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The Impact of Tryptase and Epigenetic Mechanisms on Mast Cells

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Abstract

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Mast cells have a large influence on multiple immune-mediated responses, including allergic conditions, and they have been implicated in various diseases such as arthritis and cancer. Mastocytosis is characterized by abnormal mast cell proliferation induced by mutations in KIT, the stem cell factor (SCF) receptor. Mast cell leukemia is the most aggressive form of systemic mastocytosis, with no curative treatment options. Therefore, a therapy or study that help finding a cure for this disease is urgently needed.

In paper I, we studied the effect of histone modification inhibition on mast cells. Our findings showed for the first time that mast cell leukemia cells are highly sensitive to histone methyltransferase inhibition. In paper II, we further investigated mast cell function, examining whether DNA can substitute heparin in stabilizing tryptase enzymatic activity. The mechanism by which tryptase retains its enzymatic activity in the nuclear environment is unknown. Our study demonstrated that double-stranded DNA maintained the enzymatic activity of human β-tryptase and identified that tryptase is located within the nucleus of primary human skin mast cells. The interaction of tryptase with DNA is further investigated in paper III, with the aim of determining whether tryptase can affect the formation of neutrophil extracellular traps (NETs). This study showed for the first time that tryptase of mast cells binds to DNA and it has a significant potentiating effect on the formation of NETs in reaction to neutrophil triggering stimuli. Furthermore, the study showed that DNA-stabilized tryptase has a high capacity for proteolytic modification of a variety of cytokines, implying a regulatory role for NET-bound tryptase in inflammatory processes. Finally, in paper IV, we examined the effect of mast cell apoptosis on histone processing, and the extent to which these processes are reliant on tryptase. The findings demonstrated that using a granule-mediated approach to induce mast cell death resulted in substantial processing of core histones. Additionally, they showed that tryptase is highly required for the processing and that tryptase regulates the amplitude of epigenetic core histone modifications during the process of cell death.

Taken together, the findings provide a basis for investigating histone modification inhibition as a potential therapeutic strategy for the disease. Furthermore, they reveal a previously unknown way of mediating mast cell tryptase stabilization and indicate that tryptase plays a role in the regulation of mast cell death, having the potential to influence our experience and understanding of how tryptase affects nuclear processes.

Keywords: mast cells, mastocytosis, tryptase, histone, DNA, methylation, neutrophil extracellular traps

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To my family

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Alanazi, S., Melo, F. R., & Pejler, G. (2020). Histone Methyltransferase Inhibition Has a Cytotoxic Impact on Transformed Mast Cells: Implications for Mastocytosis. *Anticancer research*, *40*(5), 2525–2536. doi.org/10.21873/anticanres.14223.

II. Alanazi, S., Grujic, M., Lampinen, M., Rollman, O., Sommerhoff, C. P., Pejler, G., & Melo, F. R. (2020). Mast Cell β-Tryptase Is Enzymatically Stabilized by DNA. *International journal of molecular sciences, 21*(14), 5065. doi.org/10.3390/ijms21145065.

III. Pejler, G. Alanazi, S., Grujic, M., Adler, J., Olsson, A. K., Sommerhoff, C. P., & Rabelo Melo, F. (2021). Mast Cell Tryptase Potentiates Neutrophil Extracellular Trap Formation. *Journal of innate immunity,* 1–14. https://doi.org/10.1159/000520972.

IV. Alanazi, S., Rabelo Melo, F., & Pejler, G. (2021). Tryptase Regulates the Epigenetic Modification of Core Histones in Mast Cell Leukemia Cells. *Frontiers in Immunology* (Vol. 12, p. 5210). https://www.frontiersin.org/artcle/10.3389/fimmu.2021.804408.

Contents

Abbreviations

MC	Mast cell
BMMC	Bone marrow mast cell
NK	Natural killer cell
CTL	Cytotoxic T lymphocyte
MMPs	Matrix metalloproteases
MMC	Mucosal type mast cell
CTMC	Connective tissue type mast cell
TNF	Tumour necrosis factor
CPA3	Carboxypeptidase A3
MCP	Mast cell protease
MRGPRX2	MAS-related G protein-coupled receptor-X2
IL	Interleukin
GAG	Glycosaminoglycan
MC_T	Mast cell tryptase type
MC_{TC}	Mast cell tryptase & chymase type
c-KIT	Receptor tyrosine kinase
SCF	Stem cell factor
HDC	Histidine decarboxylase
ECC	Enterochromaffin cell
PAR-2	Protease activated receptor 2
SM	Systemic mastocytosis
PTM	Posttransitional modification
HMC-1	Human mast cell line-1
PRR	Pattern recognition receptor
TLR	Toll-like receptor
LPS	Lipopolysaccharide
TGF	Transforming growth factor
TMT	transmembrane tryptase
PAF	Platelet activating factor
AHR	Airway hyperresponsiveness
VIP	Vasoactive intestinal peptide
HDAC	Histone deacetylase
HTMi	Histone methyltransferase inhibitor
LLME	H-Leu-Leu-OMe

Introduction

The immune system is an exceptional system that supports the growth of life and helps us to overcome hostile threats. In general, the immune system is made up of two parts, known as the innate and adaptive immune systems. Each has its unique set of characteristics that combine to generate a complicated network of reactions. *Innate immunity* is a general phrase that refers to the host's nonadaptive responses, which include structural and physical barriers such as the skin, tears, and mucosal layers. This system is comprised of a variety of leukocytes, notably phagocytic cells (neutrophils, dendritic cells, and macrophages), basophils, eosinophils, and natural killer (NK) cells. In the event of an infection, they offer immediate protection. However, when a microorganism crosses the initial line of defense, these cells collectively engage, typically resulting in measures to eliminate the body of the invader, displayed as an inflammatory reaction.

Adaptive immunity is the second part of the immune system. Adaptive immunity's elements enable the immune system to identify infections precisely as well as remember them and hence launch a robust full response. Here, the leukocytes are classified as B-cell and T-cell lymphocytes. B cells are engaged in humoral immune responses, whereas T cells are engaged in cell-mediated immunological responses. The adaptive strategy entails B cells producing antibodies that specifically target the invading pathogens. T cells are classified as adaptive immune response orchestrators comprising T-helper cells, or cytotoxic T cells. Collectively, the innate and adaptive systems contribute to the development of an effective defense against potentially dangerous pathogens.

Mast cells (MCs) are generally recognized as a component of the innate immune system. MCs are a type of leukocyte that presents throughout the body, and they are found in abundance in the areas where the body encounters the external environment: the skin, lungs, and digestive system. Once MCs identify a microbe or virus, they initiate an inflammatory reaction via releasing a wide range of mediators defending the body against pathogens and illnesses. One major type of mediators is a large family of proteins known as serine proteases (e.g., tryptases and chymases), which are prevalent in secretory granules of MCs as well as other immune cells (e.g., neutrophils, NK cells, and cytotoxic T lymphocytes [CTLs]).

Background

Mast cells

MCs are connective-tissue resident cells that contain abundant granules. They arise from hematopoietic pluripotent progenitors in the bone marrow and circulate in the blood vessels as immature MC progenitors [1-3]. MCs do not normally mature before they exit the bone marrow; instead, they travel through the vascular system as immature MC progenitors that complete their development and maturation after arriving in peripheral tissues, such as connective or mucosal tissues, in a process that may be regulated by a variety of local environmental factors. MCs are found in most tissues, typically around blood vessels and nerves, and are particularly abundant in the interfaces between the external and internal environments, such as the skin, lungs, and digestive system mucosa, as well as the mouth, conjunctiva, and nose [4-6].

MCs are known to be involved in a wide variety of physiological and pathological processes. For instance, MCs play a significant role in allergic disorders and contribute to the pathogenesis of a range of other pathological conditions, including rheumatoid arthritis, cardiovascular problems, bone disease, fibrosis, and a variety of inflammatory skin conditions [7-12]. However, it is well established that MCs also have positive functions, including a critical involvement in the degradation of a variety of toxins and in the host defense against parasite and bacterial infections [13, 14]. When MCs grow and mature in the tissue environments, an abundance of secretory granules is generated. These are densely stuffed with a variety of premade inflammatory mediators that are immediately secreted once MCs are stimulated in a manner that results in their degranulation. Various different chemicals are contained in the premade mediators, comprising biogenic amines (histamine, dopamine, and serotonin), premade cytokines, different lysosomal enzymes (e.g., -hexosaminidase), proteoglycans of serglycin-type, and vast amounts of MC-restricted proteases. Chymase, tryptase, and carboxypeptidase A3 (CPA3) are examples of the MC-restricted proteases [15-18]. Moreover, it has been demonstrated that MC granules host a variety of additional proteases that are not exclusive to MCs. For example, MC granules contain various aspartic-acid and cysteine cathepsins as well as renin, cathepsin G, and matrix metalloproteases (MMPs) [19].

Mast cell activation

MCs can be activated by different mechanisms due to their ability to express numerous activating receptors on their surface (figure 1). Because of MC activation, a wide range of preformed mediators, as well as cytokines and chemokines, are released. In addition, it is possible that the degranulatory mechanism does not play a role in this process at all. It is worth noting that MCs do not behave similarly to all stimulating signals, and the nature and severity of the MC response may vary based on the phenotype of MCs and the intensity and specific type of received stimuli [3, 20]. Moreover, the degree of this response is regulated by a balance of positive and negative intracellular molecular events [21]. These mechanisms are typically divided into two groups: IgE-dependent and IgE-independent activation pathways.

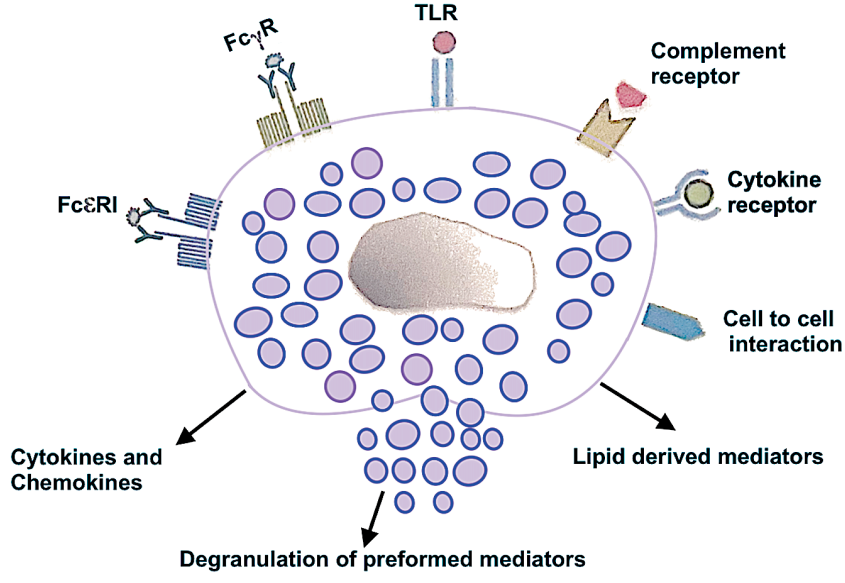

Figure 1. Different mechanism of mast cell activation.

IgE-dependent activation

The IgE-dependent pathway is a well-studied mechanism of MC activation. When an antigen binds to immunoglobulin E (IgE) bound to high-affinity IgE-receptor (FcεRI), it stimulates clustering of FcεRI, which in turn initiates downstream signaling pathways. These signaling pathways include phosphorylation of several signaling proteins, as well as lipid metabolism, transcription factor activation, and intracellular Ca2+ mobilization. Subsequently, this will result in secretion of MC mediators [3, 22]. MCs express and exhibit three subunits of FcεRI (FcεRIα, FcεRIβ, and two FcεRIγ), and they are arranged in a tetrameric organization (αβγ2). The α-chain binds to the Fc portion of IgE,

causing IgE to adhere to the cell surface [23-25]. The β-chain interacts with Lyn kinase via Syk activation or interacts through a Fyn-dependent route, which will result in signal enhancement and phosphorylation of the β- and γ-chains [26, 28]. In rodents, the FcεRI tetrameric organization is required for cell surface presentation, but in humans, a variant without the β-chain is present. Apart from MCs and basophils, other cells, including eosinophils, dendritic cells, and monocytes, also produce α- and γ-chains in their trimeric form in humans [29-31]. MC activation via this pathway is usually observed in acute allergic reactions such as anaphylaxis, and it plays a role in the host's response to several helminth parasites as well [14, 21, 32].

IgE-independent activation

MCs can also be activated by pathways that are independent of IgE/FceRI. MCs are highly effective at alerting the host defense mechanisms to the detection of pathogens. This is effectively done by directly identifying pathogens utilizing pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), which are triggered by certain molecules from pathogenic organisms that have preserved molecular patterns, known as pathogen-associated molecular patterns [34]. MCs can express TLR-2, TLR-3, TLR-4, TLR-6, TLR- 7, TLR-8, and TLR-9 [35, 36]. The PRRs also include Nod-like receptors and C-type lectins such as Dectin-1. The selective PRR involvement is considered an important mechanism in regulating this kind of MC response. For example, peptidoglycan stimulation of MCs through TLR2 results in cytokine release and degranulation, whereas lipopolysaccharide (LPS) activation via TLR4 results in cytokine release alone [37, 38]. Moreover, the binding of Dectin-1 to fungal β-glucan causes MC release of LTC4, while binding of CD48 to the Escherichia coli adhesion FimH causes TNF-α release [39]. As compared to IgE-mediated activation, TLR-mediated MC activation is often independent of degranulation.

The activation of MCs can also be induced by IgG receptors that are present on their surface. FcRIII IgG receptor is expressed only in mouse connective tissue-type MCs (CTMCs), whereas human MCs display both the FcγRI and FcγRIII (activating) as well as FcγRIIB (inhibitory) IgG receptors [40-42]. FcγRI may interact with IgG1 in humans, leading to degranulation and the release of cytokines [43]. Although the FcεRI and FcγRI receptors share γ-subunits [44], their activation processes are different. The FcγRIIB inhibitory effect on MCs leads to the downregulation of secretory responses [45, 46]. Because MCs produce FcγRI on their surface and degranulate in response to IgG cross-linking, they are an excellent contributor in TH1 type immune reactions. There, the Fc receptors (FcεR and FcγR) bind microbe-specific antibodies, facilitating the detection of pathogens by MCs and the subsequent induction of an appropriate TH1 or TH2 immune response. Moreover, it has also

been shown that complement components C3a, C4a, and C5a can cause MC degranulation, which leads to anaphylactic reactions, via particular receptors in vivo [47, 48]. Although in humans, complement compounds C3a and C5a, which are expressed on the skin, have been demonstrated to be able to activate MCs, they have no effect on MCs in the lungs [49, 50]. C5a receptors are not normally present in normal physiological situations; the C3a receptors are only expressed in systemic mastocytosis; and in mice only, CTMCs have complement receptors [51-53]. In addition, MAS-related G protein-coupled receptor-X2 (MRGPRX2) as well as its mouse ortholog MRGPRB2 are two receptors that can induce IgE-independent MC activation [54].

Other than the pathways described above, MCs can also be activated by cytokines, peptides, and physical stimuli. For example, histamine can be released in varying amounts by cytokines such as Interleukin-1 (IL-1), IL-3, IL-8, and GM-CSF [55-57]. Stem cell factor (SCF) has been shown to degranulate MCs in vivo and in vitro [58]. MIP-1α has the ability to induce MC degranulation in vivo [59]. Moreover, several peptides have been reported to cause histamine release, MC degranulation, or both. Neuropeptides such as substance P and neurotensin are capable of affecting neighbouring MCs, causing their degranulation [60]. Additionally, there are compounds that directly provoke MC degranulation. Calcium ionophores, adenosine, and endothelin are a few examples [47]. Degranulation can also be triggered by external stimulants, such as venom, following specific pathogen invasions that penetrate the skin surface [61]. Furthermore, physical stimuli such as osmolarity and pressure alterations can trigger degranulation. Further, cell-to-cell interactions can drive MC activation; evidence shows that activated T cells can activate MCs to secrete mediators and cytokines via ICAM-1 and LFA-1 [62, 63]. Indeed, MC activation via IgE-independent pathways is considered critical in the pathogenesis of numerous nonallergic inflammatory disorders in which MCs are involved [64, 65].

Heterogeneity of mast cells

MCs are known as a heterogeneous cell population with two distinct kinds of mature MCs that have been characterized in mice and humans. The characterization is based on their anatomical location, biochemical characteristics, or protease expression profile [3, 4, 5, 15]. Mouse MC populations are classified according to their anatomical location, which comprise CTMCs and mucosal MCs (MMC; Table 1) [66]. CTMCs contain glycosaminoglycans (GAGs) of the heparin type and they are found in the skin, peritoneum, gut, and submucosa of the stomach. On the other hand, MMCs contain chondroitin sulfate instead of heparin and they are present in the respiratory and gastrointestinal tracts [4, 5]. Human MCs contain both heparin and chondroitin sulfate

proteoglycans [67], and they are divided into two populations based on their protease content: those with just tryptase (MCT) and those with both tryptase and chymase (MCTC) [68]. The human MCTCs are most comparable to mouse CTMCs with respect to protease content and tissue localization, both expressing tryptase and chymase and being found in the skin, gastrointestinal, and bronchial submucosa [5]. Human MCT, on the other hand, expresses tryptase but lacks chymase and they are mostly present in the gut, bronchial mucosa, and the bronchial epithelium, generally equivalent to MMCs [5, 69, 66]. In mice, MC progenitors are c-kit+, Thy-1lo cells that contain granules and express mRNA for MC-specific proteases [70]; nevertheless, when it comes to humans, MC progenitors are nongranulated cells. They are CD34+, CD13+, and CD117+ (c-kit+) [71]. SCF, also denoted c-kit ligand, is the major cytokine implicated in the differentiation and proliferation of MCs [72]. In vitro, bone marrow-derived MCs (BMMCs) can be induced with a variety of cytokines to develop into either MMCs with the addition of transforming growth factor-beta (TGF-β) and IL-9, or CTMCs with the addition of SCF and IL-3 [73, 74].

Table 1. Mast cell Heterogeneity in human and mouse.

	Human		**Mouse**	
	MC_T	**MC_{TC}**	**CTMCs**	**MMCs**
Tryptase	+	+	mMCP-6 & mMCP-7	
Chymase	-	+	mMCP-4 & mMCP-5	mMCP-1 & mMCP-2
CPA	-	+	+	-
Proteoglycan	Heparin & chondroitin sulfate	Heparin & chondroitin sulfate	Heparin	Chondroitin sulfate

Abbreviation: MC, mast cell; CPA, carboxypeptidase A; mMCP, mouse mast cell protease; CTMC, connective tissue-type MCs; MMC, mucosal-type MCs

Mast cell granules

A distinctive morphological characteristic of MCs is their abundance of electron dense secretory granules that fill a large portion of the cytoplasm. The presence of these granules was crucial in Paul Ehrlich's discovery of MCs in 1878, when he discovered connective tissue cells that appeared "well fed" ("mastung" in German), a term that referred to the presence of loaded secretory granules [75, 76]. Since then, the presence of secretory granules has been used as the main indicator for identifying MCs, which can be easily viewed

using various cationic dyes that generate the classic metachromatic staining of MCs. MC granules contain a large variety of preformed mediators. These are released into the extracellular environment when MCs are activated and degranulated, and they can have a significant effect on many physiological or pathophysiological events.

MC degranulation could happen in response to a variety of external stimuli, as mentioned above most notably by IgE receptor cross-linking, but also in response to neuropeptides, complement activation, and certain toxins [77, 78]. However, it is crucial to highlight that in addition to causing the secretion of preformed granule components, MC activation can also result in the de novo synthesis of many bioactive compounds, which include lipid mediators and platelet activating factor, as well as various cytokines and chemokines [77, 78]. In addition, it is worth noting that activation of MCs does not always result in degranulation. MC exposure to lipopolysaccharide, for example, can result in the release of cytokines without visible degranulation [78]. Generally, these MC mediators can be classified into three categories: preformed mediators, newly synthesized mediators, or a variety of cytokines and chemokines. These mediators are generally released in one of two ways: continuously or in response to MC activation.

Preformed mediators in mast cell granules

The MC granules are filled with preformed mediators such as biogenic amines (e.g., histamine), proteases, and proteoglycans.

Biogenic amines

Histamine is likely the most important biogenic amine produced by MCs. It has been associated with a wide range of physiological and pathological conditions [79], ranging from initiating the inflammatory response in tissues to modulating gut physiology and serving as a neurotransmitter. Histamine is retained within cells in the secretory granules, under an acidic pH condition, via an ionic connection to the carboxyl groups of proteoglycans and peptides. MCs are the main producers of histamine, which is the most well-examined mediator in MCs with regard to its bioactivity and mechanism of function. Histamine is made when the enzyme L-histidine decarboxylase (HDC) reacts with the amino acid histidine. HDC-deficient mice exhibit lower MC counts and lower protease and proteoglycan levels [80]. Histamine, for instance, facilitates inflammation, raises vascular permeability, and promotes stomach acid production in the gastrointestinal system [81]. It acts via the histamine receptors (Histamine 1, Histamine 2, Histamine 3, and Histamine 4 receptors), which are members of the G–protein-coupled receptor family [82, 83]. Histamine receptor antagonist drugs have previously been effectively used. Smooth muscle cells and endothelial cells have H1 receptors, as do a

variety of other cells. The primary role of H2 receptors is to increase stomach acid production. The neurological system is the primary site of H3 receptor expression. Several types of hematopoietic cells have H4 receptors, including MCs. Additionally, MCs produce H1 and H2 receptors, and stimulation of these two types of receptors influences the release of MC mediators [84].

Another biogenic amine is serotonin, which is primarily a neurotransmitter in the central nervous system. However, it is also found in a wide variety of peripheral tissues and other cells, which include most of the immune cells such as monocytes, macrophages, lymphocytes, MCs, platelets, and enterochromaffin cells (ECCs) [85]. According to publications, ECCs and brain cells produce the majority of serotonin, which is subsequently absorbed and packaged by other cells [86]. Serotonin's key activities in the immune system are well-studied and comprise T-cell and natural killer cell activation, delayed-type hypersensitivity reactions, and the generation of chemotactic mediators (e.g., by macrophages) [87].

Mast cell proteases

MC proteases are abundantly stored in MC granules, contributing up to 25% of the total protein of an MC [16, 17, 88]. As a result, when MCs degranulate, huge quantities of proteases are released into the surrounding tissue, and they are likely to have a significant effect on any condition in which MC degranulation occurs. Notably, unlike most other proteases, the MC proteases are all found in an active form in the granules. This is why, when degranulation occurs, the proteases released into the extracellular environment have the ability to execute their actions rapidly in the tissue [16]. Proteases that are expressed exclusively by MCs are commonly denoted "MC proteases" and they encompass chymases, tryptases, and CPA3. However, it should be noted that MCs also express a variety of non–MC-specific proteases, including lysosomal cathepsins, granzymes, neurolysin, and cathepsin G [17].

Chymase

Chymases are monomeric serine proteases that have chymotrypsin-like cleavage specificity; they cleave after aromatic amino acid residues. Chymases are produced as inactive forms, and therefore they are activated by the elimination of the acidic N-terminal dipeptide via dipeptidyl peptidase I [89, 90]. Subsequently, the active form is stored in granules, where chymase is firmly linked to heparin within the acidic granules, which is considered to hinder autolysis. Following MC degranulation, chymase remains in association with heparin proteoglycan, which improves enzymatic activity and protects chymase from extracellular inhibitors [91, 92]. Humans express just one chymase, belonging to the family of α-chymases [93-95]. In mice, the MMCs express two β-chymases, including mouse MC protease 1 (mMCP-1) and mMCP-2, but mMCP-

2 does not have proteolytic activity [96-99]. CTMCs mostly express mMCP-4 (a β-chymase) and mMCP-5 (an α-chymase) [98, 101], whereas mMCP-5 appears to show more elastase-like cleavage selectivity [102 -106]. mMCP-9 is produced mainly via MCs in the mouse uterine tissue [107]. mMCP-5 is probably the closest sequence homolog to human chymase, and the only α-chymase synthesized by murine MCs. However, the human α-chymase (CMA1) has the same cleavage specificity and distribution as mMCP-4 [107]. According to this, mMCP-4 might be the human chymase's functional homolog [108]. One α-chymase (rMCP5) and four β-chymases (rMCP-1, 2, 3, and 4) are expressed in rat MCs [109-111]. One α-chymase is expressed by canine MCs [112].

MC carboxypeptidase A

CPA3 is a monomeric exopeptidase that catalyzes the cleavage of amino acids from the C-terminal end of proteins and peptides [113]. CPA3, in mice and humans, has a comparable tissue distribution and other features [114]. Although the specific mechanism by which pro-CPA3 is converted to its active form is unknown, evidence seems to suggest that cathepsin E may contribute to this mechanism [115]. Further, additional data demonstrate the important role of serglycin and its heparin-linked side chains during pro-CPA3 conversion [116,117]. When being converted, CPA3 is retained in its active state within the granules. The low pH of about 5.5 within the granules ensures that protease activities are kept to a minimal level [117].

Tryptase

Tryptase is an MC serine protease that cleaves after Lys/Arg residues, apparently similar to trypsin. It is remarkable in that it is tetrameric, with all of its active sites facing a narrow central pore [17, 118]. Tryptase is insensitive to all known physiological protease antagonists and exhibits a relatively restricted substrate cleavage characteristic as a result of its organization [88,118,119]. Serglycin, that is, proteoglycans with strongly negatively charged GAG side chains of the heparin (or chondroitin sulfate) type connected to the serglycin core protein, has been demonstrated to be crucial for the storage of tryptase within the granules [120, 121]. Heparin is known to be required for the assembly of enzymatically active tryptase tetramers in addition to enhancing tryptase storage [122, 123]. Furthermore, it has been demonstrated that heparin is required for the tetrameric, active organization of tryptase to stay stable, and therefore the absence of stabilizing heparin results in fast monomerization and loss of enzymatic activity [124]. Recently, the long-held belief that tryptase is restricted to secretory granules was challenged by the finding that tryptase could also be found in the nucleus of MCs and in tumor cells challenged with tryptase. In the cell nucleus, tryptase has been found to degrade many nuclear substances, including lamin B1, and histone proteins, and tryptase was also found to regulate gene expression and

proliferation [125-127]. Thus, these findings reveal that tryptase is capable of becoming enzymatically active inside the nucleus and exerting functional effects on nuclear events. However, because heparin is unlikely to be found within the nucleus, it is unknown how tryptase maintains enzymatic activity within the nuclear compartment.

Tryptases are maintained as active enzymes within the MC granules as a complex with serglycin proteoglycan [123, 124]. Human MCs mainly express two kinds of tryptases, known as α- and β-tryptases. The β-tryptases are classified into three subsets that include β-I, β-II, and β-III, and they are the predominant types identified in MCs with the most catalytic activity [127, 129]. The α-tryptases are divided into subtypes I and II. It is possible to detect α-tryptase subtype I in the bloodstream even when the MC is not degranulated, suggesting it is released on a constant basis [130]. An additional tryptase type was also identified, based on structural analysis, and was named gamma tryptase (γ-tryptase) or human transmembrane tryptase (hTMT). This type of tryptase is exposed to the cell surface during MC degranulation, and it is clearly distinct from the other forms [131, 132]. The δ-tryptase form of tryptase has also been found in human MCs, but its biological relevance has yet to be established [131]. Nearly 29% of people are genetically deficient in α-tryptase, exhibiting considerable differences among ethnicities [133]. In mice, mMCP-6 and mMCP-7, as well as a transmembrane γ-tryptase (known as mTMT), are expressed by MCs [131]. mMCP-6 is considered the most homologous to human β-tryptases and is expressed by CTMCs [2]. Additionally, tryptase mMCP-11 is also expressed by mouse MCs in the initial phases of development. In rat, MC tryptases have nearly identical expression profiles to their mouse equivalents, encompassing rMCP-6 and rMCP-7. Additionally, in sheep and bovine, two tryptases, as well as one tryptase in canine and one in porcine, have been discovered [134-137].

It has been suggested that tryptase degrades a variety of extracellular matrix components, such as fibrinogen, fibronectin, and type VI collagen, as well as activating pro-MMP-3 [138-141]. Moreover, it has been reported that tryptase activates the protease-activated receptor 2 (PAR-2), which could result in inflammatory responses [142]. Various studies have shown that MC tryptase can have a role in asthma and allergies. Tryptase has been shown to enhance smooth muscle cell-, fibroblast-, and epithelial cell proliferation, which may contribute to airway hyperresponsiveness (AHR) and airway remodeling events in asthma [143, 144]. Furthermore, tryptase is capable of degrading vasoactive intestinal peptide (VIP), which serves to ease bronchial smooth muscle in the lungs [145]. Studies in which tryptase inhibitors prevented airway inflammation and AHR in allergic sheep have added credence to the hypothesis that tryptase has a pathogenic function in asthma [146]. Moreover,

incubating human-derived bronchi with tryptase enhances histamine release and subsequent in vitro bronchial responsiveness to histamine [147].

Proteoglycans

Proteoglycans are widely expressed and very abundant in the body, and they have a wide range of activities. For example, proteoglycans are implicated in embryological growth and function in the majority of the body's organ systems, including the immune system [148, 149]. Proteoglycans are made up of a core protein and GAG chains that are covalently connected. Proteoglycans are generally classified into three groups: those present on the cell surface (syndecans and glypicans), those that are connected with the extracellular matrix (e.g., versican, aggrecan, decorin, agrin, and perlecan), and those that are intracellular (serglycin). Serglycin is formed by a variety of hematopoietic cells, comprising macrophages, MCs, lymphocytes, NK cells, and platelets [150]. Serglycin proteoglycan is made up of a 17.6 kDa core protein with an extended serine/glycine (Ser/Gly) amino acid repeat, with the Ser residues serving as GAG adhesion sites [151]. Heparin, heparan sulfate, and chondroitin sulfate are the major GAGs associated with serglycin proteoglycans [152]. Serglycin proteoglycan's diverse biological activities are generally due to its highly sulfated GAG chains.

In CTMCs, heparin is coupled to serglycin; heparin is a well-known GAG and one of the body's most negatively charged molecules [153]. Heparin can interact with a variety of proteins due to its high negative charge, particularly the prestored MC proteases. MMC serglycin, as opposed to CTMCs, contains GAGs of the chondroitin sulfate type that are less negatively charged [154]. Both heparin and chondroitin sulfate GAG chains can bind to the serglycin core protein in human MCs. Serglycin proteoglycan has been shown, by assessing mutant mice, to function as storage meshes for various proteases in the MC granules [41]. CTMCs lacking serglycin proteoglycan show poor cationic dye staining and impaired storage of a variety of granule components, which include CPA3, mMCP-4, mMCP-5, mMCP-6, histamine, and serotonin [41, 155, 156]. Due to a deficiency of highly negatively charged heparin, MCs lacking N-deactylase/N-sulfotransferase 2, an enzyme involved in the early phase of heparin sulfation, exhibit altered secretory granule protease retention [40, 157]. Altogether, serglycin proteoglycan and negatively charged heparin are essential for storing of a variety of positively charged MC-granule components.

Serglycin is exocytosed in association with molecules that rely on serglycin for storage as well as mediators that do not rely on serglycin for storage [158]. Histamine storage is also dependent on serglycin but dissociates from serglycin during secretion due to the increase in pH outside the granules. However, certain proteases stay in combination with serglycin-proteoglycan after

release, which could enhance protease activity by allowing the enzymes to be in close proximity to their heparin-binding targets [159]. CPA3 and chymase, for example, stay attached to the serglycin-proteoglycan following degranulation and may work synergistically to perform biological tasks at the site after MC activation. Apart from the biological activity of serglycin-dependent proteases, serglycin acts as a ligand for CD44, which is a transmembrane glycoprotein implicated in a number of cellular activities, such as differentiation, growth, and survival [160]. Serglycin may also have additional biological activities such as helping to protect serglycin-interacting proteins from proteolysis, binding inflammatory substances such as immune response regulators, and transporting molecules to target cells. The latter hypothesis was advanced in cytotoxic T lymphocytes, where granzyme B is secreted in association with serglycin-proteoglycan and transported to target cells, therefore facilitating eventual death (apoptosis) [161].

Newly synthesized lipid mediators

Several lipid mediators can be newly synthesized by activated MCs. Eicosanoids are arachidonic acid-derived lipid mediators that are newly generated and released directly from activated cells without being stored. Upon activation, MCs can release enormous quantities of arachidonic acid metabolites, including prostaglandin D2, leukotriene C4, and platelet activating factor (PAF) [162-164]. These mediators exhibit broncho-constricting and vasodilatory capabilities as well as contribute to host immunity, inflammatory processes, and allergic pathologies through various activities including effector cell transportation, immune cell activation, and antigen presentation [165-167].

Cytokines and chemokines

Cytokines are immunomodulatory compounds expressed by certain immune cells that act as messengers between cells and even impose the intended effect on certain cells. Both cytokines and chemokines exhibit a wide range of biological activities, having important roles in inflammatory processes, infection, immune responses, cell survival, and growth. MCs synthesize a diverse number of cytokines, both anti- and pro-inflammatory cytokines, and MCs are implicated in the storage and secretion of TNF-α, which is a pro-inflammatory cytokine [68, 168]. MCs are also able to make a variety of other cytokines, including IL-3, IL-4, IL-5, IL-6, IL-10, IL-13, b-FGF-2, TGF-β1, and GM-CSF [168-175]. IL-3 is highly critical in the growth and development of MCs [176] and can cause bone marrow cell differentiation, in vivo, into functional MCs. Moreover, MCs express several chemokines. They can release MCP-1 and RANTES, which attract monocytes and macrophages to the target-tissue sites, as well as IL-8, which attracts neutrophils [177, 178].

Human MCs are also known to synthesize IL-16 and lymphotactin, both of which have been shown to recruit lymphocytes to areas of MC degranulation [179, 180]. Although cytokine release is often triggered by receptor clustering followed by degranulation, it can also be induced without degranulation (e.g., by SCF and LPS).

Apoptosis

Apoptosis is the process through which cells are instructed to die, and it is commonly called programmed cell death [181]. During apoptosis, biochemical processes result in distinctive cell alterations and death of cells. These alterations include membrane blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, DNA degradation, and mRNA fragmentation [182]. Each day, the normal adult person loses on average about 60 billion cells undergoing apoptosis [183]. In contrast to necrosis, which is a type of catastrophic cell death caused by acute cellular damage, apoptosis is a carefully controlled and regulated mechanism. Apoptosis causes the formation of cell fragments known as apoptotic bodies, which phagocytic cells can engulf and clear before the remnants of the cells can cause damage to nearby cells [184]. Apoptosis is strictly controlled and regulated by several pathways, because once initiated, it always results in cell death [185, 186]. The intrinsic pathway, which is known as the mitochondrial pathway, and the extrinsic pathway are the two main activation mechanisms of apoptosis [187, 191]. The intrinsic pathway is induced by intracellular signals produced upon cell stress and it depends on the release of proteins from the mitochondrial intermembrane space [188]. Cytotoxic drugs, DNA damage, and cytokine depletion can all activate the intrinsic route. For example, MCs have been demonstrated to suffer apoptotic cell death through the intrinsic route when SCF is depleted [193]. The Bcl-2 family of pro-apoptotic proteins, such as Bcl-xs, Bax, Bad, and Bik, and anti-apoptotic proteins, such as Bcl-XL, Bcl-2, Mcl-1 and A1, are involved in the intrinsic route's downstream signaling [193]. The Bcl-2 family members regulate the release of apoptotic mediators upon mitochondrial membrane permeabilization. Moreover, weak signals from external stimuli might also activate the intrinsic apoptotic route [189]. However, the extrinsic pathway is induced when external ligands connect to death receptors on the surface of cells, resulting in the development of the death-inducing signaling complex [190].

Both mechanisms result in cell death via activation of caspases, which are proteases (protein-degrading enzymes) [192]. These mechanisms activate initiator caspases, which subsequently induce executioner caspase activation that then destroys the cell by degrading proteins. Many disorders have been linked to defective apoptosis, including cancer, heart disease, and Parkinson's

disease [194, 195, 196]. Excessive apoptosis results in atrophy, whereas inadequate apoptosis causes abnormal cell proliferation, which potentially can lead to cancer. Certain factors, such as Fas-receptors and caspases, induce apoptosis, whereas others, such as members of the Bcl-2 protein family, block apoptosis [193]. SCF, the agonist for the c-kit receptor, is crucially important for MC viability in vivo and in vitro [197]. During normal circumstances, the number of tissue MCs does not change. Mastocytosis can be caused by an imbalance in the apoptosis of MCs, which can lead to an excessive MC accumulation. As a result, inducing MC apoptosis might be an effective treatment for MC-related pathologies.

Mastocytosis

Mastocytosis is a disorder characterized by aberrant MC proliferation in one or more tissues [300]. In the majority of cases, abnormal MC expansion is driven by mutations in KIT (mainly KITD816V), the receptor for SCF (the main growth factor for MCs), which causes the MCs to become self-sufficient in terms of growth. Mastocytosis subtypes range clinically from cutaneous to systemic, with the second being classified as indolent, smoldering, or aggressive, as well as MC leukemia [300-302]. The most serious form of systemic mastocytosis (SM) is MC leukemia, which is defined by bone marrow involvement [300-302]. Organ damage is also present in SM, and the average survival period for individuals with aggressive SM variants is generally less than 6 months. At the moment, there are just a few treatment choices for SM, and allogeneic stem cell transplantation is appearing as a relatively promising therapeutic approach [303]. Nevertheless, a considerable number of aggressive SM patients are elderly, and these individuals are often ineligible for stem cell transplantation. As a result, treatment choices are generally restricted to symptomatic relief, and there is no known cure [300-303]

Mast cells and the immune system

Mature MCs have a lengthy half-life and are located in many sites in the body. They are especially common at body-environment interfaces, which provide entrance routes for infections and hazardous chemicals, and at nerve endings and blood vessels [3]. MCs' development, phenotype, and functionality are regulated by the surrounding microenvironment factors in the tissues. Thus, these factors modulate MCs' capacity to identify and respond precisely to a range of diverse stimuli via the production of a variety of powerful mediators [3]. Because of these characteristics, MCs can function as the first layer of protection against pathogens. Besides that, MCs can transmit signals to other immune cells across the body to encourage the recruitment of various immune

cells to the damaged tissues in response to such assaults (e.g., infections, allergens, toxins, and so forth) [3]. It is clear that MCs perform a crucial role in starting inflammation and mediating innate or adaptive immune responses [267].

Actually, MCs can play both beneficial and detrimental roles for the organism. It has been indicated that MCs can play an important function in protecting the body against animal venoms and some parasitic helminth diseases [268-270]. As an example of a beneficial role of MCs, they can directly eliminate microbes by phagocytosis and the generation of reactive oxygen species [271]. They can also synthesize antimicrobial peptides such as cathelicidins, both constitutively and in response to lipoteichoic acid or LPS exposure [272]. In vitro and in vivo, these peptides were determined to facilitate the killing of Group A streptococci (GAS) [272]. Furthermore, MCs, similar to neutrophils, have been shown in vitro to generate extracellular traps that enclose and eliminate microbes such as GAS [273].

Through the release of granular and secretory mediators, MCs can influence the host's innate immunological responses [274, 275]. Histamine and other vasodilators can enhance vascular permeability and local blood circulation, and they can act on smooth muscle cells to promote parasite ejection. Furthermore, histamine causes the production of mucus by epithelial cells, which could also assist in pathogen neutralization and cell protection. Additionally, MC-derived chemotactic substances can facilitate the attraction of a variety of inflammatory cells, such as NK cells (IL-8), eosinophils (eotaxin), and neutrophils (TNF-α and IL-8) [268, 274].

MC mediators are also essential in adaptive immune response modulation [268, 274]. For example, dendritic cells (through CCL20 and TNF-α) and effector T cells (through RANTES and CXCL10) can be recruited to the infection site and to draining lymphatic nodes by cytokines and chemokines generated by MCs. In addition, MCs, particularly for CD8+ T cells, can serve as antigen-presenting cells directly. MC products can further promote dendritic cell maturation, antigen presentation, and co-stimulatory molecule production. While MC histamine has been shown to promote Th2 polarization of naïve T cells by decreasing dendritic cell IL-12 synthesis and boosting IL-10 secretion in response to LPS [275], direct MC interaction could also prime dendritic cells, in vitro, to enhance Th1 and Th17 polarization [276].

MC responses, although enhancing host defense at the infected area, may also exacerbate the outcome of some infections by causing further damage to host tissues. MCs are unquestionably best recognized for their adverse effects on allergies, especially potentially deadly anaphylaxis, and other associated disorders such as asthma and atopic dermatitis [22, 277]. Multiple lines of

evidence indicate MCs' essential involvement in the pathophysiology of allergic disorders. For instance, asthmatic patients exhibit an increased quantity of lung MCs, particularly in the smooth muscle layer of the airway, the lung mucosa, and the alveoli [278, 280].

Additionally, accumulating evidence indicates that MCs are involved in the development of various other diseases. MCs have been shown to be overrepresented and activated at inflammatory sites in a variety of diseases. These include several autoimmune disorders and mastocytosis, many types of cancer, and atherosclerosis [3, 281-286]. Indeed, the continuous activation of MCs and release of different pro-inflammatory mediators such as certain proteolytic enzymes, growth factors, cytokines, and chemokines can lead to serious inflammatory responses [3, 287, 288]. For example, previous research has demonstrated that even though intraperitoneal MCs were shown to protect against experimental intra-abdominal sepsis, extraperitoneal MCs caused higher mortality, associated with pro-inflammatory IL-6 release [289]. In addition, increased levels of histamine in the blood were also shown to be related to a higher death rate, indicating that both sepsis-induced systemic MC degranulation and an overproduction of pro-inflammatory mediators were responsible. Generally, an unregulated rise in the number and activation of MCs, as well as changes in the location of MCs throughout the body, might have negative effects for the organism.

Mast cells against microorganisms

Recent studies have demonstrated that MCs can protect against a wide range of diseases. Even though remarkable progress has been made using MC-deficient animals, the fundamental mechanisms by which MCs repress many of these microorganisms remain mostly unclear. Initial evidence for a role of MCs in protection against microbes arose from investigations of parasitic illnesses such as those elicited by protozoa and helminths [268, 274]. Studies with MC-lacking mice revealed that MCs promote hook-worm expulsion from the intestine by expressing mMCP-2. Related findings from Strongyloides and Trichinella-spiralis infection approaches have revealed that MCs drive nematode gut expulsion and restrict the effect of the parasite on tissues. Further, MC-deficient mice had raised parasitic load and larger lesions when infected with Leishmania, as well as a decrease in inflammatory responses and IL-12 generation at the infection site. Finally, reconstituting MC-deficient mice with wild-type MCs and TNF-a-lacking mice revealed a significant function for MC-produced TNF-a in reducing parasitemia, the quantitative content of parasites in the blood, in a mouse model for malaria [290].

MCs' involvement in antibacterial defense has been demonstrated more recently, notably in relation to gram-negative bacteria [268, 274]. MCs have been shown to protect against experimental pulmonary infection caused by many pathogens, including Mycoplasma pneumoniae, Klebsiella pneumoniae, GAS skin infection, Pseudomonas aeruginosa, and Hemophilus influenzae otitis media as well as urinary infections and peritoneal E. coli infection [291]. In contrast, there is less evidence that MCs influence antiviral immunity. By activating MCs using synthesized viral dsRNA, CD8+ T cells were recruited to the infection site, which was not seen in mice lacking MCs [292]. MC-deficient mice exposed to dengue virus showed an elevated viral load in draining lymph nodes caused by an absence of NK and NK T-cell recruitment towards the infected area [293]. MCs, on the other hand, might have a role as a viral reservoir in the case of HIV infection, particularly during the latent phase [294].

Even less is known about the involvement of MCs in the pathogenesis of fungal infection. MCs secreted LTC4 in vitro in response to zymosan, a S. cerevisiae cell-wall component [38]. In one study, it was shown that Aspergillus fumigatus hyphae caused MC degranulation via an IgE-independent pathway [295]. Extending these investigations to the in vivo setting is essential for understanding the involvement of MCs in fungal infections because there may be considerable differences in how MCs defend against fungi versus other eukaryotic microbes and parasites. While inducing a MC-associated Th2 response is often beneficial during parasite infection, a Th2 response is typically unfavorable during fungal infection [296].

MCs could also be both pro- and antitumorigenic, dependent on the type of cancer and on the stage of the disease [297]. Even though MC activity is linked to the recruitment of immune cells that can limit tumor growth, MCs may also promote tumor progression in some malignancies, such as prostate cancer [298]. The latter is accomplished by the release of angiogenesis-promoting growth factors such as VEGF and FGF-2. Additionally, tumor-derived peptides can attract and stimulate MCs in the tumor, favoring tumor development [299]. As a result, MCs have been identified as potential targets for cancer treatment [297]. Furthermore, MCs can contribute to wound healing processes by inducing inflammation and healing of injured tissue. MCs can become activated to respond to damage and secrete VEGF, FGF-2, PDGF, and NGF, in addition to tryptase and histamine, which assist in wound healing [299].

Epigenetics

Epigenetics is defined as the study of effects on gene expression that occur without changing DNA sequence. It includes a variety of mechanisms such as

DNA methylation, posttranslational modification (PTM) of core histone proteins, and the effects of non-coding RNAs [198, 199]. In a nucleosome, which is the basic unit of chromatin structure, 147 bases of DNA are wrapped around an octamer of histone proteins (H3, H4, H2, A, and B) (Figure 2) [258-260]. The N-terminal ends of core histones can be modified by a variety of post-translational modifications (PTMs), including methylation, acetylation, phosphorylation, and ubiquitination [200-203]. Such epigenetic modifications, it is thought, alter the transcriptional processes and the structural organization of chromatin, which consequently have an impact on expression of genes, either in combination with other modifications or on their own [201]. Epigenetic changes, such as DNA methylation and histone PTMs, are frequently altered in myeloid malignant disorders, including myelodysplastic syndromes and myeloproliferative neoplasms [204]. Numerous genes are mutated in these diseases, and a number of point mutations in genes encoding epigenetic regulators (e.g., DNMT3A, EZH2, and TET2) have been identified in these malignancies, with implications for disease development, etiology, and prognosis [205, 206]. Similar patterns of these mutations are also detected in SM [207, 208] and increasing evidence shows that more understanding of these issues can help in prediction of development towards aggressive disease phenotypes [209-212].

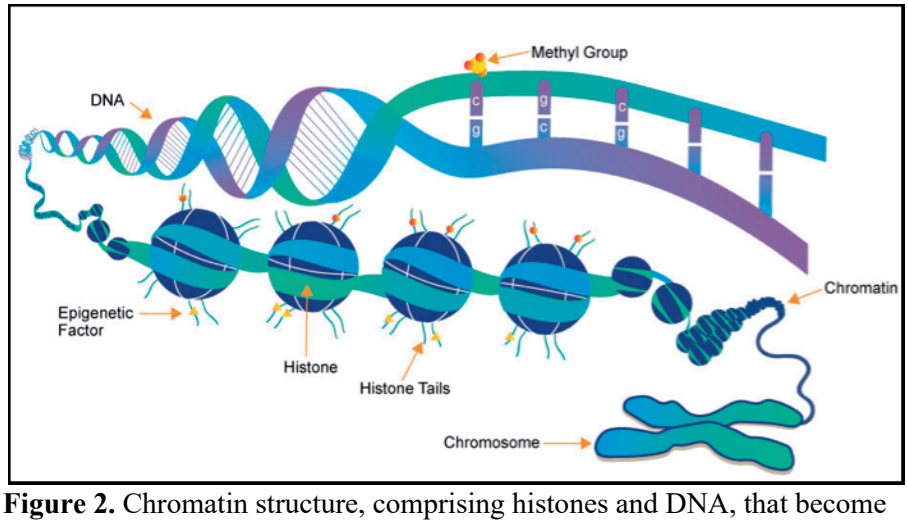

Figure 2. Chromatin structure, comprising histones and DNA, that become exposed to epigenetic marks is represented in this figure. Epigenetic Modifications. (2019). [Graph]. https://scienceofhealthy.com/wp-content/uploads/2019/04/Epigenetics.jpg

Posttranslational modification

PTM refers to generally enzymatic and covalent processes that alter the characteristics of a protein via proteolytic cleavage or the addition of a modifying

group to one or more amino acid, such as acetyl, phosphoryl, glycosyl, or methyl groups [213]. PTMs are important in a wide range of biological activities because they have a profound influence on the structure and activity of proteins [214, 215]. PTMs may be reversible or irreversible [216]. Covalent modifications are reversible processes, whereas proteolytic modifications are irreversible [213]. PTMs can takes place in one or multiple types of amino acids, and they can cause alterations in the chemical characteristics of the sites that have been modified [218]. PTMs are typically found in proteins that have important structural and functional properties, including membrane proteins, secretory proteins, and histones [219]. These alterations have an effect on a variety of protein activities and properties, such as enzymatic activity and synthesis, lifespan of the protein, protein–protein communication, cellular interactions, molecular trafficking, protein folding, and solubility or localization [219-228]. As a result, these modifications have a role in a number of biological processes, including signal transmission, gene expression, regulation of activity, DNA maintenance, and cell cycle regulation [229-231]. PTMs are found in a variety of sites of the cell, such as the cell nucleus, the cytoplasm, Golgi apparatus, and endoplasmic reticulum [217]. In the following subsections, three of the most studied PTMs are described. However, other PTMs also exist, including ubiquitylation, myristylation, sulfation, prenylation, SUMOylation, palmitoylation, and glycosylation.

Phosphorylation

Phosphorylation is an important reversible regulatory process that is required for the activity of a large number of enzymes, membrane channels, and numerous other different proteins in eukaryotic and prokaryotic cells [232-233]. The target sites for phosphorylation include Tyr, Arg, His, Ser, Thr, Pro, Cys, and Asp residues [218], but such modifications occur most frequently on Thr, Ser, His, and Try residues [234]. These PTMs are introduced by kinase enzymes, by adding a phosphate group from ATP onto the acceptor residues. Phosphatases are responsible for the dephosphorylation of such residues [235]. Phosphorylation is by far the most known PTM among the various types of PTMs, occurring often on target proteins in the cytosol or nucleus [236]. This alteration has the potential to modify the protein function significantly in a relatively short period through binding to interaction domains or binding an effector molecule to a site other than the active site of the enzyme [237]. Phosphorylation is essential for several cellular activities, including replication, transcription, response to stress, metabolic functions, immune responses, and apoptosis [224, 238, 239]. Moreover, the disruption of phosphorylation pathways has been linked to a wide range of disorders, including cancer, Alzheimer's disease, Parkinson's disease, and heart diseases [239- 241].

Acetylation

Acetylation is a type of PTM that is mediated by histone acetyltransferases and lysine acetyltransferases. In this type of modification, Acetyl CoA is used as a cofactor by acetyltransferases to add an acetyl group to the ε-amino group of lysine side chains, whereas acetyl groups can be erased from lysine side chains by deacetylases [242]. Acetylation can occur at varying frequency on Lys, Ala, Asp, Arg, Cys, Gly, Met, Glu, Pro, Val, Thr, and Ser residues, with acetylation being most common on lysine residues. (218) Acetylation is important for several biological activities, including chromatin integrity, cell cycle regulation, protein–protein associations, cellular metabolism, actin formation, and nuclear trafficking [243-245]. Based on previous studies, acetylated lysine is essential for cell growth, therefore its disruption could result in, for example, cancers, immunological disorders, cardiovascular diseases, and neurological diseases [243, 246-248].

Methylation

Methylation is a reversible PTM that frequently occurs in the cell nucleus and on nuclear proteins such as histones [213, 250]. In target proteins, methylation generally occurs on Arg, Lys, Asn, Ala, Asp, Gly, Cys, Glu, Gln, Leu, His, Phe, Pro, or Met residues [6]. Nevertheless, at least in eukaryotic cells, Lys and Arg are the major target residues for methylation [251, 252]. Among the most important biological functions of methylation is the modification of histone proteins. Following their synthesis, histone proteins may become methylated at Arg, Lys, His, Asn, or Ala residues [253]. Nε-lysine methylation is generally the most frequently occurring histone modification in eukaryotic chromatin, and it involves the transfer of methyl groups from S-adenosylmethionine to histone proteins by methyltransferase enzymes. Methylated Arg has also been reported in histone and nonhistone proteins in eukaryotes [254]. Lys can undergo either mono-, di- or trimethylation, whereas Arg can be either mono- or dimethylated [262]. According to the methylated residue and modification state, histone methylation can activate or repress transcription [263]. For instance, transcriptional activity is related with methylation of the Lys residues on H3K4, H3K36, and H3K79. On the other hand, gene suppression is associated with H3K9, H3K27, and H4K20 methylation [264]. The processes behind active and suppressive actions, as well as why they operate in such a tightly regulated fashion, remain unclear [201-203]. Recent research has demonstrated that methylation plays a role in a variety of cellular processes, from transcriptional regulation to epigenetic suppression mediated by heterochromatin formation [255]. A dysregulation in this modification can therefore result in a variety of illnesses, including diabetes, cancers, occlusive disease, and mental disorders [242, 256, 257].

Present investigations

Aims

The aim of the studies in the thesis was to investigate MC function, with a particular emphasis on the role of tryptase and histone modifications. The group has previously demonstrated that MC tryptase can influence the core histone processing, and a part of the present work builds on those earlier research findings. Specifically, the aims of each study was as follows:

Paper I

The purpose of this study was to determine the effect of histone modification inhibitors on MCs, with a particular focus on MC leukemia cells.

Paper II

To extend the focus on MCs by investigating whether tryptase can be regulated by DNA

Paper III

To investigate whether tryptase can influence the formation of neutrophil extracellular traps (NETs).

Paper IV

To determine the impact of MC tryptase on apoptosis, histone processing and epigenetic modification, as well as to investigate whether such activities are dependent on tryptase.

Results and Discussion

This section contains a summary of the most important findings from each paper.

Paper I

Histone Methyltransferase Inhibition Has a Cytotoxic Impact on Transformed Mast Cells: Implications for Mastocytosis.

Mastocytosis represents an abnormal growth of MCs. Systemic mastocytosis can progress to MC leukemia, which is an uncommon and severe condition with limited treatment options. Transformed MCs are sensitive to core histone-modifying enzyme suppression, which has implications for mastocytosis treatment. Previously, these effects were restricted to histone deacetylase (HDAC) inhibitors. Therefore, in Paper 1, the aim was to investigate whether other histone-modifying enzyme inhibitors could affect the growth and viability of transformed MCs. To test this possibility, we examined the influence of posttranslational modification (PTM) inhibitors, beyond HDAC inhibitors, on the growth and viability of transformed MCs (HMC-1 cells). Hence, HMC-1 cells were preincubated with or without the relevant inhibitors at varying concentrations and times, followed by an assessment of cytotoxicity and cell counts. The results show that HMC-1 cells are sensitive to both histone methyltransferase (HMT) inhibitors and HDAC inhibitors. We also examined these chemicals on LUVA MCs, another transformed MC population. This showed that histone methyltransferase inhibition also affects the growth and viability of LUVA MCs and that the LUVA MCs were HDAC-sensitive.

Next, we examined the mechanism by which histone methyl transferase inhibition causes cell death. Using flow cytometry, we found that blocking histone methyltransferase in HMC-1 cells increases caspase-3 activation. The activation of caspase-3 by HMT inhibition indicates apoptotic cell death. However, although caspase-dependent DNAase fragmentation is a typical event occurring in classical apoptosis, inhibition of histone methyltransferase did not result in DNA fragmentation in the HMC-1 cells. The cells were then labeled with Annexin V and DRAQ7 to improve the understanding of how HMT inhibition causes cell death. In HMC-1 cells treated with histone methyltransferase inhibitors, cells double positive for Annexin V and DRAQ7 predominated, indicating cell death with signs of necrosis (i.e., with membrane permeabilization). Moreover, a small number of Annexin V+/DRAQ7 cells were also observed, indicating minor classical apoptosis. Next, we used western blot analysis to assess the inhibitors' capacity to inhibit histone methylation, focusing on H3K4me1, H3K9me2, and H3K27me3 methylation. Both inhibitors decreased H3K9me2 levels in HMC-1 cells.

Previously, it was not known whether inhibition of histone-modifying enzymes other than HDAC could limit transformed MC growth and/or viability. Here, our findings show that transformed MCs are sensitive to histone methyltransferase inhibitors, suggesting that adequate histone methylation (e.g., at H3K9 or H3K27) is important for their survival. Our findings open the door to further exploration into histone modification inhibition as a potential therapeutic option for the disease.

Paper II

MC β-Tryptase Is Enzymatically Stabilized by DNA.

In Paper 2, we expanded our focus on MCs to examine whether tryptase activity might be influenced by DNA. Previously, the group has revealed that MC tryptase can be located in the MC nucleus in addition to its conventional location within secretory granules. Additionally, our group's findings also showed that tryptase maintains enzymatic activity in the nuclear environment. Importantly, tryptase's enzyme activity depends on anionic proteoglycans. Because proteoglycans are not typically found in the nucleus, we hypothesized that DNA, a polyanion, may potentially replace heparin in maintaining tryptase activity. This study's major aim was to determine whether DNA could stabilize tryptase's enzymatic activity. To address this, we used recombinant human ß-tryptase and double-stranded DNA isolated from bone marrow-derived MCs to study tryptase stabilization. Tryptase was tested at 37°C and at room temperature with or without DNA, heparin, or cDNA in neutral pH buffer. The incubations were carried out for set periods. Then, the residual tryptase enzyme activity was determined by measuring the absorbance at 405 nm immediately after adding a chromogenic substrate for tryptase (S-2288). Moreover, to determine the presence of tryptase in the cell nucleus of human MCs, we stained MCs from human eyelid skin with tryptase and a nuclear marker, followed by confocal microscopy examination.

The findings indicate for the first time that tryptase interacts with DNA and that the interaction stabilizes its enzymatic activity. Our findings further reveal that DNA-stabilized tryptase has a strong ability to degrade core histones, suggesting that tryptase is involved in the regulation of epigenetic processes mediated by core histone modification. Finally, the study shows that tryptase is present in the nucleus of human MCs.

Paper III

MC Tryptase Potentiates Neutrophil Extracellular Trap Formation.

In Paper 3, we further studied the interaction of tryptase with DNA. Activated neutrophils are recognized for their release of extracellular DNA-containing traps (NETs) and based on the recognized potential of tryptase to interact with

negatively charged polymers, we hypothesized that tryptase may interact with DNA contained within NETs and thus regulate NET formation. To study this, neutrophils were purified from human peripheral blood, and NET formation was induced by treating the neutrophils with phorbol myristate acetate (PMA). To assess whether tryptase can affect NET formation, we conducted experiments in which NETs were induced in the absence or presence of recombinant human β-tryptase. Tryptase alone did not induce NET formation, but when it was added together with PMA, NET formation was more pronounced in comparison with PMA treatment only. Moreover, neutrophils treated with PMA + tryptase displayed a completely disintegrated nuclear architecture in comparison with PMA treatment only. These results suggest that tryptase has a strong potentiating effect on NET formation induced by PMA treatment of human neutrophils.

After that, we investigated the mechanism underlying the effect of tryptase on NET formation. To this end, we assessed whether tryptase could interact with NETs by labeling tryptase with Alexa-488 and then introducing it to PMA-activated human neutrophils, followed by confocal microscopy analysis. Indeed, the labeled tryptase largely overlapped with NETs in the neutrophil population, implying that tryptase binds to NETs efficiently. We furthermore examined whether tryptase may cause proteolysis of core histones in PMA-treated neutrophils. The tryptase + PMA treatment resulted in a significant core histone truncation with distinct proteolytic fragments of H2B, H3, and H4. Moreover, the levels of citrullinated H3 (H3cit), which is a hallmark event during NET formation, were significantly decreased following tryptase treatment, indicating that tryptase can cleave these modifications from the H3 N-terminal tail. Next, we tested whether DNA influences tryptase function. First, we investigated whether DNA could preserve the tetramerization of tryptase and, indeed, these experiments revealed that the tryptase tetramer was maintained in the presence of DNA (and as expected, by heparin as well). We then tested whether DNA-stabilized tryptase can degrade cytokines and other pro-inflammatory substances to examine if tryptase interaction with DNA can affect its proteolytic action on macromolecular targets. Only eight proteins (IL-20, Gal7-His, TSLP, IFNg, TRAIL, KGF-2, OSM, and IL-2) out of more than 70 showed proteolytic susceptibility to tryptase, with different effects on tryptase catalytic activity between DNA and heparin.

Finally, we assessed the role of tryptase in NET formation in vivo using tumors from tryptase-deficient mice. It is known that melanomas are linked to significant inflammatory infiltrates, including MCs and neutrophils, and MCs in melanomas can secrete tryptase into the tumor environment. In this way, tryptase from tumor-associated MCs might interact with NETs formed in the tumor's inflammatory environment. Gr1 (Ly-6G/6C) staining revealed a high number of neutrophils in the tumor stroma, and extracellular DNA structures

appearing as NET-like filaments were commonly seen in association with the tumors. However, tryptase-deficient mice displayed markedly fewer NETs than did wild-type mice. In fact, no NET formation was found in tumor sections examined from tryptase-deficient mice. These results imply that tryptase in vivo can interact with NETs and has a profound effect on NET formation in melanoma.

In conclusion, the findings of this paper show for the first time that MC tryptase binds to NET-contained DNA and that tryptase has a considerable potentiating effect on the production of NETs in response to neutrophil-activating triggers. Moreover, the findings show that DNA-stabilized tryptase has a strong ability to modify different cytokines proteolytically, indicating that NET-bound tryptase is involved in the regulation of inflammatory processes.

Paper IV

Tryptase Regulates the Epigenetic Modification of Core Histones in MC Leukemia Cells.

In Paper 4, we studied the effect of MC (HMC-1) apoptosis on histone processing and modification and, in addition, whether such processes are dependent on tryptase. To investigate this issue, we first assessed how various cytotoxic agents affect HMC-1 cells. HMC-1 cells were treated with H-Leu-Leu-OMe (LLME), staurosporine, or histone methyltransferase inhibitors (HMTi). All these compounds were shown to be cytotoxic to HMC-1 cells, and all of them caused mixed apoptotic and necrotic cell death. Notably, LLME induced predominantly apoptotic cell death, in line with prior observations in primary mouse MCs. We then utilized western blot analysis to assess the influence of cell death on core histone processing in HMC-1 cells. A general decrease in the respective core histone (H3, H2B, H4, and H2A) levels during cell death was observed in response to HMTi. Intriguingly, H3 cleavage was observed in response to HMTi, indicating that histone processing may accompany cell death. Staurosporine caused limited H3 cleavage, whereas LLME caused profound H3 cleavage. Overall, these findings show that multiple cytotoxic agents cause HMC-1 cell death accompanied by histone cleavage. LLME showed the most substantial effect on core histone cleavage in HMC-1 cells.

On the basis that apoptosis is associated with significant histone modifications, we evaluated whether cell death in HMC-1 cells affects the levels of epigenetic histone marks. The effects of these compounds on H3K4me1, H3K9me2, and H3K27me3 were studied. Both HMT inhibitors induced substantial reductions of the levels of H3K4me1, H3K27me3, and H3K9me2. LLME also caused reduced H3K4me1 and H3K9me2 levels. Staurosporin caused a limited reduction in H3K4me1 and H3K9me2 levels, whereas neither staurosporin nor LLME caused a decrease of H3K27me3. As shown above,

cell death in HMC-1 cells is followed by substantial core histone processing and effects on the levels of epigenetic histone mark. Tryptase has been found to influence these mechanisms in primary murine MCs. The effects on core histone processing/epigenetic modification in HMC-1 cells may thus be tryptase-dependent. To investigate this, we tested whether inhibiting tryptase by Pefabloc or nafamostat can affect the cell death process in HMC-1 cells. Since LLME was the cytotoxic agent that caused the greatest core histone cleavage and epigenetic change, we focused on LLME in the following experiments. In HMC-1 cells, LLME caused apoptotic cell death, but in the presence of either Pefabloc or nafamostat, the proportion of apoptotic cells decreased markedly, and the number of late apoptotic/necrotic cells increased. Nafamostat and, to some extent, Pefabloc were not cytotoxic to HMC-1 cells. Thus, tryptase affects the mechanism of cell death in HMC-1 cells in response to LLME.

Further, we tested whether LLME-induced H3 cleavage involved tryptase. LLME treatment was associated with significant cleavage of H3, but the H3 processing was eliminated when cells were treated with Pefabloc or nafamostat. This was not the case when assessing broad-spectrum inhibitors of cysteine proteases (E-64d), aspartic acid proteases (Pepstatin A), or metalloproteases (EDTA). Thus, tryptase has a major role in H3 cleavage during cell death in HMC-1 cells. The observed effect of tryptase on nuclear histones indicates that it is physically associated with these proteins, either at baseline or during apoptosis. To address this, we stained HMC-1 cells for tryptase before and after LLME treatment. HMC-1 cells stained strongly for tryptase in the cytoplasm, consistent with the abundance of tryptase in secretory granules. Tryptase positivity was also found after LLME-induced cell death. However, after inducing cell death, tryptase staining decreased, most likely due to tryptase release or degradation. The nuclear compartment also showed tryptase staining. This was seen both before and after LLME-induced cell death. Further, the LLME treatment caused substantial disintegration of the HMC-1 nuclear compartment, but the levels and distribution of nuclear tryptase were similar at baseline and cell death conditions.

In conclusion, our findings show that induction of MC apoptosis by a granule-mediated pathway results in extensive processing of core histones, and that such processing is dependent on tryptase. Furthermore, tryptase was found to regulate the extent of epigenetic modification of core histones throughout the cell death process. Tryptase was also found in the nucleus of both dying and viable MC leukemia cells, and we show that its blockade results in enhanced MC leukemia cell proliferation. These findings are highly novel and show that tryptase has a role in the regulation of MC death.

Concluding remarks and continuation of studies

MCs are implicated to have detrimental functions in various pathologies, such as asthma and other allergic conditions. MC leukemia is the most aggressive form of the systemic mastocytosis, for which therapeutic options are limited to dampening the symptoms, and there is still no curative therapy. Thus, there is an urgent need for novel insight that may pave the way for finding a curative therapy for this disease. In this context, it is noteworthy that our studies have established that tryptase can be detected in the cell nucleus in an active form in MC leukemia cells. Possibly, this finding may have the potential to be exploited for therapeutic strategies, but extensive further work will be needed to elaborate this possibility.

The studies presented in this thesis demonstrate previously unknown effects of histone modifying enzymes on MC leukemia cells and introduce a novel mechanism of maintaining tryptase enzymatic activity. In addition, the studies reveal a new perspective of tryptase function during cell death and in the regulation of inflammatory processes. The first study provides a valuable input regarding histone-modifying enzymes' effects on transformed MCs. Except for histone deacetylase (HDAC), almost nothing was known about the inhibitory effects of other histone-modifying enzymes on the viability and growth of transformed MCs prior to this work. Here, we demonstrate for the first time that the inhibition of histone methyltransferases (HMT) is cytotoxic to MC leukemia cells. This implies that HMT inhibitors may have a therapeutic potential in the treatment of MC transformation-related diseases and opens the way for potential utilization of these inhibitors in the treatment of mastocytosis. Additionally, we can consider the application of methyltransferase inhibition in malignant and other diseases where MCs may be harmful.

In studies 2, 3, and 4, we further investigated MC's function by focusing on tryptase. We examined the effects of tryptase on the epigenetic modification of core histones in human MC leukemia cells. Our findings show that DNA preserves the enzymatic activity of human β-tryptase with a similar efficiency

as heparin, and that DNA-stabilized-tryptase is capable of degrading core histones. Further, we show that tryptase is located within the nucleus of primary human skin MCs. In fact, these findings reveal a novel mechanism of stabilizing enzymatic activity of MC tryptase and provides a reasonable explanation for how tryptase enzymatic activity is maintained in the nucleus of various types of cells. Together, these findings have an important impact on our understanding of how tryptase regulates nuclear events.

Moreover, the studies show that MC tryptase can regulate NET formation, introducing a novel mechanism of interaction between neutrophils and MCs. Our findings reveal that, using a granule-mediated strategy to trigger MC death, substantial modification of core histones is seen. Moreover, tryptase was shown to be responsible for this, indicating that tryptase modulates the amplitude of epigenetic core histone changes during the process of cell death. Indeed, the significant capacity of DNA-stabilized tryptase to conduct cytokine proteolysis indicates that its action on NETs could potentially result in a wide range of consequences. Consequently, it is plausible that NET-associated tryptase is able of modifying the cytokine profiles under pathological situations where both neutrophils and MCs are implicated. However, the latter scenario will need to be addressed experimentally.

Based on our findings, we speculate that tryptase may have the capacity to stimulate the secretion of neutrophil granule components into the external environment, which may exert an influence on the inflammatory or immune - mediated processes. For example, since tryptase has the capacity to promote NET formation, we may speculate that tryptase thereby has the ability to enhance bacterial clearance. However, this hypothesis will need to be tested systematically.

In the studies of this thesis, our findings reveal hitherto unknown effects of histone modifying enzymes on MC leukemia cells and introduce a unique approach for maintaining tryptase enzymatic activity, both of which were previously unidentified. Furthermore, we show a potentially important role of tryptase in both cell death and inflammatory responses. Although we identified a previously unknown mechanism of how tryptase enzymatic activity is maintained in the nucleus, it is still unknown how tryptase enters the nucleus? This would be a main area of investigations in the continuation of this project. Additionally, we show here that MC tryptase can regulate NET formation, and this led us to ask if tryptase is also capable of influencing the formation of MC-derived extracellular traps. Clearly, this would also be an important focus of upcoming investigations.

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