Published in final edited form as:
Drug Discov Today Dis Models. 2011 ; 8(1): 47–55. doi:10.1016/j.ddmod.2011.06.004.

Mast Cell Proteases and Inflammation

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Abstract

Mast cells are best known for their role in allergic reactions but are also now recognized for their important contributions to a number of disparate inflammatory conditions through the release of inflammatory mediators, serglycin and other proteoglycans, and proteases. Because these tissue resident inflammatory cells express proteases in such great abundance and their enzymatic activity results in cleavage of a multitude of proteins and peptides, which in turn modify tissue function, their substrate specificity, tissue distribution, and mode of action have become the subjects of great interest. Although mast cell protease-dependent proteolysis is critical to host defense against invading pathogens, regulation of these hydrolytic enzymes is essential to limiting self-induced damage as well. Indeed, dysregulated release of mast cell proteases is now recognized to contribute to the pathogenesis of a number of inflammatory conditions including asthma, abdominal aortic aneurysm formation, vessel damage in atherosclerosis and hypertension, arthritis, and ischemia/reperfusion injury. Understanding how mast cell proteases contribute to inflammation will thus help unravel molecular mechanisms that underlie such immunologic disorders and will help identify new therapeutic targets for drug development.

INTRODUCTION

Mast cells are best known for their role in producing the harmful effects of allergic reactions. However, a growing body of evidence indicates that these immune cells exhibit a complex set of functions and contribute to a diverse array of disorders including asthma, arthritis, formation of abdominal aortic aneurysms, intimal hyperplasia after balloon injury, and ischemia/reperfusion (I/R) injury (1–9). In addition to their adverse effects, mast cells also participate in beneficial functions, most notably with regard to their role in innate immune responses to parasitic and bacterial infections, downregulation of adaptive immunity, and contributing to cell survival in tissues exposed to short bouts of ischemia and reperfusion (ischemic preconditioning) prior to the onset of prolonged cessation of flow (1–9). The dual nature of mast cell responses suggest that these inflammatory cells can alter their phenotype depending upon the prevailing immunologic context and thereby exert protective or deleterious effects. Given the growing body of evidence implicating mast cell proteases in disease, the purpose of this review is to summarize our current understanding of these enzymes with regard to their substrate specificity, distribution, storage, and mechanism of action and their roles in inflammation and I/R.

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MAST CELL PROTEASES

Mast cells are derived from bone marrow precursor cells that circulate in the blood and differentiate into mature immunologic cells after entering tissues. Although they are long-lived cells, surviving for months or years in the tissues, that are terminally differentiated, they can proliferate in response to specific signals. Their close proximity to blood vessels, lymphatics, and nerves, when coupled with the vast array of mediators they can release, allows mast cells to function as sentinels which are able to rapidly respond to humoral and neural stimuli.

A prominent morphologic feature of mast cells is their rich investment with electron-dense granules, which contain enormous amounts of preformed compounds that are collectively referred to as mast cell mediators. These include cytokines (eg, tumor necrosis factor), biogenic amines (eg, histamine and serotonin), serglycin and other proteoglycans such as heparin and chondroitins, acid hydrolases, lysosomal enzymes, renin, angiotensin II, and a number of mast cell-specific proteases, including the neutral proteases chymase, tryptase, and carboxypeptidase A (MC-CPA) (4,7,8). Upon activation, mast cells degranulate and release these mediators into the extracellular space. Interestingly, an emerging body of evidence indicates that mast cells can also undergo alterations in mediator phenotype when exposed to changing conditions (1,2,5). In other words and in contrast to the widely held notion that mast cell degranulation is a non-specific process in which many mediators are released, it is now apparent that specific mediators are secreted in response to particular stimuli or pathologic states.

Mast cell proteases are stored in exceptionally high levels, accounting for 25% or more of the total protein in these immune cells (8,10,11). Because the stored mast cell proteases are present as fully active enzymes, they can immediately contribute tissue injury when released on degranulation. Cleavable substrates include a variety of inflammatory mediators that can be either activated or inactivated by the hydrolytic activity of mast cell proteases, extracellular matrix constituents, inactive proenzymes, and cell surface proteins (8). In addition, the release of angiotensin II formed by the proteolytic activity of mast cell chymase may serve to recruit neutrophils to sites of inflammation (12). These infiltrating phagocytes can release their own complement of hydrolytic enzymes, as well as cytotoxic oxidants, and thereby amplify the destructive effects initiated by mast cell degranulation.

Substrate specificity

Although we will focus on mast cell-specific proteases (the chymases, tryptases, and MC-CPA) in this review, it is important to recognize that non-mast cell-specific proteases are also expressed by these immune cells (including neurolysin, granzymes, and cathepsins) because they influence cellular function when released. However, because they are released by other cell types, it is difficult to determine which cell types are the most important source of these non-mast cell-specific proteolytic enzymes in disease. Of the mast cell-specific proteases (see references 4,7,8 for review), MC-CPA is a zinc-dependent metalloproteinase whereas the tryptases and chymases are serine proteases. MC-CPA is a monomeric exopeptidase that severs amino acid residues from the C-terminal end of peptides and proteins. Chymases are also monomeric in structure and exhibit chymotrypsin-like specificity, cleaving proteins after aromatic amino acid residues. As their name implies, tryptases exhibit trypsin-like substrate specificity and preferentially sever proteins after lysine or arginine residues. The mast cell tryptases are tetrameric in structure, with all active sites facing a narrow central pore. This structural feature not only limits access to potential substrates but also restricts the accessibility of large protease inhibitors to the active sites in the narrow pore of the tetramer, rendering tryptase resistant to inhibition by the macromolecular protease inhibitors expressed in mammals. Depending on the substrate

affected, mast cells proteases and mediators may influence the function of a large number of cells in inflammatory states (Figure 1).

Distribution

Mast cells differ from other hematopoietic cells (eg, neutrophils, basophils, and eosinphils) in that they are present in tissues under normal physiologic conditions. They reside between parenchymal cells, but also in close proximity to blood vessels, lymphatics, and nerves that are embedded in the parenchyma. This perivascular, perilymphatic, and perineuronal location, when coupled with the vast array of mediators they can release, allows mast cells to function as sentinels which are able to rapidly respond to humoral and neural stimuli. In humans, mast cells are classified according to their protease content (see references 4,7,8 for review). MC_T mast cells express only tryptases (α and β), whereas MC_{TC} mast cells express all three types of mast cell-specific proteases. Murine mast cells are also classified by their protease constituents, being subdivided into the connective tissue (CTMC) and mucosal (MMC) subtypes in this species. Although CTMC mainly express two different chymases (an α chymase designated mouse mast cell protease 5 or mMCP-5 and a β chymase designated mMCP-4), this murine mast cell subtype also expresses two different tryptases (mMCP-6 and mMCP-7) and MC-CPA. On the other hand, MMC predominantly express two types of β chymases designated mMCP-1 and mMCP-2.

Storage

Mast cell proteases are initially synthesized as preproenzymes, with subsequent intracellular cleavage of the signal (pre-) and activation (pro-) peptides accomplished by a dipeptidyl peptidase I/cathepsin C-dependent mechanism (see references 4,7,8 for review). Efficient storage of many mast cell proteases (eg, tryptases, the chymases mMCP-4, -5, -6, and MC-CPA) relies on tight packaging via electrostatic interactions with the proteoglycan serglycin. However, not all mast cell proteases (eg, mMCP-1 and -7) are stored in complexes with serglycin, which probably reflects different surface positive charges on the various mast cell proteases. The serglycin-protease complex remains intact initially after mast cell degranulation, but dissociates after exposure to extracellular pH.

MODELS TO STUDY MAST CELL PROTEASE-DEPENDENT PATHOPHYSIOLOGY

Intravital microscopy represents a particularly powerful approach to examine inflammatory responses involving mast cells. By this approach, mast cell degranulation, leukocyte rolling, adhesion and emigration, vascular permeability, and oxidant production can be readily assessed in the living microcirculation in real time (6,12,13). Adhesion molecule expression can also be readily measured in the same tissues used for intravital microscopic observation of the aforementioned inflammatory responses (14,15). Use of in situ zymography allows for assessment of protease activity (16,17). Although intravital microscopy has been applied primarily to thin tissues that allow transillumination of the preparation, inflammatory events in microvessels and accessory inflammatory cells may also be observed on the surface of thick tissues (eg, epicardial surface in the heart, tracheal and bronchial circulations) by conventional microscopy using epiillumination. However, application of multiphoton confocal microscopy allows visualization deeper into the surface in intact but thick tissues (17). Use of mast cell-deficient mouse models have proven to be very useful to the mechanistic exploration of the role of these cells in inflammatory events, while application of knockout and transgenic models affords the opportunity to examine the role of specific gene products of interest. Specific fluorescent probes may be employed in intravital fluorescence microscopic studies to monitor changes in cell membrane potential, intracellular calcium and cell signaling events, mitochondrial function, cell pH, apoptosis

and necrosis, site-specific oxidant generation, mitochondrial function, and endothelial cell-vascular smooth muscle communication in the microcirculation of inflamed tissues.

In vitro cell co-culture models have also proven useful in the study of mast cell-dependent mechanisms of inflammation. This reductionist approach allows for assessment of the role for individual cell components and inflammatory mediators in a more controlled environment than intact tissues that is not influenced by neural or humoral systems that complicate data interpretation in studies conducted in intact tissues (18). Mast cells have also been seeded into three-dimensional constructs of the extracellular matrix, together with cells comprising tissue parenchyma, to examine tissue remodeling in response to inflammatory mediators. This approach is particularly appealing as it more accurately reflects the in vivo situation than standard individual or cell co-culture systems, but retains the ability to study isolated cell populations and mediators in a more controlled environment (19).

Mast Cell Proteases and Inflammation

Although the different mast cell proteases exhibit stringent requirements regarding the specificity of the amino acid cleavage sites in proteins and peptides, these enzymes are relatively promiscuous with regard to protein substrates because they may contain lysine/arginine and/or aromatic amino acid residues at accessible locations. Thus, each mast cell-specific protease is capable of degrading a multitude of different proteins. Moreover, depending on the repertoire of amino acid residue cleavage sites contained in a given protein, these proteases may cleave the substrate at single or multiple sites. It is also likely that the availability of preferred substrates varies at different stages of a given pathologic process. This important concept applies to the regulation of inflammatory processes, which allows mast cell-specific proteases to exert dual roles that on the one hand are proinflammatory, but on the other are protective (Figure 2). For example, a large number of proinflammatory chemokines and cytokines are substrates for mast cell-specific proteases (see references 1–4,7,8 for review). In many instances, the severing activity leads to activation of inflammatory mediators, as is the case for chymase-mediated cleavage of CCL6, -9, -15, and -23, pro-IL-1β, pro-IL-18, and CTAP-III. On the other hand, the enzymatic activity of chymase inactivates CCL3, CCL5, IL-6, and IL-13, while tryptase and MC-CPA have been reported to cleave IgE and endothelin-1, respectively. Interestingly, mast cell β-tryptase selectively degrades RANTES and eotaxin, thereby interfering with eosinophil recruitment (see references 1–4,7,8).

Mast cell chymase appears to play an important role in increasing microvascular permeability by severing adhesion proteins comprising junctional complexes between endothelial cells (6,20–24). This may contribute to edema formation in inflammatory states involving release of mast cell chymase. By attacking components of the extracellular matrix, mast cell-specific proteases may also alter the compliance characteristics of the interstitium, which facilitates the formation of edema in inflammatory states by limiting the increase in interstitial fluid pressure that would otherwise occur and oppose fluid filtration if the extracellular matrix had remained intact (24). Disruption of the intestinal mucosal barrier by mast cell chymase-dependent scission of junctional proteins may allow contents of the bowel lumen to gain access to the intestinal interstitium, even to the point of allowing bacterial translocation and development of sepsis (20,21). In addition to these inflammatory changes, disruption of the three-dimensional reticular structure of the extracellular matrix by mast cell proteases expedites the influx of inflammatory cells such as neutrophils, owing to reduced resistance to their amoeboid movement through the tissue spaces (4,24). Release of their own cytotoxic arsenal of reactive oxygen species and hydrolytic enzymes by these inflammatory leukocytes can amplify the tissue damage initiated by mast cell degranulation.

Indeed, the enzymatic activity of mast cell proteases, as well as the release of hydrolytic enzymes by infiltrating neutrophils, may also lead to release of matrix bound chemoattractants, thereby facilitating autologous chemotaxis (24,25). Chymase also indirectly influences remodeling of the extracellular matrix via its ability to convert pro-matrix metalloproteinase-2 and -9 to the active forms of the enzymes (26). Similar effects have been reported for other matrix metalloproteinases (27,29).

Tryptase catalyzes the degradation of type IV collagen while chymase cleaves vitronectin and procollagen. Both proteases also cleave fibronectin. These extracellular matrix proteins are important constituents of basement membranes and serve as scaffolds to support cells comprising blood vessel walls by providing attachment sites (focal adhesions) for vascular cells. Thus, inappropriate cleavage of proteins in basement membranes and the extracellular matrix between vascular cells contribute to diminished structural integrity of blood vessels and a propensity for aneurysm formation. Thus, it is not surprising that chymase inhibitors have proven effective in limiting the development of abdominal aortic aneurysms (30–32). It is well-known that tryptase activates protease receptor 2 (PAR2) thereby modulating the activity of immune cells (33–35). PAR2 activation signals through MAPK and PI3K (36), which may activate cell survival programs in the development of resistance to I/R injury induced by preconditioning (37). However, the role of mast cell tryptase in preconditioning has not been evaluated. Chymase targets angiotensin I for cleavage, resulting in the generation of the powerful vasoconstrictor angiotensin II (38,39). However, mast cell-dependent, chymase mediated regulation of angiotensin II levels is complex in that this protease also possesses angiotensin II degrading activities (40). Angiotensin II also induces the activation of NADPH oxidase, an oxidant producing enzyme that has been implicated in the pathogenesis of numerous disease states (41). Indeed, we have shown that I/R-induced leukocyte infiltration is dependent on angiotensin II formation secondary to the enzymatic activity of mast cell chymase, which subsequently acts to activate NADPH oxidase (12).

In addition to effects on angiotensin II levels, mast cell-derived chymase is involved in the synthesis or degradation of other vasoactive and proinflammatory agents, such as bradykinin, endothelin 1 and 2, neurotensin, and substance P (4,42). Taken together, these observations suggest that the enzymatic activity of mast cell chymase may alter the availability of vasoactive agents and promote oxidant production during inflammatory states, leading to changes in blood pressure and the distribution of blood flow and infiltration of inflammatory leukocytes. The activity of mast cell proteases also plays a role in atherothrombosis via tryptase-dependent effects to cleave thrombin, fibrinogen and urokinase-type plasminogen activator (43). Chymase activity has been implicated in the pathogenesis of atherosclerosis and hypertension. This may be mediated by chymase-induced formation of angiotension II, which activates NADPH oxidase, degradation of high-density lipoprotein, induction of endothelial cell apoptosis, and disruption of the fibrous collagen cap in atherosclerotic plaques (30,41,43). Indeed, chymase may be the predominant source of angiotensin II in injured tissues (39). Importantly, this enzyme can account for as much as 80–90% of angiotensin II formation in the heart and is a significant source of the peptide in damaged arterial vessels (39).

MAST CELL PROTEASES CONTRIBUTE TO I/R INJURY

I/R is now recognized as one form of acute inflammation in which mast cells play an important role. Moreover, it is now appreciated that reperfusion can initiate a cascade of deleterious processes, including mast cell degranulation, that exacerbate the tissue injury induced by ischemia (reviewed in 44). Indeed, work conducted over the past 20 years has led to the development of the concept that oxidant-induced leukocyte/endothelial cell interactions are largely responsible for the microvascular dysfunction induced by

reperfusion. Reactive oxygen species generated by xanthine oxidase and other enzymes (eg, NAD(P)H oxidase) promote the formation of proinflammatory stimuli, modify the expression of adhesion molecules on the surface of leukocytes and endothelial cells, and reduce levels of the potent anti-adhesive agent nitric oxide (NO). This latter effect is exacerbated by a postischemic decline in endothelial nitric oxide synthase activity and oxidation of soluble guanylyl cylase (sGC), which serves to amplify the intense inflammatory responses elicited by I/R by reducing the bioavailability of NO and ability of downstream signaling elements to respond to this antiadhesive signaling molecule. Coincident with these changes, perivascular cells (eg, macrophages, mast cells) become activated and release other inflammatory mediators (eg, proteases, histamine, TNFα and other cytokines, PAF, LTB_4). Mast cells also participate in the regulation of adhesion molecule expression (45). As a consequence of these events, leukocytes begin to form adhesive interactions with postcapillary venular endothelium. Platelets also play an important role in the adhesion of leukocytes to the postischemic microvasculature. The activated leukocytes emigrate into the tissues, inducing microvascular barrier and mucosal dysfunction via release of oxidants and hydrolytic enzymes. In addition to these changes, leukocytes also contribute to postischemic nutritive perfusion failure (fewer perfused capillaries, ie, capillary no-reflow), endothelium-dependent vasoregulatory dysfunction in arterioles, and mucosal dysfunction. Thus, mast cell degranulation and leukocyte-endothelial cell adhesive interactions, which precipitate the development of arteriolar, capillary, and postcapillary venular dysfunction in the microcirculation, is one of the earliest signs of tissue dysfunction and injury elicited by I/R.

Tissue resident mast cells degranulate during reperfusion (6,13), liberating mediators that promote adhesion molecule expression and thereby contributing to postischemic leukocyte infiltration. Interestingly, mast cell degranulation provokes the expression of endothelial cell adhesion molecules to promote leukocyte infiltration, mucosal barrier disruption, and increased vascular permeability in postischemic tissues, effects that are abolished by treatment with mast cell stabilizers (6,13,44). Because mast cell density is very high in gastrointestinal tissues (and the respiratory tract) (46), it is not surprising that these perivascular cells play a much more prominent role in the inflammatory response to I/R in the small bowel vs skeletal muscle and other tissues that do not come in direct contact with the environment. For example, arteriolar endothelium-dependent vasodilator responses are not influenced by mast cell degranulation in skeletal muscle (47). Work conducted in mast cell-deficient mice indicates that these perivascular cells play a prominent role in I/R-induced inflammatory responses in the small bowel, but contribute very little in skeletal muscle (13). However, when mast cell-deficient Wf/Wf mice were engrafted with bone marrow-derived mast cells, I/R induced significant injury (48). This latter result is important in that it indicates that compensatory gene alterations or other gene deficiencies that may result in creating the mast cell-deficient mice do not contribute to the lack of effect in mast cell-deficient mice. It is important to emphasize that other studies provide equally compelling support for mast cells in skeletal muscle I/R injury (49). Not only is mMCP-5 is abundantly expressed in wild-type muscle mast cells, mice deficient in this murine chymase (mMCP-5 null mice) are far less susceptible to muscle I/R injury than their wild-type counterparts (49). Interestingly, since mMCP-5 null mice also lack MC-CPA, the latter protease may also be involved in skeletal muscle I/R. A role for chymase has also been invoked in myocardial I/R (39,50,51).

We have recently implicated mast cell chymase in postischemic arteriolar endothelium-dependent vasodilator dysfunction (44). As shown in Figure 3, I/R induces leukocyte rolling, adhesion and emigration, an effect that is partially dependent on the formation of angiotensin II by mast cell-derived chymase (12). Infiltrating neutrophils release matrix metalloproteinase-9, which cleaves extracellular matrix components, thereby exposing

matricryptic sites and releasing matricryptins that can now interact with αvβ3 integrin on the surface of mast cells. This promotes further mast cell degranulation, chymase release and angiotensin II formation. Angiotensin II interacts with AT1 receptors to promote the activation of NADPH oxidase, thereby fueling the generation of superoxide. This reactive oxygen species interacts with NO released in response to acetylcholine receptor stimulation or flow-dependent increases in wall shear rate to form reactive nitrogen oxide species (RNOS). This effectively reduces the bioavailability of NO and prevents subsequent activation of guanylyl cyclase/protein kinase G-dependent relaxation of vascular smooth muscle.

ROLE OF MAST CELL PROTEASES IN ISCHEMIC PRECONDITIONING

Ischemic preconditioning refers to an adaptive process that occurs in tissues exposed to brief periods of vascular occlusion and reperfusion prior to the onset of prolonged ischemia. Brief exposure to mildly noxious stimuli such as these short bouts of preconditioning ischemia and reperfusion evoke the expression of cell survival programs that limit the deleterious effects of prolonged I/R. It has been suggested that mast cells may participate in ischemic preconditioning, a notion that arose from observations that mast cells degranulate in response to ischemic preconditioning and because they are responsive to a variety of nonimmunologic stimuli, including those that instigate preconditioned states, such as adenosine (3,5). As summarized by Walsh et al in 2008 (9), the prevailing view at that time was that mast cell degranulation induced by ischemic preconditioning protected the heart by depleting these cells of mediators prior to the onset of subsequent prolong I/R. However, Koda et al (5) have recently presented evidence indicating that mast cells may play a more proactive role in ischemic preconditioning (Figure 4). In this study, preconditioning prevented I/R-induced mast cell degranulation, thereby limiting renin release from these cells. As a consequence, tissue angiotensin II levels are reduced in preconditioned hearts, an effect which limits norepinephrine release from sympathetic nerves terminals, and thus prevents the subsequent development of ventricular arrhythmias. These effects of ischemic preconditioning to stabilize mast cells during I/R appeared to be mediated by an adenosine A2b/A3 receptor-/protein kinase C-ε–/aldehyde dehydrogenase-dependent mechanism. Mast cell stabilization by this mechanism prevented mast cell renin release and the dysfunctional sequelae to local activation of the renin-angiotensin system, which include angiotensin II-induced norepinephrine release and consequent development of lethal arrhythmias. Since inhibition of mast cell degranulation would also be expected to decrease chymase release, local generation of angiotensin II would also be suppressed in preconditioned hearts, which may also contribute to protection.

Acknowledgments

This work was supported by grants from the National Institutes of Health (HL-082816, HL-095486, and AA-014945)

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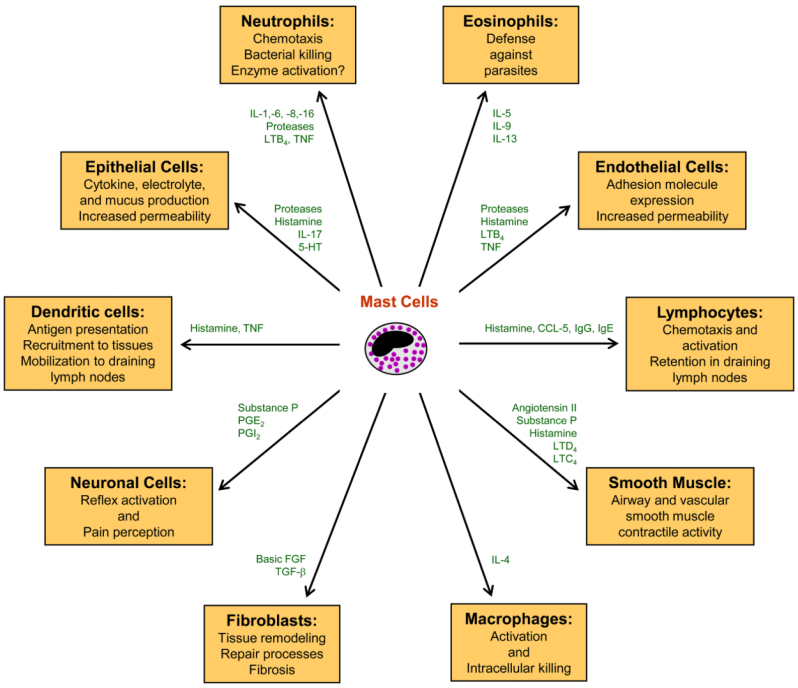

Figure 1.
Mast cells communicate with a large number of cells via mediator release to influence inflammatory processes. Modified from reference 1.

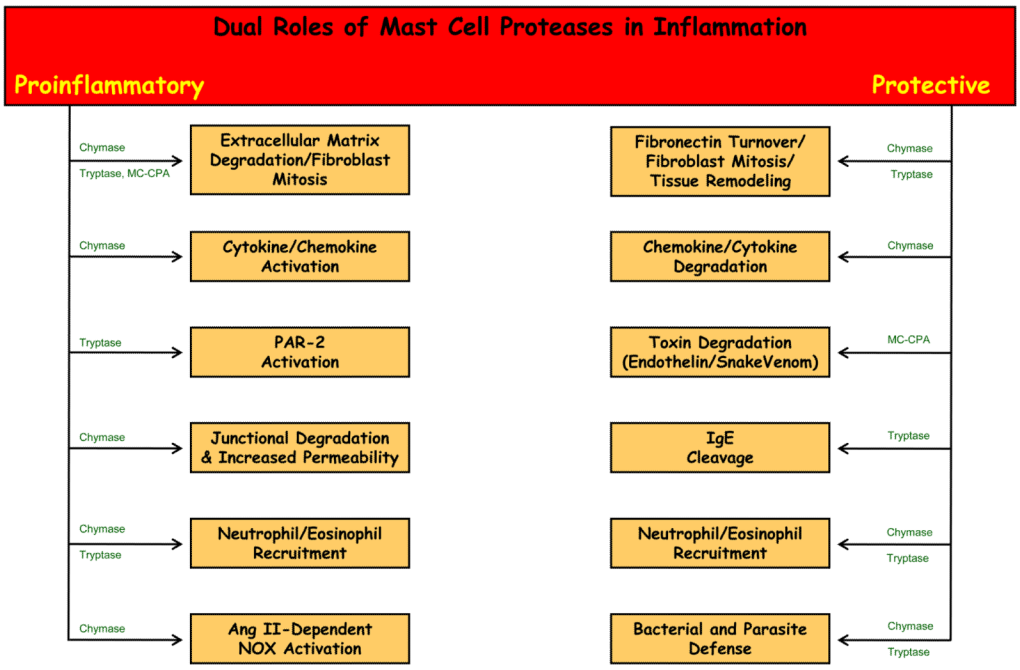

Figure 2.
Mast cells may play dual roles in inflammatory states depending on immunologic context, exerting proinflammatory and protective effects. Modified from reference 1.

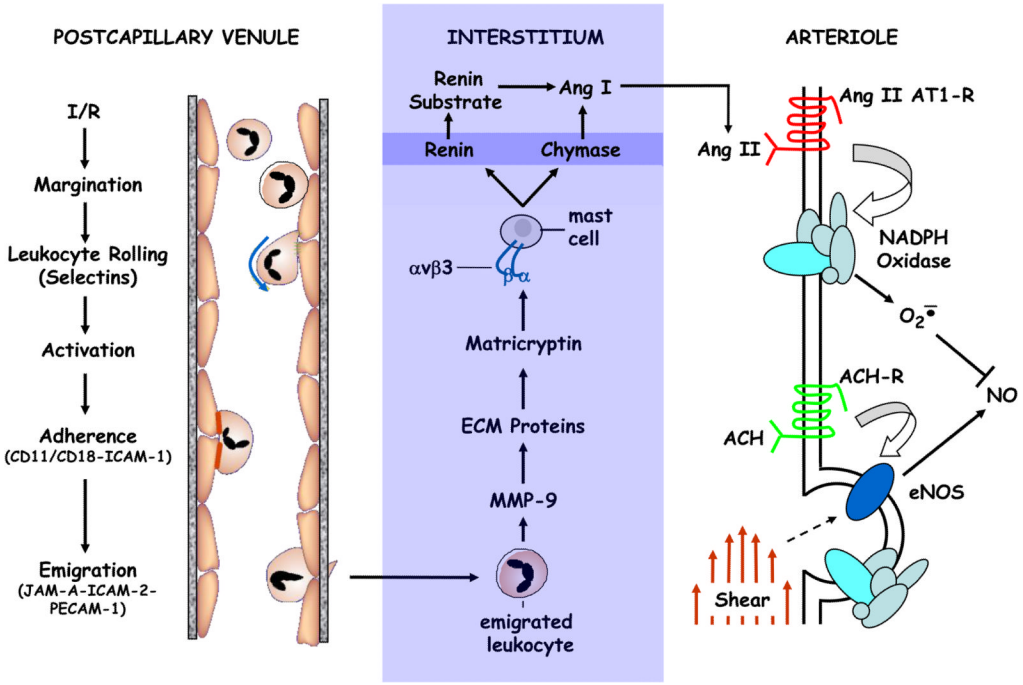

Figure 3.

Mast cell-derived chymase promotes the formation of angiotensin II during ischemia/reperfusion, which in turn activates NADPH oxidase via an AII receptor type 1 (AII-R) dependent mechanism. Superoxide (O_2^-) formation fueled by the enzymatic activity of NADPH oxidase quenches nitric oxide (NO) produced by endothelial nitric oxide synthase (eNOS) in response to acetylcholine (ACH) receptor (ACH-R) or flow-dependent stimulation, leading to impaired endothelium-dependent vasodilator responses in postischemic tissues. Interestingly, mast degranulation is dependent on infiltrating leukocytes, which release matrix metalloproteinase-9 (MMP-9). The proteolytic activity of MMP-9 degrades extracellular matrix components, exposing matricryptic sites and releasing matricryptins, which in turn activate mast cells by interacting with αvβ3 integrin. Mast cell-derived chymase release may facilitate further mast cell degranulation by cleaving neutrophil-derived pro-MMP-9 to the active form of the enzyme, thereby creating a positive feedback cycle that generates additional angiotensin II. Modified from reference 44.

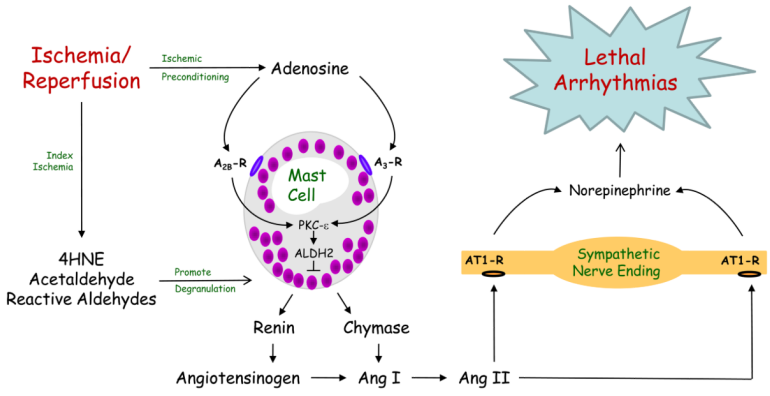

Figure 4.

Ischemia/reperfusion (I/R) causes postischemic arrhythmias via stimulation of mast cell degranulation secondary to generation of acetaldehyde, 4-hydroxynonenol (4HNE), and other reactive aldehydes. Mast cell degranulation is associated with renin and chymase release. I/R-induced renin release results in the cleavage of angiotensin I (Ang I) from angiotensinogen. Subsequent removal of two amino acids from Ang I by the action of mast cell-derived chymase, as well as angiotensin converting enzyme (ACE), results in the formation of angiotensin II (Ang II), which acts on sympathetic nerves terminals to potentiate norepinephrine release by interacting with angiotensin type 1 receptors (AT1-R). Both Ang II and NEPI promote arrhythmias. Ischemic preconditioning prevents postischemic renin release from mast cells by upregulation of aldehyde dehydrogenase-2 (ALDH2), an effect that is initiated by activation of adenosine A_{2B} (A_{2B}-R) and A_3 (A_3-R) receptors, which subsequently induce the translocation and activation of protein kinase C-epsilon (PKCε). Modified from reference 5.