as well as reduced levels of apoptosis and angiogenesis; suggesting thus that mast cells are important effectors in these mechanistic changes inside the diseased vessel.

Furthermore, one of the above mentioned studies described increased Ang II formation in aneurysmal and atherosclerotic aortic samples, assigning chymase an

important role in both syndromes[111]. The most remarkable observation in this study was the increased numbers of activated mast cells in aneurysmal aortas, which were even higher than in atherosclerotic samples.

The effect of chymase in this disease was also underlined in an experimental hamster model in which aneurysm formation was inhibited upon treatment with the chymase inhibitor, NK3201[175]. Additional evidence implicates chymase in the progression of aneurysm formation, as high levels of chymase-positive mast cells in human aortas directly associate with the development of the disease[176]. This study also provided a causal relation between mast cells and aneurysm formation, by showing reduced aneurysm incidence in both chymase deficient and $Kit^{W\text{-}sh/W\text{-}sh}$ mice, but also after reconstitution of the $Kit^{W\text{-}sh/W\text{-}sh}$ mice with chymase deficient mast cells. The authors suggested that chymase exerts its function through cathepsin expression, ECM degradation and angiogenesis.

Yet, once more, tryptase was also proven to directly participate in the development of abdominal aneurysms, after lack of experimental aneurysm formation in tryptase-deficient mice[177]. These observations were again combined with data acquired from human aortic samples, showing tryptase accumulation inside aneurysmal tissues while its circulatory levels directly correlated with aneurysm progression rate. Similarly to atherosclerosis, aortic aneurysms display increased expression of chymase, tryptase and cathepsin-G, together with angiogenic proteins expression levels and with the number of adventitial mast cells, all correlating with the degree of neovascularization[178]. The critical influence of mast cells has also been established after chemical stabilization with tranilast in $apoE^{-/-}$ mice and mast cell deficient rats, with both models presenting a reduction in aneurysm formation[179].

Finally, a mouse study uncovered a role for IgE in abdominal aortic aneurysms, showing aggravated disease progression by IgE mediated activation of not only mast cells, but also T cells and macrophages[180]. Moreover, FcεR1-deficient $apoE^{-/-}$ mice failed to develop abdominal aneurysms and presented lower plasma IL-6 levels[180]. With reference to this study, it is important to note that although FcεR-mediated effects are generally considered mast cell specific, increasing evidence supports the notion of an inducible FcεR-expression pattern in additional immune populations, such as T cells and macrophages, depending on the environmental stimuli, emphasizing thus the plastic nature of disease-specific immune responses.

An example of this plasticity comes from a study examining the role of the peptide hormone adrenomedullin in human abdominal aortic aneurysms, and revealing that this peptide can be released from mast cells and act as an anti-fibrotic agent[181]. These results contradict the previously discussed pro-fibrotic role of mast cells in aneurysm

formation, and illustrate the diverse, and possible immune-regulatory, responses of this immune-effector cell type upon excessive inflammation levels.

8. Therapeutic potential

Mast cells are rightly regarded as potent inflammatory cells in cardiovascular diseases. The massive amount of evidence gathered so far mainly relates to their harmful pathophysiological events, thereby leading to the perception that global inhibition of mast cell activation could save the local microenvironment from their disadvantageous effects. For that very reason, as well as to examine the overall influence of mast cells on disease progression, general mast cell inhibitors, such as cromolyn, are widely used in experimental models; mainly suggesting that mast cell stabilization can mitigate the inflammation levels in acute cardiovascular syndromes. Furthermore, pharmacological mast cell stabilizers, already widely used in allergic disorders, were considered as promising therapeutic agents in humans suffering from vascular diseases, a characteristic example being the use of tranilast in a large patient study for the prevention of restenosis; without however presenting any beneficial clinical outcome[182]. The adverse effects of this application can be predicted by recognizing that mast cells do not only reside in the heart and vessels, but also comprise the first line of host defense against pathogens. Therefore, a possible systemic inhibition may compromise the protective responses exerted by mast cells as part of immune protection.

Therefore, new technologies are applied, in order to scan for novel targeting molecules that may avert from the risks of systemic mast cell inhibition[183]. An appealing alternative would be to focus on the type and magnitude of the differential activation and inhibition signals, to manage the sort of mast cell reaction needed per occasion[184]. In an attempt to concentrate on the action of specific mast cell mediators, rather than the general activation pathway, chemical inhibitors of serine proteases and histamine receptor antagonists have been designed and tested in animal models, and they have, indeed showed some very promising results[185]. However, the inhibition of molecules with such a broad spectrum of effects can also increase the risk of hindering multiple downstream mechanisms, not all of them being harmful. For instance, both proteases are able to degrade pro-inflammatory cytokines[186], probably upon highly inflammatory conditions, an effect which may prove beneficial. Thus, elucidation of the net effect, beneficial or harmful, in such highly complex *in vivo* conditions requires rigorous experimental testing in suitable animal models of the disease conditions in question.

Mast cells have also been described to be important mediators in immune suppression. An intriguing paradigm is an allograft study in which these cells were directed to enter tolerant grafts, after responding to high IL-9 levels secreted by T

regulatory lymphocytes[187]. In favor of this immune regulatory role, mast cells have been found to possess an interesting ability to communicate with distant targets *via* secretion of small membrane vesicles containing cytokines and other mediators, named exosomes and microparticles[188]. These vesicles are proven to carry molecules *via* which they elicit immune-regulatory mechanisms[189]; suggesting that enhancement of immune tolerance, rather than chemical inhibition of mast cell activation, may prove more advantageous as a therapeutic method.

The above illustrated examples on the importance, as well as the complexity of mast cells, suggest that manipulation of this cell type should be approached in a delicate way, superseding the general consideration of them being detrimental effector cells that need to be blocked at any cost. After all, it is not by accident that a very recent review on the field of cancer immunology has addressed mast cells as "sentinel cells" that may be alternatively modulated[190], rather than inhibited, in order to provide beneficial immune reactions and contribute to improved prognosis.

9. Conclusion

This review aimed to highlight the potential effects of mast cells in cardiovascular diseases, which are characterized by increased morbidity and mortality rates worldwide. In conclusion, mast cells can be considered to be important effector cells of the immune system, acting as a driving force of the local response, either by their direct cell-cell interaction with adjacent vascular cell types (**Figure 2**), or *via* their mediator release; resulting in targeted stimulation of cells residing in the microenvironment in which the mast cells have been activated. The plethora of secreted molecules, together with the multiple activation pathways, allows the identification of specialized therapeutic interventions focusing on the distinct features of each disease, while refraining from interfering with their beneficial characteristics. Therefore, modulation of individual pathways in mast cell function, rather than systemic mast cell blockade, may serve as a promising strategy to alleviate the progression of cardiovascular diseases, with an ultimate aim to reduce the excess morbidity and mortality associated with these disorders.

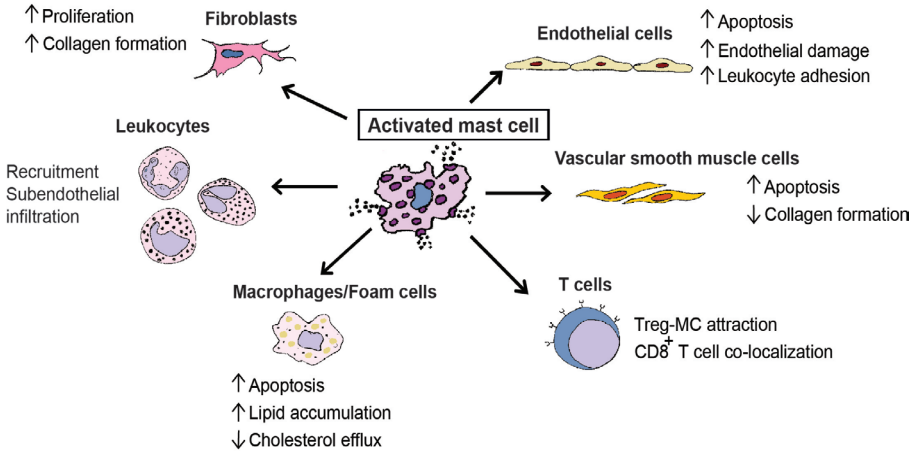

Figure 2: Mast cell interaction with adjacent cell types in the vasculature. This schematic overview illustrates the major cell interaction mechanisms between mast cells and various cell types present in the vessels. Activated mast cells are able to affect key biological processes, such as proliferation and apoptosis, of resident cell types in the cardiovascular system.

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