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Induction of Mast Cell Apoptosis via Granule Permeabilization

A Novel Approach to Target Mast Cells

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Abstract

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Mast cells are densely granulated tissue-resident immune cells that play an important role in orchestrating inflammatory responses. Dysregulated increases in the numbers and activation status of mast cells can have deleterious consequences for the body in various inflammatory diseases. Mast cells are best-known for their detrimental roles in allergic diseases, e.g., asthma. Thus, strategies that target mast cells and their harmful activities in such pathological conditions are potentially attractive therapeutic options. An efficient strategy to accomplish a full blockade of the harmful events mediated by various mast cell mediators is to locally eliminate mast cell populations altogether by inducing their apoptosis.

Using *in vitro*-cultured mast cells, we identified that mefloquine, an antimalarial drug with lysosomotropic activity, causes permeabilization of secretory granules, increased production of reactive oxygen species (ROS), release of granule-localized proteases into the cytosol and apoptotic cell death (**Paper I**). Moreover, intraperitoneal injections of mefloquine in mice resulted in a reduced peritoneal mast cell population *in vivo*.

To evaluate the possibility of using lysosomotropic agents for selectively depleting human lung mast cells by induction of apoptosis, human lung specimens were used. Exposure of either intact human lung tissue, purified lung mast cells or mixed populations of lung cells to mefloquine revealed that human lung mast cells are highly susceptible to ROS-induced apoptosis in this setting. In contrast, other cell populations of the lung were largely refractory (**Paper II**).

Lastly, in an attempt to gain a deeper insight into the mechanism underlying ROS production and the downstream events in response to lysosomotropic challenge, we identified that the mast cell secretory granules comprise major subcellular compartments for ROS production in response to mefloquine (**Paper III**). Lysosomal iron, granzyme B and the ERK1/2 MAP kinase signaling pathway were found to contribute to production of ROS in response to mefloquine. Furthermore, granule acidification was shown to be essential for mefloquine-mediated effects in mast cells, i.e., granule permeabilization, ROS production and cell death. Collectively, the present thesis introduces the possibility of inducing selective mast cell apoptosis via granule permeabilization as a novel strategy to target mast cells. Thus, this strategy has a potential to be used therapeutically to ameliorate mast cell-mediated detrimental effects in inflammatory diseases, such as asthma.

Keywords: Mast cells, Granules, Apoptosis, Reactive oxygen species, ROS, Lysosomotropic agents, Mefloquine

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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 **Paivandy A**, Calounova G, Zarnegar B, Öhrvik H, Melo FR and Pejler G. (2014) Mefloquine, an anti-malaria agent, causes reactive oxygen species-dependent cell death in mast cells via a secretory granule-mediated pathway. *Pharmacology Research & Perspectives*, 2(6):e00066. doi: 10.1002/prp2.66.

II **Paivandy A**, Sandelin M, Igelström H, Landelius P, Janson C, Melo FR and Pejler G. (2017) Induction of human lung mast cell apoptosis by granule permeabilization: a novel approach for targeting mast cells. *Frontiers in Immunology*, 8:1645. doi: 10.3389/fimmu.2017.01645.

III **Paivandy A**[#], Eriksson J, Melo FR, Sellin ME and Pejler G[#]. (2019) Lysosomotropic challenge of mast cells causes intra-granular reactive oxygen species production. *Cell Death Discovery*, 5:95. doi: 10.1038/s41420-019-0177-3.

[#] Corresponding authors

List of additional papers not included in this thesis

I **Paivandy A**[*], Grujic M[*], Rafati N and Pejler G. (2020) DNA demethylation regulates gene expression in IgE-activated mouse mast cells. *Allergy (In revision).*

II Akula S, **Paivandy A**, Fu Z, Thorpe M, Pejler G and Hellman L. (2020) Quantitative in-depth analysis of the mouse mast cell transcriptome reveals organ-specific mast cell heterogeneity. *Cells,* 9(1), 211. doi: 10.3390/cells9010211

III Grujic M, **Paivandy A**, Gustafson AM, Thomsen AR, Öhrvik H and Pejler G. (2017) The combined action of mast cell chymase, tryptase and carboxypeptidase A3 protects against melanoma colonization of the lung. *Oncotarget*, 8(15):25066-79. doi: 10.18632/oncotarget.15339.

IV Pejler G, Hu Frisk JM, Sjöström D, **Paivandy A** and Öhrvik H. (2017) Acidic pH is essential for maintaining mast cell secretory granule homeostasis. *Cell Death & Disease*, 8(5):e2785. doi: 10.1038/cddis.2017.206.

V Melo FR, Wallerman O[*], **Paivandy A**[*], Calounova G, Gustafson AM, Sabari BR, Zabucchi G, Allis CD and Pejler G. (2017) Tryptase-catalyzed core histone truncation: A novel epigenetic regulatory mechanism in mast cells. *Journal of Allergy and Clinical Immunology*, 140(2):474-485. doi: 10.1016/j.jaci.2016.11.044.

VI Hagforsen E, Lampinen M, **Paivandy A**, Weström S, Velin H, Öberg S, Pejler G and Rollman O. (2017) Siramesine causes preferential apoptosis of mast cells in skin biopsies from psoriatic lesions. *British Journal of Dermatology*, 177(1):179-187. doi: 10.1111/bjd.15336.

VII Hagforsen E, **Paivandy A**, Lampinen M, Weström S, Calounova G, Melo FR, Rollman O and Pejler G. (2015) Ablation of human skin mast cells in situ by lysosomotropic agents. *Experimental Dermatology*, 24(7):516-21. doi: 10.1111/exd.12699.

* Equal contribution

Contents

Abbreviations

AHR	Airway hyperresponsiveness
AIF	Apoptosis-inducing factor
BAF	Bafilomycin
BAL	Bronchoalveolar lavage
BMMC	Bone marrow-derived mast cell
BTK	Bruton's tyrosine kinase
CBMC	Cord blood-derived mast cell
CD	Cluster of differentiation
CLR	C-type lectin receptor
CML	Chronic myeloid leukemia
COPD	Chronic obstructive pulmonary disease
CPA3	Carboxypeptidase A3
CTMC	Connective tissue mast cell
DAMP	Damage-associated molecular pattern
DFO	Deferoxamine mesylate
DISC	Death-inducing signaling complex
ELISA	Enzyme-linked immunosorbent assay
FcR	Fragment crystallizable receptor
FDA	Food and drug administration
GAG	Glycosaminoglycan
GPR35	G-protein-coupled receptor 35
HMC-1	Human mast cell line-1
Hsp70	Heat shock protein 70
Ig	Immunoglobulin
IL	Interleukin
LAMP	Lysosomal-associated membrane protein
LDCD	Lysosome-dependent cell death
LIMP	Lysosomal integral membrane protein
Lin	Lineage
LLME	L-leucyl-L-leucine methyl ester
LMP	Lysosomal membrane permeabilization
LPS	Lipopolysaccharide
LT	Leukotriene
MC	Mast cell
MCp	Mast cell progenitors
MMC	Mucosal mast cell
mMCP	Mouse mast cell protease

MOMP	Mitochondrial outer membrane permeabilization
MRGPR	MAS-related G protein-coupled receptor
MSDH	O-methyl-serine dodecylamide hydrochloride
NAC	N-acetylcysteine
PAMP	Pathogen-associated molecular pattern
PBS	Phosphate-buffered saline
PCMC	Peritoneal cell-derived mast cell
PDGFR	Platelet-derived growth factor receptor
PG	Prostaglandin
PGN	Peptidoglycan
PI3K	Phosphatidylinositol 3-kinases
PRR	Pattern recognition receptor
RA	Rheumatoid arthritis
RLR	RIG-like receptor
ROS	Reactive oxygen species
SCF	Stem cell factor
SCG	Sodium cromoglycate
SG	Serglycin
Siglec	Sialic acid-binding immunoglobulin-like lectin
ssRNA	Single-stranded RNA
SYK	Spleen tyrosine kinase
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand
TSLP	Thymic stromal lymphopoietin
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
V-ATPase	Vacuolar ATPase
VEGF	Vascular endothelial growth factor
WT	Wild type
XIAP	X-linked inhibitor of apoptosis protein

Introduction

Our immune system is a complex network of tissues, cells and molecules that protects us against foreign invaders such as microbes, non-infectious environmental substances and toxins. When an invader successfully breaches the physical barriers of the host, the immune system recognizes this intruding agent and the associated tissue damage and subsequently launches a set of coordinated reactions, called immune responses, to eliminate it. As a crucial part of this protective response, inflammation is triggered which allows recruitment of immune cells to the affected tissue to not only eliminate the cause of damage, but also to initiate the healing and repair process. Under normal conditions, after eradication of the invader by the host immune system, the inflammation is resolved in order to return the tissue to homeostasis. However, unresolved or excessive inflammatory responses can lead to persistent tissue damage, thereby giving rise to development of chronic inflammatory diseases.

Mast cells are among the effector immune cells that participate in the host inflammatory responses. Mast cells are present in the tissues that are portals of entry for external stimuli, and are equipped with a broad range of sensors that enable the recognition of various stimuli. Upon recognition, mast cells respond to these stimuli by releasing a panel of inflammatory compounds. These particular properties enable mast cells to play a key role in orchestrating inflammation. Nevertheless, dysregulated mast cell activation contributes to pathogenesis of chronic inflammatory diseases including asthma, atopic dermatitis, cutaneous mastocytosis and psoriasis. Thus, strategies that target mast cells are potentially attractive for treatment of such inflammatory diseases.

Here, several available mast cell-directed therapies and their limitations are briefly discussed. More importantly, the present thesis introduces a novel and efficient approach to target mast cells selectively via permeabilization of their granules.

Background

Mast cells

Mast cells are long-lived tissue-resident cells that originate from hematopoietic pluripotent progenitors in the bone marrow[1-3]. As opposed to most other hematopoietic cells, mast cells leave the bone marrow to enter the blood circulation as immature progenitors and complete their development only after they arrive at peripheral tissues[4, 5]. As mast cell progenitors (MCp) differentiate into mature mast cells in the tissue under the influence of local cytokines and growth factors, e.g., stem cell factor (SCF), they acquire more and more filled secretory granules. In fact, the most unique morphological feature of mature mast cells is their high content of the secretory granules which occupy most of the cytoplasmic space[6]. The secretory granules are filled with large quantities of various preformed mediators, including biogenic amines, mast cell-specific proteases, lysosomal enzymes, certain cytokines, chemokines, growth factors and serglycin proteoglycans[3, 6]. As will be discussed later, the preformed mediators are released into the extracellular environment when mast cells are activated to degranulate. In addition to the release of preformed granule constituents, mast cell activation can lead to the *de novo* synthesis and release of a diverse array of bioactive mediators[3, 6, 7] (*Figure 1; Table 1*).

Based on their anatomical location, biochemical properties or protease expression profile, two major subtypes of mature mast cells have been described in mice and humans[3, 6]. In mice, these two subtypes are connective tissue mast cells (CTMCs) and mucosal mast cells (MMCs). CTMCs mainly have glycosaminoglycans (GAGs) of the heparin type and are located in the skin, peritoneal cavity and submucosa of stomach and gut. On the contrary, MMCs have chondroitin sulphate as the main species of GAG attached to the serglycin core protein[6] and are predominantly found in the gut and respiratory mucosa[4, 5]. Given that human mast cells contain both heparin and chondroitin sulfate proteoglycans[8], they were subdivided based on their profile of protease expression into MC_{TC} and MC_T[6, 9]. In terms of protease content and tissue localization, human MC_{TC} correspond to mouse CTMCs, expressing both tryptase and chymase and being located in the skin as well as gastrointestinal and bronchial submucosa[5]. On the other hand, human MC_T, which express tryptase but lack chymase, are found mainly in the gastrointestinal and bronchial mucosa as well as bronchial epithelium, roughly corresponding to MMCs[5, 9, 10].

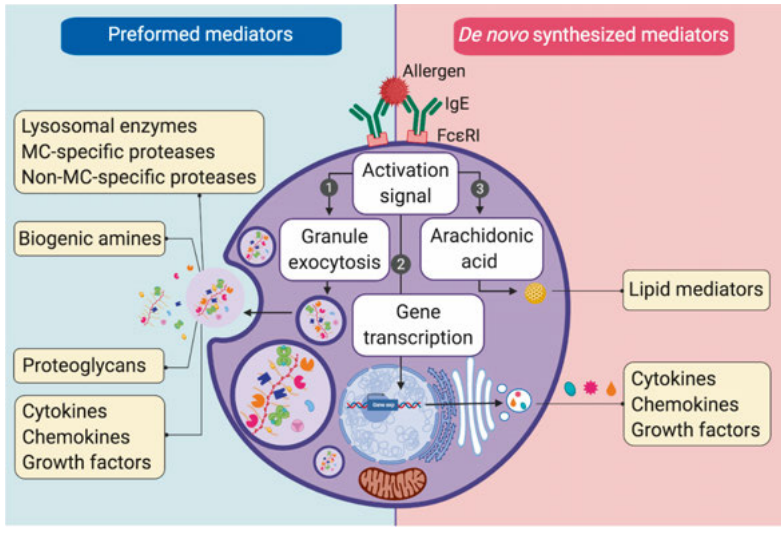

Figure 1. **Mast cell activation and mediator release.** Mast cells become activated when IgE molecules bound to surface FcεRI are cross-linked by antigens (e.g., allergen). Such activating signals lead to the release of preformed mediators through degranulation as well as *de novo* production and release of several other mediators.

Table 1. Examples of mast cell-derived mediators (human or mouse).

Mediator Class	Mediators (e.g.)	Ref
Preformed (immediate release)		
Lysosomal enzymes	Cathepsins (B, C, D, E, L), β-hexosaminidase	3, 6
Non-MC-specific proteases	Cathepsin G, Granzyme B*, Active caspase 3	3, 6
MC-specific proteases	Tryptases*, Chymases*, CPA3*	3, 6
Proteoglycans	Serglycin (heparin, chondroitin sulphate)	3, 6
Biogenic amines	Histamine*, Serotonin*	3, 6
Cytokines and chemokines	TNF, IL-4, CCL5, CXCL8	3, 6
Growth factors	SCF, VEGF, FGF, NGF, TGF-β	3, 6
De novo *synthesized (delayed release)*		
Lipid mediators	PGD2, PGE2, LTB4, LTC4, PAF	3
Cytokines	TNF, IFNγ, IL-1, -2, -3, -4, -5, -6, -9, -10, -13, -33	3, 7
Chemokines	CCL1, 2, 3, 4, 5, 7, 11, 17, 20, 22; CXCL2, 8, 10	7
Growth factors	SCF, VEGF, FGF, NGF, TGF-β, PDGF, GM-CSF	3, 7

Abbreviations: CPA3, carboxypeptidase A3; FGF, fibroblast growth factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; LT, leukotriene; MC, mast cell; NGF, nerve growth factor; PAF, platelet activating factor; PDGF, platelet-derived growth factor; PG, prostaglandin; SCF, stem cell factor; TGF-β, transforming growth factor-β; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor. *Examples of mediators that are dependent on serglycin for their storage[6, 11].

Mast cell receptors

Mast cells express a broad set of receptors, on the cell surface and intracellularly, that provide them with a remarkable ability to sense and respond to various exogenous and endogenous stimuli[3, 12]. These receptors are either activating or inhibitory, and operate to regulate the biological responses of mast cells. Examples of major activating receptors present in mast cells are Fc receptors, pattern recognition receptors (PRRs), cytokine, chemokine and growth factor receptors and peptide receptors such as MAS-related G protein-coupled receptor-X2 (MRGPRX2)[12-15]. Examples of major inhibitory mast cell receptors are FcγRIIB, sialic acid binding Ig-like lectins (e.g., Siglec-8), receptors for anti-inflammatory or immunomodulatory cytokines (e.g., IL-10R and TGF-βR) and G-protein-coupled receptor 35 (GPR35)[12, 16-21] (*Table 2*).

Mast cell activation

Owing to the expression of various activating receptors, mast cells can become activated through diverse pathways that are generally classified into IgE-dependent and IgE-independent activation pathways. The direct interaction between specific stimuli and their respective activating receptors in mast cells generates activating signals that give rise to induction of a mast cell response. Generally, a mast cell response consists of two distinct phases: (1) immediate degranulation, resulting in the release of preformed granule-stored mediators within seconds, and (2) delayed secretion of *de novo* synthesized mediators that are released within minutes (e.g., lipid mediators) or hours (e.g., cytokines, chemokines and growth factors)[22, 23] (*Figure 1; Table 1*). Of note, mast cells do not respond uniformly to all activating stimuli. More precisely, depending on the phenotype of mast cells as well as the type, property, strength, and combination of the incoming stimuli, the nature and intensity of the mast cell response might vary in range[3, 24]. For example, stimulation through some receptors, such as high affinity IgE receptor (FcεRI), launches a full-scale mast cell response, i.e., release of various preformed mediators through degranulation as well as release of *de novo* synthesized lipid mediators (e.g., leukotrienes [LTs] and prostaglandins [PGs]), numerous cytokines, chemokines and growth factors via degranulation-independent events. On the contrary, stimulation of mast cells through certain PRRs (e.g., TLR4) or cytokine receptors (e.g., IL-33 receptor and thymic stromal lymphopoietin [TSLP] receptor) induces the selective release of *de novo* synthesized cytokines and chemokines in the absence of degranulation[25-28]. There are also instances where stimulation of mast cells through certain receptors, mainly those that bind to growth factors (e.g., c-kit), primarily induce mast cell survival, proliferation or differentiation rather than induction of mediator release[3].

Table 2. Examples of activating and inhibitory receptors expressed by mast cells (human or mouse).

Ligands	Activating receptors (e.g.)	Ref
Microbial products (PAMPs)		
Bacterial lipopeptides, PGN, dsRNA, LPS,	Toll-like receptors (TLR1–9)	12, 13, 29
Flagellin, LTA, ssRNA, CpG-DNA	C-type lectin receptors (Dectin-1)	29, 30
	RIG-like receptors (RIG-I)	30
FimH, *S. aureus*	CD48	13, 31
Endogenous products (DAMPs or alarmins)		
IL-33, TSLP	IL-33R (ST2), TSLPR	31
Products of the innate immune system		
Cytokines and growth factors (GFs)	Cytokine/GF receptors (IL-1R, -3R, -4R, -12R, -15R, -18R, IFNγR, c-Kit)	12, 31
Chemokines	Chemokine receptors (CCR1, 3, 4, 5, 7; CXCR1, 2, 3, 4, 6; CX3CR1)	12-14
Complement components	Complement receptors (CR1, 2, 3, 4, 5; C3aR, C5aR)	12, 13
Products of the adaptive immune system		
IgE	FcεRI	12, 13
IgG	FcγRs (FcγRI, FcγRIII)	12, 13
Endogenous and exogenous peptides/compounds		
Endogenous: Neuropeptide (Substance P, VIP), Antimicrobial peptides (β-Defensin)	$MRGPRX2^{(human)}$/$MRGPRB2^{(mouse)}$	15
Exogenous: Insect toxins (Mastoparan), Compound 48/80, Icatibant		
Endogenous: Bioactive peptides (Endothelin-1)	ET_A	13, 15
Exogenous: Animal toxins (Sarafotoxin-B)		

Ligands	Inhibitory receptors (e.g.)	Ref
Products of the adaptive immune system		
IgG	FcγRIIb	13, 17, 32
Anti-inflammatory/immunomodulatory cytokines		
IL-10, TGF-β	IL-10R, TGF-βR	12
Other ligands		
Sialic acid	Siglec-8	17, 32
MC stabilizing drugs (SCG, nedocromil sodium)	GPR35	21

Abbreviations: DAMP, damage-associated molecular pattern; dsRNA, double-stranded RNA; GPR35, G-protein-coupled receptor 35; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MC, mast cell; MRGPR, MAS-related G protein-coupled receptor; PAMP, pathogen-associated molecular pattern; PGN, peptidoglycan; SCF, stem cell factor; SCG, sodium cromoglycate; Siglec-8, sialic acid binding Ig-like lectins; ssRNA, single-stranded RNA; TGF-β, transforming growth factor-β; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin; VIP, vasoactive intestinal polypeptide.

IgE-dependent activation

The best studied mechanism of mast cell activation is the IgE-dependent mechanism. The IgE receptor, FcεRI, is abundantly present on the mast cell plasma membrane. Binding of an antigen to FcεRI-bound IgE antibodies results in cross-linking of FcεRI, which in turn initiates a complex intracellular signaling cascade. The key components of this signaling cascade include phosphorylation of various signaling proteins, lipid metabolism and phosphorylation, intracellular Ca^{2+} mobilization and transcription factor activation that ultimately trigger a full mast cell response (*Figure 1*)[3, 33]. The IgE/FcεRI-mediated mast cell activation is typically seen in immediate hypersensitivity (allergic) reactions, with the most serious manifestation being the potentially life-threatening anaphylaxis[34, 35]. This type of mast cell activation is also considered to contribute to host defense against certain helminth parasites[36].

IgE-independent activation

Mast cells can become activated independently of IgE/FcεRI when stimulated via innate mechanisms. The major stimuli that can trigger IgE-independent mast cell activation include (1) microbial products known as pathogen-associated molecular patterns (PAMPs), (2) endogenous products released upon tissue damage known as damage-associated molecular patterns (DAMPs) or alarmins, (3) products of the innate immune system such as cytokines, chemokines and complement components, (4) products of the adaptive immune system (e.g., IgG) and (5) various endogenous and exogenous peptides (*Table 2*). In the following section, some of these IgE-independent mast cell activation pathways are briefly described.

Mast cells express various PRRs such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs) and RIG-like receptors (RLRs)[29, 30] (*Table 2*), which enable them to recognize different microbes and to participate in the host response during infection[23]. Different mast cell phenotypes display distinct expression patterns of the PRRs. Moreover, in some cases, stimulation of mast cells with various PAMPs can induce the expression of certain PRRs that are otherwise undetectable or detected at low levels[30]. The mast cell PRRs are located in the plasma membrane, endosomes or cytosol and can bind to distinct conserved molecular structures present in microbes. Such ligations trigger signaling cascades leading to production and release of inflammatory cytokines, chemokines, antiviral interferons (when the stimuli are viral PAMPs) and in some instances lipid mediators[29]. In general, stimulation of mast cells through interaction between PAMPs and PRRs do not appear to efficiently activate the degranulation machinery[29]. However, degranulation is detectable in a few cases; for example, when mast cells are stimulated with ligands of TLR2 or Dectin-1[25, 37, 38], the latter being a member of CLRs. Importantly,

stimulation of mast cells through interaction between PAMPs and PRRs can potentiate various aspects of IgE-mediated mast cell activation. For example, prolonged stimulation of mast cells with a TLR4 ligand, i.e., lipopolysaccharide (LPS), has been found to enhance degranulation and secretion of leukotrienes, cytokines and chemokines in mouse mast cells following IgE/FcεRI cross-linking. On the other hand, TLR3 ligation only enhanced degranulation and secretion of leukotrienes under the same experimental conditions[39].

Another receptor that mediates IgE-independent mast cell activation is MAS-related G protein-coupled receptor-X2 (MRGPRX2) and its mouse ortholog MRGPRB2. Although it was initially thought that these receptors are only expressed in sensory neurons[40], later investigations revealed that MRGPRX2 and MRGPRB2 are also expressed in human skin mast cells expressing both tryptase and chymase (MC_{TC}) and mouse CTMCs, respectively[41-43]. Mast cells can become activated through MRGPRX2 when binding to various endogenous and exogenous peptide stimuli including neuropeptides, antimicrobial peptides and components of insect venom[15] (*Table 2*). Additionally, compound 48/80 was found to mediate its effect on mast cells through MRGPRX2[44]. Activation of mast cells through MRGPRX2 can induce degranulation in a process that is dependent on Ca^{2+} mobilization[42, 45-47]. Interestingly, the IgE-mediated degranulation has been found to cause a sustained release of larger and more heterogeneously shaped granule structures[45]. On the other hand, in the same experimental settings, MRGPRX2-mediated activation by ligands such as substance P, compound 48/80 and icatibant (a peptide drug), has been shown to induce mast cells to rapidly secrete small and relatively spherical granule structures, a pattern consistent with the secretion of individual granules[45]. Furthermore, unlike IgE-mediated activation that induces strong *de novo* secretion of lipid mediators (e.g., PGD2 and PGE2) and several inflammatory cytokines, chemokines and growth factors, MRGPRX2-mediated activation by substance P results in secretion of only low amounts of the aforementioned lipid mediators and vascular endothelial growth factor (VEGF) by mast cells[45]. These differences in the pattern of degranulation and in the *de novo* expression of mast cell mediators upon stimulation through FcεRI and MRGPRX2 may contribute to the observed differences in mast cell-dependent inflammation[15]. In line with this, pseudoallergic skin reactions induced by peptide drugs, which are mediated mainly through MRGPRX2/MRGPRB2[43], are often transient (e.g., icatibant[48]), while IgE-dependent allergic skin reactions are usually sustained and lead to more persistent local inflammation[49].

The activation of mast cells via IgE-independent pathways is believed to play an important role in the pathophysiology of various non-allergic inflammatory diseases, in which mast cells are implicated[50, 51]. Moreover, the fact that multiple non-IgE stimuli (e.g., PAMPs, IL-33, etc) have been found to have

synergistic effects with IgE-mediated mast cell activation[39, 52], suggests that IgE-independent pathways can also contribute to the mast cell-driven pathological effects in allergic inflammatory diseases by aggravating them. In line with this notion, respiratory infections are frequently found to be the major cause of exacerbations in asthmatic patients[53-55].

Mast cells in health and disease

Mast cells are present in virtually all vascularized tissues. In particular, they are abundantly located at junction points of the body and external environment, which are portals of entry for pathogens and harmful substances (e.g., skin, gastrointestinal tract and airways), as well as near nerve endings and blood vessels[3]. Within the tissues, local microenvironmental stimuli control maturation, phenotype and function of mast cells. Therefore, these stimuli regulate the ability of mast cells to specifically recognize and respond to numerous different stimuli via secretion of a variety of potent mediators[3]. All these features enable mast cells to serve as immune sentinel cells and as the first responders following encounter with a tissue insult. In addition to their ability to respond rapidly and directly to such insults (e.g., pathogens, allergens, toxins, etc), mast cells can communicate with other immune cells by sending signals throughout the body to promote the recruitment of different immune cells to the affected tissues[3]. Therefore, mast cells play an important role in initiating inflammation, modulating both innate and adaptive immune responses, as well as launching a program of repair and homeostatic maintenance[3, 23]. However, if the tissue insult is repeated or persistent, mast cells can become potentially harmful. This is because the sustained mast cell activation and release of various proinflammatory mediators such as proteases, cytokines, chemokines and growth factors can give rise to severe inflammation, tissue damage and remodeling[3, 56, 57]. Moreover, while the number of tissue mast cells is tightly regulated by migration, proliferation and survival in steady state conditions, mast cell homeostasis could be disturbed under pathological conditions, leading to rapid alterations in the number and distribution of mast cells[58]. Altogether, uncontrolled increase in the number and activation of mast cells as well as alterations in their tissue distribution can have deleterious consequences for the organism.

Based on the points discussed above, mast cells can play both beneficial and detrimental roles for the organism. Examples of potentially beneficial roles of mast cells include their involvement in protection against certain animal venoms[59-61] and infections, particularly those caused by certain parasitic helminths[22, 62, 63]. However, mast cells are undoubtedly best known for their detrimental roles in allergies (including potentially fatal anaphylaxis) and related diseases, such as asthma, allergic rhinitis and atopic dermatitis[35, 64]. Moreover,

a growing body of evidence frequently reports the increased numbers and enhanced activation of mast cells at the sites of inflammation in several other human diseases including various autoimmune disorders[65, 66] and cancers[3, 67], mastocytosis[68, 69], chronic obstructive pulmonary disease (COPD)[70, 71], and atherosclerosis[3, 72]. This indicates that mast cells are implicated in these pathologies.

The central role of mast cells in the pathogenesis of allergic diseases, e.g., asthma, is supported by several lines of evidence. For example, asthmatic patients have increased numbers of lung mast cells, especially in locations such as the airway smooth muscle layer, lung epithelium, and alveolar parenchyma[73-75]. Moreover, a higher number of mast cells has been found in the distal airways of individuals with non-fatal and fatal asthma compared to non-asthmatic controls[76]. Of note, the abnormal accumulation of mast cells in these lung compartments has been associated with enhanced asthma symptoms[73, 75, 77, 78]. In line with these observations, an increased percentage of degranulated mast cells has been found in the mucous glands in cases of fatal asthma compared to non-fatal asthma and controls[79]. The extensive mast cell degranulation in fatal asthma suggests that mast cells are highly activated in severe asthma[9, 79]. Importantly, a role for mast cells in asthma is also supported by a number of studies conducted on mice[80-82]. In mouse models of allergic asthma, elevated numbers of airway mast cells are found and mast cells have been demonstrated to contribute in a major way to several symptoms associated with experimentally induced allergic airway inflammation, including eosinophilic airway inflammation, enhanced airway hyperresponsiveness (AHR) to methacholine or antigen, goblet cell hyperplasia and enhanced mucus production[80].

The inflammatory reactions that occur in allergic disorders are divided into three temporal phases, namely early, late and chronic[64]. Importantly, mast cells are recognized as effector cells that participate in all these phases[64]. With regard to allergic asthma, mast cells drive early-phase asthmatic reactions by releasing mediators such as histamine, PGD2 and LTC4 immediately after allergen challenge. These vasoactive and spasmogenic mediators induce symptoms of asthma including bronchoconstriction, respiratory mucosal edema and mucus secretion[3, 57]. The relative rate of mediator release from human lung mast cells *in vitro* is histamine > PGD2 > LTC4 with half maximal release occurring at 2, 5 and 10 minutes respectively[83]. Interestingly, this is paralleled *in vivo* by the recovery of these mediators in bronchoalveolar lavage (BAL) fluid within 5–10 min after local bronchial allergen challenge[84-90]. The central role of these mast cell mediators is supported by the observations indicating that potent and selective receptor antagonists of histamine[91, 92], LTC4[93, 94] and to a lesser degree PGD2[95], can markedly attenuate early-phase asthmatic reactions. In late-phase asthmatic reactions, proinflammatory mediators,

including cytokines and chemokines, which are released by mast cells contribute to the recruitment of inflammatory cells such as eosinophils, basophils, $CD4^+$ T cells and macrophages to the airways. Recruitment and activation of these inflammatory cells is followed by mediator release and tissue damage, leading to airway obstruction and AHR which accompanies the late-phase asthmatic reactions[57]. Given that many cell types are recruited and activated during late-phase asthmatic reactions, it is not an easy task to determine the contribution of mast cells as a source of bronchospastic mediators during this phase[57]. Nevertheless, it has been found that anti-IgE therapy markedly attenuate the late-phase asthmatic reactions[96], indicating that mast cell activation during the early-phase initiates events leading to the late-phase reactions. Thus, mast cell-derived mediators released upon allergen challenge likely contribute to development of late-phase asthmatic reactions and associated symptoms[57]. Lastly, when allergen exposure is continuous or repetitive, early- and late-phase reactions develop into a chronic phase that is associated with persistent inflammation, tissue remodeling and fibrosis[64]. In chronic allergic asthma, ongoing mast cell activation and degranulation is observed[57]. In line with this, increased levels of mast cell products, such as histamine and tryptase, have been found in BAL fluid from asthmatics compared to healthy controls[86, 97-100]. Additionally, mast cells within the bronchial mucosa in allergic asthma produce various cytokines (mRNA and/or protein) including IL-4, IL-5, IL-6, IL-13, TNF-α and TSLP, with disease-related increases in the expression of many of these[101-106]. Based on these findings and the fact that anti-IgE therapy has been successful for treating a subgroup of allergic asthmatics with severe or persistent symptoms, it is clear that mast cells and their interaction with allergens and IgE play a central role in the pathogenesis of allergic asthma[57].

Therapeutic approaches to target mast cells in disease

Given the well-recognized harmful role that mast cells play in allergic disorders, and their emerging detrimental functions in non-allergic diseases, there is an urgent need to identify efficient strategies that can limit the harmful effects of mast cells in such pathological settings. Currently, there are several therapeutic approaches available for counteracting mast cells' unfavorable actions. In general, the aims of these approaches are to (1) inhibit mast cell-derived mediators or their effects, (2) inhibit mast cell activation, or (3) reduce mast cell numbers. In this section, some of the therapeutic anti-mast cell options that are used in the clinic or being considered for future use are briefly reviewed (*Figure 2*), and their advantages and limitations are summarized. Furthermore, the concept of inducing mast cell apoptosis, particularly via granule permeabilization, as a novel therapeutic strategy is discussed.

Approaches aimed at inhibiting mast cell-derived mediators

Given that mast cells are capable of releasing a large array of diverse biologically active preformed and newly synthesized mediators, one possible therapeutic option is to target individual mast cell mediators that are responsible for the respective mast cell-associated pathology. For example, several mast cell mediators such as histamine, leukotrienes, prostaglandins and cytokines are currently targeted effectively by available drugs[107] (*Figure 2*). Moreover, multiple mast cell-specific proteases, e.g., chymase and tryptase, can be selectively inhibited[107-111]. In general, targeting any given mast cell mediator can be achieved either by direct inhibition of the mediator, for example when the enzymatic activities of mast cell proteases are blocked by inhibitors, or by inhibition of mediator's effects. Examples of the latter are neutralizing antibodies or receptor antagonists that are used to block the mediator–receptor interaction and thereby inhibiting the mediator's effect.

Drugs that target histamine (e.g., ketotifen), leukotrienes (e.g., montelukast) and prostaglandin D2 (e.g., fevipiprant) function as receptor antagonists for these mediators (*Figure 2*). Interference with the effects of these mediators reduces symptoms of allergies and related disorders such as asthma in clinical settings[112-115]. Mast cell-produced proinflammatory cytokines and growth factors, such as TNF-α, IL-4, IL-13, IL-17 and VEGF, can be targeted by neutralizing antibodies. Although these proinflammatory compounds are not exclusively produced by mast cells, in several pathological settings mast cells are an important source of these compounds[116]. For example, mast cells are the predominant cell type producing IL-17 in patients with inflammatory skin and joint diseases including psoriasis[117], rheumatoid arthritis (RA)[118] and spondyloarthritis[119, 120]. Therefore, monoclonal antibodies directed to IL-17 (*Figure 2*) are being investigated in patients suffering from the aforementioned conditions[121].

Other mast cell-derived cytokines that are therapeutic targets include IL-4 and IL-13. These two cytokines play key roles in promoting several hallmark pathological features of Th2 inflammation including IgE production, smooth muscle contractility, mucus production and recruitment of inflammatory cells to the inflammation site[122-124]. In asthma patients, mast cells were found to produce and release IL-4[102]. IL-4 is also found as a preformed meditator stored in mast cell granules[125]. Strikingly, the ability to produce IL-4 has also been demonstrated in MCp[126]. Furthermore, IL-4– and IL-13–expressing mast cells accumulate within the airway smooth muscle of asthmatics, suggesting an important role for these cytokines in mast cell–airway smooth muscle interactions[127]. Mast cells also produce IL-13 in response to various stimuli including IgE/antigen, IL-1β, IL-4, IL33, LPS and peptidoglycan (PGN)[37, 128-132]. Dupilumab is a monoclonal antibody directed against the α subunit of the IL-4

receptor (IL-4Rα) that blocks signal transduction pathways activated by IL-4 and IL-13 (*Figure 2*). Of note, dupilumab has shown striking beneficial effects in improving several disease features in patients suffering from mast cell-associated allergic diseases such as asthma[133] and atopic dermatitis[134, 135]. Dupilumab is the first and currently only approved monoclonal antibodies for atopic dermatitis in Europe and the US[136]. More recently, dupilumab has been approved by the US Food and Drug Administration (FDA) for treatment of moderate-to-severe asthma[137].

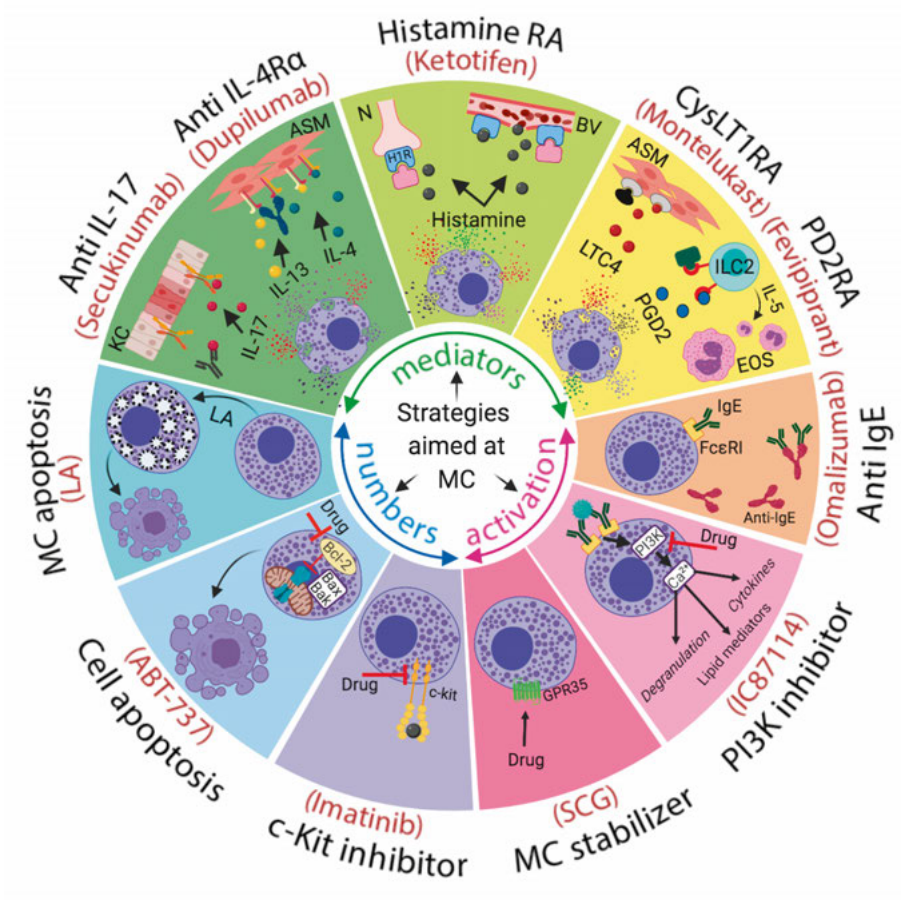

Figure 2. **Simplified overview of therapeutic strategies aiming at the inhibition of mast cell mediators or activation, and reduction of mast cell numbers**. (⊣ denotes inhibition). Abbreviations: ASM, airway smooth muscle; BV, blood vessel; CysLT1RA, cysteinyl leukotriene receptor 1 antagonist; EOS, eosinophils; GPR35, G-protein-coupled receptor 35; H1R, histamine H1 receptor; Histamine RA, histamine receptor antagonist; ILC2, type 2 innate lymphoid cells; KC, keratinocytes; LA, lysosomotropic agent; LTC4, leukotriene C4; MC, mast cell; N, neuron; PD2RA, prostaglandin D2 receptor 2 antagonist; PGD2, prostaglandin D2; PI3K, phosphatidylinositol 3-kinase; SCG, sodium cromoglicate.

Although targeting the individual mediators of mast cells can reduce their ability to mediate the related inflammatory responses, the beneficial therapeutic effects of such treatments are often regarded as limited. There are many patients suffering from mast cell-associated diseases who do not gain adequate relief from their symptoms, despite receiving higher doses of the aforementioned drug classes[116]. One major reason could be that the overall impact of mast cells on any given pathological setting is likely a result of combined effects from several different mast cell mediators, rather than being due to a single mediator acting on a single target[107]. Thus, targeting a single mast cell mediator will only partly interfere with detrimental mast cell effects. In line with this notion, therapeutic regimens with, for example, anti-leukotrienes alone have shown a limited efficiency in the management of asthma. Thus, these drugs are often recommended as add-on therapy to inhaled corticosteroids[114, 138]. As another example, targeting only IL-13 by lebrikizumab or tralokinumab, two anti-IL-13 monoclonal antibodies, has shown a very limited beneficial effect without reducing the exacerbations in asthma patients, whereas targeting both IL-4 and IL-13 by dupilumab effectively improved lung function and symptoms and resulted in reduced asthma exacerbations[139].

In addition to this, mast cells release their mediators differentially when exposed to distinct activating stimuli as discussed elsewhere (see "*Mast cell activation*"). Thus, it is difficult to predict what mediators are released in response to any given stimulus, either alone or in combination with other stimuli. Altogether, the difficulty in identifying targets and the necessity to combine drugs to achieve higher efficacy, limit the applicability of this approach.

Approaches aimed at inhibiting mast cell activation

Considering that mast cells express a large number of activating and inhibitory receptors of various types, one anti-mast cell therapeutic option is to use drugs that interfere with mast cell activation. Many of the drugs targeting mast cell-derived mediators are commonly used to control the disease symptoms associated with those mediators, whereas the inhibition of mast cell activation provides an opportunity to counteract with the immune dysfunction underlying mast cell-associated diseases with a more direct approach.

One approach to inhibit mast cell activation is to use monoclonal antibodies to target IgE molecules and thereby block the interaction of IgE with FcεRI (*Figure 2*). Omalizumab is a humanized IgG1 antibody against IgE that is approved for clinical use and was found to reduce asthma symptoms in adults and children[140, 141]. Moreover, beneficial therapeutic effects of omalizumab have been observed in persistent allergic rhinitis[142], atopic dermatitis[143], urticaria[144, 145] and food allergies[146]. The effect of omalizumab is mainly attributed

to its ability to reduce plasma IgE levels and FcεRI expression on mast cells and basophils[116].

Although anti-IgE therapy represents a successful approach to inhibit mast cell activation, there are several disadvantages that limit its use in treatment of mast cell-associated disorders. For example, anti-IgE therapy is beneficial in treatment of allergy-related disorders but has very limited efficacy, if any, in the treatment of non-allergic mast cell-driven diseases, in which mast cell are activated by IgE-independent pathways[107]. Another limitation with this treatment is its unpredictable efficacy, i.e., some asthmatic patients show considerable improvement, whereas most patients experience little or no signs of clinical improvement[147]. Additionally, similar to other treatments that involve the use of humanized monoclonal antibodies, anti-IgE therapy is associated with high economic costs, and therefore it cannot be used for all patients suffering from any given allergic condition[116].

Another group of compounds that display inhibitory effects on mast cell activation are mast cell stabilizers that have the ability to inhibit mast cell degranulation and mediator release in response to various stimuli[148]. Sodium cromoglycate (SCG) and nedocromil sodium are the most common mast cell stabilizers used for treating asthma and other diseases that involve mast cell activation, including allergic rhinitis, allergic conjunctivitis, atopic dermatitis, and mastocytosis[65]. Despite being in clinical use for decades, the mechanisms by which these drugs inhibit mast cell activation and degranulation are still not well-defined[107]. However, it has become more evident during the recent years that the effect of SCG and nedocromil sodium is mediated via GPR35, an inhibitory mast cell receptor[21] (*Figure 2*).

Although mast cell stabilizers are generally well-tolerated, their inhibitory effects are moderate or negligible[107]. In fact, comparative studies suggest that the beneficial effect of SCG in controlling asthma symptoms is rather small in both children and adults[149, 150]. One possible reason for this could be that mast cell stabilizers do not inhibit human lung mast cells effectively. In support of this notion, SCG has been found to be a weak inhibitor of histamine release from freshly isolated human lung mast cells in response to IgE-mediated activation, even when high concentrations of SCG were used[151]. Another disadvantage of mast cell stabilizers is that, due to their low potency and short half-life, high concentrations of the drug need to be given at frequent intervals to have an effective inhibitory impact. Moreover, local administrations are preferred to maximize the concentration of the drug in the target tissue[148].

An alternative approach to inhibit mast cell activation is to interfere with the intracellular signaling pathways that are essential for mast cell degranulation and mediator release[107, 116, 152]. This can be achieved, for example, by using

pharmacologic inhibitors to block the function of key cytoplasmic signaling proteins such as spleen tyrosine kinase (SYK), phosphatidylinositol 3-kinases (PI3K) and Bruton's tyrosine kinase (BTK). Since these proteins are involved in early signaling events induced by IgE–FcεRI interaction, their inhibition could theoretically result in effective suppression of antigen-induced degranulation and mediator release[107]. In line with this, several inhibitors of the aforementioned signaling proteins exhibited beneficial effects when tested in preclinical *in vivo* models. For example, IC87114, a selective inhibitor of PI3Kδ, was found to have a therapeutic potential for the treatment of allergic asthma and rheumatoid arthritis in the relevant disease models[153, 154] (*Figure 2*). Furthermore, a number of different inhibitors are being tested in clinical trials for diseases such as allergic rhinitis, asthma, urticaria and rheumatoid arthritis[107, 116]. Although some of these inhibitors have been able to reduce certain disease symptoms in patients during initial phases of clinical trials, so far none of these inhibitors have been approved for routine treatment of mast cell-related diseases in the clinic[107]. For example, the SYK inhibitors, R112 and R343, both failed in clinical phase II studies for treatment of allergic rhinitis or asthma[116].

One major problem with approaches targeting signaling pathways is that they are not exclusive to mast cells. Indeed, the fact that the signaling proteins are widely expressed by many different cell types gives rise to an increased risk of adverse effects when signaling protein inhibitors are used[107]. Moreover, the majority of inhibitors that are available or being considered for clinical development, are directed against signaling pathways that operate downstream of classical IgE-mediated mast cell activation[57]. Thus, they predominantly suppress mast cell activation in allergic settings and have limited effectiveness in other contexts. This is a very important issue to consider when developing new inhibitors of signaling proteins, because in many non-allergic inflammatory disorders and in chronic conditions such as asthma, mast cell activation can be mediated via different IgE-independent mechanisms whose signaling pathways are not well understood in mast cells[57].

Approaches aimed at reducing mast cell numbers

The overall impact of mast cells on any pathological setting is most likely multifaceted, i.e., it is mediated by multiple activating mechanisms and a large number of secreted mediators. Thus, targeting the individual mediators or single activation pathway in mast cells, i.e., the IgE-mediated pathway, may not be sufficient to prevent the full panel of mast cell-driven pathological effects. Theoretically, a more effective strategy for global inhibition of mast cells and their harmful activities might therefore be to reduce mast cell numbers in diseases, for example by blocking mast cell survival or inducing their apoptosis[116, 155, 156]. However, in order to avoid harmful side effects, it is essential to develop a strategy that is selective to mast cells and causes minimal

interference with other cell types. In the following section, the major strategies that can be employed for blocking mast cell survival or induction of mast cell apoptosis are briefly discussed.

Strategies to block mast cell survival

Tissue-resident mature mast cells are long-lived and primarily depend on SCF for their survival[3]. Additionally, the interaction between SCF and its receptor, c-kit (CD117) which has tyrosine kinase activity[157], induces an intracellular signaling cascade which in turn promotes mast cell differentiation, proliferation, chemotaxis and maturation[3]. The pivotal role of SCF for mast cell survival and development *in vivo* is highlighted by the finding that mice having deficiency in SCF or c-kit essentially lack mast cells[2, 158, 159]. Moreover, glucocorticoid-induced reduction of local SCF levels results in decreased numbers of tissue mast cells in mice[160]. Additionally, administration of SCF to primates, mice and rats promotes *in vivo* expansion of tissue mast cells[161, 162]. The ability of the SCF–c-kit axis to induce mast cell survival appears to be mediated, at least partly, through downregulation of the pro-apoptotic proteins such as Bim[163].

It is thought that SCF and c-kit may contribute to the mast cell accumulation and survival in mast cell-driven disorders. For example, in humans, gain-of-function mutations in c-kit leads to mastocytosis, a disorder characterized by mast cell population expansion and accumulation in patients due to the constitutive SCF-independent activation of c-kit[164]. Furthermore, in individuals with various allergic diseases including asthma, allergic rhinitis and atopic dermatitis, an increased production of SCF[165-169] and elevated mast cell numbers are commonly seen[170]. In patients with asthma or atopic dermatitis, the increased SCF levels correlate with disease severity[166, 169].

Based on these findings, blocking mast cell survival and development through inhibition of the SCF–c-kit axis has been considered as a potential treatment option to decrease mast cell numbers in certain pathological conditions[107] (*Figure 2*). Imatinib, a tyrosine kinase inhibitor initially developed for targeting BCR-ABL (breakpoint cluster region-Abelson murine leukemia viral oncogene homolog 1) in patients with chronic myeloid leukemia (CML), was recently shown to reduce mast cell numbers in endobronchial biopsy samples, and to reduce serum tryptase levels and AHR in patients with severe asthma[171]. In addition to CML, imatinib is approved by FDA for the treatment of adult patients with aggressive systemic mastocytosis that lack the KitD816V mutation[172]. In a phase IV clinical trial, imatinib caused a reduction in mast cell numbers in mastocytosis patients that had Kit mutations other than KitD816V[173]. In addition to imatinib, several other tyrosine kinase inhibitors including nilotinib, dasatinib, midostaurin and masitinib are being evaluated for their effect in several mast cell-driven diseases[116]. However, none of these

inhibitors are specific for c-kit. In fact, they are capable of inhibiting platelet-derived growth factor receptors (PDGFR) and multiple other proteins with tyrosine kinase activity[116]. Thus, the effects of available tyrosine kinase inhibitors extend far beyond mast cells, resulting in high risk of adverse effects[107]. This in turn limits the use of available inhibitors primarily to the treatment of cancers (e.g., CML) and other neoplastic diseases such as aggressive mastocytosis.

Strategies to induce mast cell apoptosis

Historically, cell death has been classified into two major forms, apoptosis and necrosis[174]. Apoptosis, also termed as "programmed cell death", is a highly regulated mode of cell death in multicellular organisms and plays an essential role in development, morphogenesis and maintaining homoeostasis through the removal of damaged, aged and potentially dangerous cells[175]. Upon activation of apoptosis, a series of intracellular signaling events is initiated that leads to activation of apoptotic caspases (there are also inflammatory caspases, but these do not contribute to apoptosis[176]). These caspases belong to a family of evolutionarily conserved proteases whose activation in a cell results in structural changes in the cytoplasm and nucleus. These changes include cell shrinking, chromatin condensation, DNA fragmentation, plasma membrane blebbing and formation of apoptotic bodies[177-179]. In contrast, necrosis is a less controlled cell death mode characterized by loss of cell membrane integrity and release of numerous cellular contents, such as danger signals, into the extracellular environment. Therefore, unlike apoptosis, necrosis can potentially induce an inflammatory response[174]. Apoptotic cell death can be induced via two major classical pathways: the intrinsic and extrinsic pathways[179]. The intrinsic pathway is initiated in response to cell stress stimuli such as DNA damage, oxidative stress, growth factor deprivation and cytotoxic substances through activating the pro-apoptotic proteins (e.g., BH3-only proteins)[180-182]. Once activated, these proteins inhibit anti-apoptotic proteins (e.g., Bcl-2 and Bcl-XL) leading to mitochondrial outer membrane permeabilization (MOMP). This results in release of apoptogenic factors such as cytochrome c and apoptosis-inducing factor (AIF) that can execute apoptotic cell death through caspase-dependent and/or -independent mechanisms, respectively[179, 183]. The extrinsic pathway of apoptosis is triggered when cell surface death receptors bind to their ligands[184]. The death receptors include tumor necrosis factor receptor (TNFR), Fas (also known as CD95) and TNF-related apoptosis-inducing ligand receptor (TRAIL-R) which all belong to TNFR superfamily[185]. Interaction of these death receptors with their cognate ligands, i.e., TNF, FasL and TRAIL, provokes the intracellular assembly of a multiprotein complex known as death-inducing signaling complex (DISC) and recruitment of adaptor proteins. This, in turn, activates caspases and leads to the induction of apoptosis[182, 184].

The concept of selectively inducing mast cell apoptosis as a means to intervene with mast cell-driven diseases is emerging as an attractive future therapeutic approach[155]. To achieve mast cell apoptosis, one strategy could be to activate pro-apoptotic pathways, for example by using agonists of surface death receptors (e.g., TRAIL-R). Currently, several TRAIL-R agonists are being tested in preclinical and clinical studies for their therapeutic beneficial effects in different cancers[186]. Although human mast cells express TRAIL-R and were found to undergo apoptosis through engagement by TRAIL[187], the selectivity of TRAIL-mediated apoptosis for mast cells is questionable. This is due to the fact TRAIL-R is widely expressed among many human tissues and cell types[186, 188, 189].

Another approach to induce mast cell apoptosis would be to interfere with the function of anti-apoptotic proteins. In line with this scenario, small molecule compounds known as BH3 mimetics were found to induce apoptosis in mast cells through inhibiting the effect of anti-apoptotic proteins such as Bcl-2, Bcl-XL and Mcl-1[190-192]. However, due to the ubiquitous expression of the anti-apoptotic proteins, such compounds are not likely to act selectively on mast cells. In line with this notion, the BH3 mimetic ABT-737 was shown to induce apoptosis in mast cells (*Figure 2*) and a variety of other cell types, including B lymphocytes, neuronal cells and transformed cells of various origin[190, 193, 194]. Due to differences in the chemical structures and properties, various BH3 mimetic compounds can target individual anti-apoptotic proteins[191]. On the other hand, different mast cell types were found to express distinct levels of individual anti-apoptotic proteins, which results in differential sensitivity toward a certain BH3 mimetic compound[190]. For example, mast cells with lower expression of Mcl-1 and higher expression of Bcl-2 are more sensitive to apoptosis induced by ABT-737, whereas mast cells with an opposite expression profile of Mcl-1 and Bcl-2 were more resistant[190]. These findings suggest that, in order to ensure an efficient mast cell apoptosis by BH3 mimetic compounds, a combination of several different compounds is likely required. This, in turn, increases the risk of having unwanted side effects, i.e., causing apoptosis in other cell types alongside mast cells.

Given that most pro-apoptotic pathways are ubiquitously present among different cell types, it has not been possible, prior to the present investigation, to identify a cell death pathway selective to mast cells. If a pro-apoptotic strategy is to be selective for mast cells, it must be established based on their unique properties. Since *the* unique feature of mast cells is their abundant cytoplasmic secretory granules, they may represent a potential target for selective mast cell apoptosis by the induction of granule permeabilization. This notion is based on a well-established concept of lysosomal membrane permeabilization (LMP)[182]. In fact, LMP, which can be induced by various stimuli, is a potentially fatal event for the cells because it is followed by ectopic translocation of

many lysosomal enzymes into the cytosol[195]. Proteases, especially cathepsins, are among these lysosomal enzymes whose release into cytosol leads to apoptosis through proteolytic activation of several pro-apoptotic proteins as well as degradation of anti-apoptotic proteins[196-198].

Interestingly, mast cell granules have striking similarities with lysosomes and are therefore also called "secretory lysosomes"[199]. For example, both compartments have an acidic pH, similar membrane composition and contain typical lysosomal enzymes such as cysteine- and aspartic acid cathepsins, arylsulfatase A, β-glucuronidase and β-hexosaminidase[6, 199, 200]. Based on such similarities, it is possible that compounds capable of inducing lysosome permeabilization, e.g., lysosomotropic agents, also cause granule permeabilization in mast cells resulting in release of potent granule enzymes, e.g., proteases, into the cytosol where they potentially may induce apoptosis. In support of this notion, it has been shown that lysosomotropic agents L-leucyl-L-leucine methyl ester (LLME) and siramesine induce mast cell apoptosis by causing permeabilization of the granule membrane[201, 202] (*Figure 2*). However, the mechanism of apoptotic cell death in response to such agents remained to be fully determined. In addition, neither LLME nor siramesine is approved for use in humans.

Lysosomes and cell death

Lysosomes are cytoplasmic organelles found in virtually all eukaryotic cells[203] that were originally described by Christian de Duve[204], a finding that yielded a Nobel Prize[205]. The primary function of these membrane-enclosed acidic organelles is the degradation of extracellular material as well as intracellular components that are delivered to them. The degradative capacity of lysosomes is attributed to their high content of various hydrolytic enzymes, including proteases, peptidases, lipases, nucleases, glycosidases, phosphatases, and sulfatases[206, 207]. In general, these enzymes exert maximal enzymatic activity at low pH (<5), which is maintained by a vacuolar ATPase (V-ATPase), pumping protons into the lysosomal lumen[208]. The lysosomal membranes are protected from the activity of lysosomal hydrolases due to high abundance of heavily glycosylated membrane proteins, which form a protective coat on the luminal surface of the these membranes[209].

Loss of the lysosomal membrane integrity is generally manifested as LMP, which can have lethal consequences for the cell, i.e., cell death[195]. The concept of cell death as a result of lysosomal rupture was first described by Christian de Duve himself, who introduced the term "suicide bags" for lysosomes, to highlight their involvement in cell autolysis, owing to their content of powerful hydrolytic enzymes[203, 210].

Lysosomal membrane permeabilization and its regulation

Lysosomal membrane permeabilization (LMP) is a perturbation of the lysosomal membrane that results in the release of the lysosomal contents into the cytosol[211]. Although the exact molecular mechanisms that mediate LMP remain enigmatic, a large number of factors have been shown to regulate LMP through promoting or reducing the lysosomal membrane stability (*Figure 3*).

Factors that promote lysosomal membrane stability, and thus prevent LMP from happening, include antioxidants, highly glycosylated lysosomal proteins, such as lysosomal-associated membrane protein 1 and 2 (LAMP-1 and LAMP-2) and lysosomal integral membrane protein 2 (LIMP-2), heat shock protein 70 (Hsp70) and the lipid composition of the lysosomal membrane, i.e., cholesterol and sphingomyelin levels[205, 212, 213] (*Figure 3*). Degradation of lysosomal proteins such as LAMPs and Hsp70, as well as alteration of lipid composition, i.e., reduction of cholesterol and sphingomyelin, decrease lysosomal membrane stability leading to LMP[205, 213-215].

Factors that reduce lysosomal membrane stability and, as a result, induce LMP are reactive oxygen species (ROS), lysosomotropic agents, certain proteases (e.g., cathepsins and calpains), as well as bacterial and viral products[195, 212] (*Figure 3*). Among these LMP inducers, ROS and lysosomotropic agents are further discussed in the following section since these are more relevant to the present thesis.

ROS. The mechanism of ROS-induced LMP is well-studied. As opposed to mitochondria, lysosomes do not contain the most common antioxidant enzymes, such as superoxide dismutase, catalase or glutathione peroxidase. Thus, when high levels of oxidative stress are induced by various stimuli (e.g., drugs, ionizing radiation, etc), the generated ROS can cause damage to the lysosomal membrane[211]. Due to autophagic degradation of iron-containing protein complexes, lysosomes contain a comparatively high level of redox-active iron (Fe^{2+})[211, 216, 217]. Upon oxidative stress, excess hydrogen peroxide (H_2O_2), which readily diffuses into the lysosomes, can react with intralysosomal redox-active iron in the Fenton reaction, giving rise to formation of hydroxyl radicals. Hydroxyl radicals are very reactive and have a high capacity to destabilize lysosomal membranes and induce LMP through peroxidation of membrane lipids and proteins[205, 218]. In support of the lysosome-destabilizing effect of ROS and intralysosomal iron, various antioxidants and iron chelators confer protection against ROS-induced LMP or cell death. For example, deferoxamine mesylate (DFO), an iron chelator that specifically accumulates inside lysosomes by fluid-phase endocytosis, can inhibit LMP-mediated cell death induced in response to oxidative challenge[205, 211, 219]. In addition to iron chelation, chelation of other intralysosomal metals such as copper (Cu^{2+}) and

zinc (Zn^{2+}) has been found to prevent cell death mediated by LMP in several experimental settings[211].

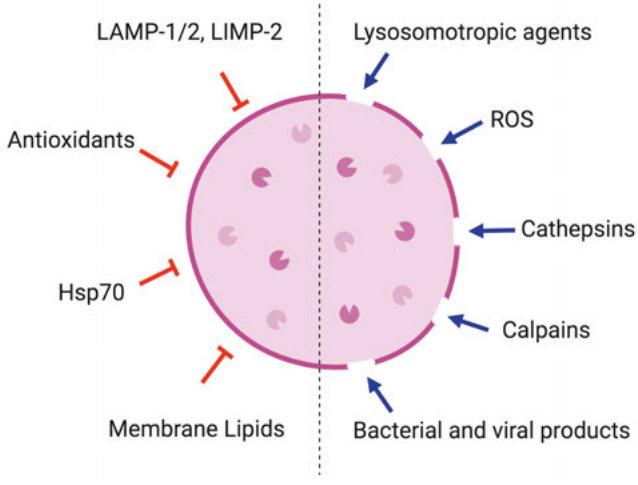

Figure 3. **Regulation of lysosomal membrane permeabilization (LMP).** Factors that promote lysosomal membrane stability and safeguard the lysosomal integrity, include lysosomal membrane proteins, such as LAMP-1 and -2, LIMP-2 as well as Hsp70. In addition, membrane lipids (e.g., cholesterol and sphingomyelin) and antioxidants enhance the integrity of lysosomal membranes. In contrast, factors that reduce lysosomal membrane stability promote the release of lysosomal contents. Examples of such factors include lysosomotropic agents, ROS, cathepsins, calpains, as well as bacterial and viral products.

Lysosomotropic agents. Another class of compounds that induce LMP are lysosomotropic agents. These compounds are weak bases that are able to freely diffuse across the membranes in their uncharged form at neutral pH (e.g., in the cytosol). Once inside the acidic compartments, namely lysosomes, they become trapped and non-diffusible as a result of protonation. When the accumulation of lysosomotropic agents in their protonated form exceed a certain concentration threshold, they acquire detergent-like properties capable of inducing lysosomal membrane damage and LMP[195]. Unlike other detergents that cause cell death through acting at the plasma membrane, lysosomotropic agents with detergent-like properties mediate cell death by disrupting lysosomes from the inside. In support of the notion of this mechanism, lysosomotropic agents are unable to lyse red blood cells, which lack lysosomes[220, 221]. Additionally, compounds that raise the lysosomal pH are able to prevent lysosomotropic agent-induced LMP and cell death[221, 222]. The latter observation further suggests that intralysosomal accumulation of these compounds is essential for their toxic effects[205]. Examples of lysosomotropic agents are lysosomotropic detergents (e.g., siramesine, LLME and *O*-methyl-serine dodecylamide hydrochloride [MSDH])[210, 223, 224], several antimalarial (e.g.,

chloroquine and mefloquine)[224-226] and anti-psychotic drugs (e.g., chlorpromazine, thioridazine and aripiprazole)[224] and multiple anti-depressants (e.g., imipramine and desipramine)[224] and quinolone antibiotics (e.g., ciprofloxacin and norfloxacin)[225].

Downstream events activated upon LMP

As described above, several different stimuli can induce LMP, resulting in release of lysosomal contents, such as hydrolytic enzymes, into the cytosol. This in turn leads to induction of lysosome-dependent cell death (LDCD; also called LCD). Lysosomal cathepsins play a prominent role in initiation and execution of LDCD and their inhibition can, at least partially, block LDCD in many experimental settings[227]. A growing body of evidence has revealed that, depending on the cellular context, LDCD can adopt apoptosis, necrosis, ferroptosis or pyroptosis-like features (*Figure 4*), suggesting that LDCD represents a more complex cell death pathway than previously thought[227]. Several factors can determine the downstream events and mode of cell death triggered by LMP, including the type and intensity of the stimulus, the amount and type of cathepsins and other lysosome-derived components released into the cytosol, the type and immortalization (transformation) status of the cell, the extent of the damage and number of permeabilized lysosomes, and the levels of endogenous inhibitors (e.g., cathepsin inhibitors and antioxidants)[195, 205, 227].

Apoptosis. One major and well-studied cell death pathway induced downstream of LMP is the classical caspase-dependent apoptosis (*Figure 4*). In this pathway, lysosomal cathepsins that are released into the cytosol play a major role in execution of apoptosis. Although lysosomal proteases are generally inactive at neutral pH, some cathepsins (e.g., cathepsins B, D, and L) remain active at neutral pH and can promote apoptotic cell death by proteolytic modification of molecules involved in apoptosis. For example, these cathepsins mediate the activating cleavage of pro-apoptotic protein, Bid, or degrade the anti-apoptotic proteins, Bcl-2, Bcl-XL and Mcl-1. These modifications result in activation of other pro-apoptotic proteins, e.g., Bax and Bak, leading to MOMP. Upon MOMP, an apoptotic cascade is triggered leading to the release of cytochrome c into cytosol, apoptosome formation and activation of caspases that are responsible for execution of apoptosis[195, 210]. Alternatively, cathepsins can induce caspase-dependent apoptosis via activating cleavage of apoptotic caspases (e.g., caspase 8) or by inhibiting cleavage of caspase 3, 7 and 9 inhibitor, X-linked inhibitor of apoptosis protein (XIAP)[210] (*Figure 4*).

In addition to inducing caspase-dependent apoptosis, cathepsins that are released upon LMP can induce apoptosis with minimal or no caspase activation[210]. In such instances, cell death is not affected by caspase inhibition (even when LMP causes a slight caspase activation), but instead is largely

suppressed by cathepsin inhibition[205, 210]. It is now known that cathepsins themselves are able to execute apoptosis through cleavage of different substrates. For example, cathepsins can induce release of AIF from mitochondria into the cytosol, which upon subsequent nuclear translocation mediates DNA fragmentation in a caspase-independent fashion[195, 205, 210] (*Figure 4*). Interestingly, it has been demonstrated that high levels of ROS generated in association with LMP causes a shift from caspase-dependent to caspase-independent cell death, perhaps due to ROS-induced oxidation of caspase catalytic sites[205]. Furthermore, although cathepsins play a central role in execution of cell death downstream of LMP, their inhibition can confer a partial protection against apoptosis induced by LMP in some experimental settings, suggesting a role for additional factors such as other lysosomal hydrolases and lysosome-derived second messengers (e.g., ROS, iron and Ca^{2+}) in cell death pathways induced by LMP[210].

Necrosis. Under certain circumstances, LMP may lead to necrotic cell death. As described previously, necrosis is generally considered an accidental and less controlled mode of cell death that is associated with loss of plasma membrane integrity and induction of inflammation[174]. Of note, it has been demonstrated that complete LMP and massive release of lysosomal contents can cause uncontrolled damage to cellular components and the plasma membrane leading to induction of necrotic cell death. However, partial LMP leads to limited and selective release of lysosomal contents, e.g., proteases, into the cytosol, where they activate apoptosis or apoptosis-like cell death, i.e., controlled modes of cell death without causing inflammation[195] (*Figure 4*).

Ferroptosis. Another form of cell death that can occur downstream of LMP is ferroptosis. Lysosomes contain high levels of iron and this iron is a major source of ROS generation inside the lysosomes that can induce LMP[195, 211]. Upon LMP, the lysosomal iron and ROS can be released into the cytosol where they can induce massive lipid peroxidation and subsequently trigger ferroptosis[211] (*Figure 4*). Importantly, the sequestration of lysosomal iron through the use of iron chelators such as DFO, inhibits ferroptosis in response to the classical inducers of ferroptosis, i.e., erastin and RSL3[211]. Furthermore, the deleterious effects of lipid peroxidation in the induction of ferroptosis are efficiently blocked by lipophilic antioxidants such as vitamin E, ferrostatin-1 and liproxstatin-1[227].

Pyroptosis. In certain conditions, cathepsins released after LMP can participate in pyroptosis. This mode of cell death is mainly implicated in host defense against intracellular microbes and tissue injury[227, 228]. Hence, pyroptosis is triggered when PAMPs and DAMPs are detected by the cell, leading to activation of a cytosolic multiprotein complex called the inflammasome, and subsequent activation of inflammatory caspases, such as caspase 1 and 11. These caspases

mediate the maturation and release of inflammatory cytokines (e.g., IL-1β) and gasdermin-D into the extracellular space, a process followed by rupture of the plasma membrane and pyroptotic cell death[205, 211, 227]. In recent years, a link between LMP, lysosomal cathepsins and inflammasome activation has been suggested (*Figure 4*). For example, microbial products (e.g., flagellin and LPS), cholesterol crystals and protein aggregates (e.g., β-amyloid) have been shown to induce LMP and activation of the inflammasome, leading to cytokine release and cell death. In these settings, inhibition of cathepsins, such as cathepsin B, blocks the activation of inflammasome and pyroptotic cell death[205, 211]. However, it is still not clear how and through which pathways cathepsin activity is linked to inflammasome activation[205].

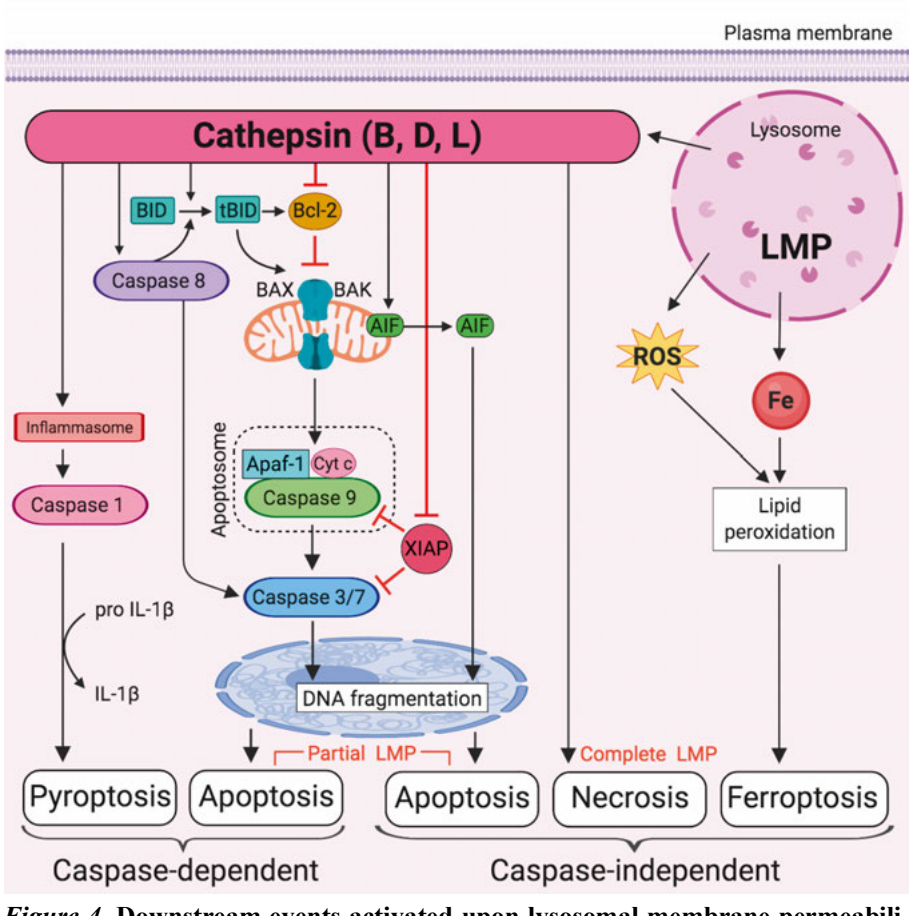

Figure 4. **Downstream events activated upon lysosomal membrane permeabilization (LMP).** Release of lysosomal contents (e.g., cathepsins, ROS and free iron) that occurs after LMP can induce activation of several cell death pathways, including caspase-dependent and -independent apoptosis, necrosis, ferroptosis and pyroptosis.

As discussed here, lysosomes are involved in the regulation and execution of cell death in response to various LMP-inducing stimuli. Given that mast cell granules are similar to lysosomes, LMP inducers may be capable of inducing granule permeabilization in mast cells leading to cell death. This could potentially be exploited for therapeutic purposes in efforts to alleviate the detrimental impact of mast cells in various pathological settings.

Present investigations

Aims

The general aim of the studies included in this thesis was to explore the possibility of inducing selective mast cell apoptosis via granule permeabilization as a novel strategy to target mast cells. Such a strategy has a potential to be used therapeutically to ameliorate mast cell-mediated detrimental effects in inflammatory diseases, such as asthma.

The specific aims were:

Paper I
To determine whether mefloquine, an FDA-approved antimalarial drug with reported lysosomotropic activity, has the ability to induce granule permeabilization and consequently cell death in mast cells *in vitro* and *in vivo*. Moreover, we sought to determine the mechanism responsible for execution of cell death in response to mefloquine.

Paper II
To evaluate the possibility of using lysosomotropic agents for selectively depleting human lung mast cells by induction of apoptosis.

Paper III
To gain deeper insight into the mechanism underlying ROS production and the downstream events in response to lysosomotropic challenge of mast cells.

Results and discussion

Paper I

Mefloquine, an anti-malaria agent, causes reactive oxygen species-dependent cell death in mast cells via a secretory granule-mediated pathway

Based on the known lysosomotropic nature of mefloquine and the fact that mast cells are exceptionally rich in lysosome-like secretory granules, we hypothesized that mefloquine might be cytotoxic for mast cells by inducing permeabilization of their secretory granules. To test this, the effect of mefloquine on viability of bone marrow-derived mast cells (BMMCs) was assessed in dose- and time-response experiments. The results showed that mefloquine causes cell death of mast cells *in vitro* in a dose-dependent fashion. The cytotoxic effect of mefloquine on mast cells was extended after prolonged incubation. Cytotoxic effects of mefloquine on other types of mast cells (i.e., peritoneal cell-derived mast cells [PCMCs], cord blood-derived mast cells [CBMCs] and the human mast cell line-1 [HMC-1]) and several transformed or primary cell types were next evaluated by comparing their IC_{50} values. Such comparisons revealed that mefloquine has greater cytotoxicity towards mast cells than towards other transformed or primary cells and that PCMCs were markedly more sensitive to mefloquine than other types of mast cells. The latter observation is in line with the notion that mefloquine-induced cell death might occur via a granule-mediated pathway because PCMCs have characteristic features of mature mast cells and contain a higher density of granules in comparison to other mast cell types tested[202, 229, 230].

To further corroborate that mefloquine causes permeabilization of mast cell granules, granule integrity and possible release of granule-contained proteases into the cytosol was assessed. Incubation of mast cells with mefloquine resulted in rapid loss of signals from acridine orange and LysoTracker, which in normal conditions accumulate selectively within intact acidic organelles. This was further accompanied by detection of granular cysteine cathepsin enzymatic activity and presence of granular tryptase (mMCP6) and CPA3 in the cytosol, strongly indicating a granule-permeabilizing activity for mefloquine. Importantly, the loss of granule integrity and release of granular proteases into the cytosol was observed at earlier time points than when profound loss of viability was seen. This finding is clearly in favor of the notion that granule permeabilization is an important step in the mechanism of mefloquine-induced cell death rather than granule permeabilization being a secondary effect of cell death.

Having identified a cytotoxic effect of mefloquine on mast cells, we next sought to determine the type of cell death triggered by mefloquine and the

mechanism of action. Flow cytometric analysis, which enables distinguishing apoptotic and necrotic cell death, showed that mefloquine induces predominantly apoptotic cell death in wild type (WT) mast cells. This was an important finding with regard to a potential future clinical use of the drug, since apoptotic cell death occurs without causing inflammation while necrotic cell death is accompanied with extensive release of proinflammatory compounds[174]. Interestingly, mast cells lacking serglycin or tryptase died to a greater extent by necrosis. This observation is in line with previous findings indicating that the serglycin-tryptase axis plays a key role in regulating apoptotic versus necrotic cell death in response to various lysosomotropic agents[201, 202, 231].

We next sought to determine the mechanism responsible for execution of cell death in response to mefloquine. Since mefloquine-treated mast cells died by apoptosis, we reasoned that caspase activation might be responsible for mefloquine-induced cell death. However, pharmacological caspase inhibition failed to rescue mast cells from cell death, indicating that caspases do not have a major contribution to cell death execution. As an alternative to a caspase-dependent mechanism, we hypothesized that granule proteases, e.g., cysteine cathepsins, aspartic acid proteases and serine proteases, may play a role in mefloquine-induced cell death. The rationale behind this was that cytosolic translocation of granule proteases in mefloquine-treated mast cells could potentially contribute to certain types of cell death[195, 232]. However, inhibition of these groups of proteases using their respective inhibitors did not restore cell viability in response to mefloquine, suggesting that granule-localized proteases are not accountable for execution of cell death. Interestingly, although serine protease inhibition did not prevent cell death in response to mefloquine, it altered the mode of cell death dramatically from apoptosis to necrosis. This finding is in line with our earlier observation and reinforces the notion that tryptase, which belongs to the serine protease family, plays a key role in promoting apoptotic versus necrotic cell death. In an effort to determine the actual mechanism of cell death execution in response to mefloquine, the contribution of oxidative stress was evaluated, based on the known involvement of reactive oxygen species (ROS) generation in apoptosis[233, 234]. Measurement of intracellular ROS levels revealed increased ROS production in mefloquine-treated mast cells. Pretreatment of mast cells with N-acetylcysteine (NAC), a hydrophilic ROS scavenger, resulted in complete blockade of ROS elevation and cell death, whereas pretreatment with α-tocopherol, a lipophilic ROS scavenger, did not rescue mast cells from mefloquine-induced cell death.

Lastly, the cytotoxic effect of mefloquine on mast cells was assessed *in vivo* by injection of mefloquine into the peritoneal cavity of mice. Mice that received mefloquine at a daily dose of 10 or 30 mg/kg for four consecutive days exhibited a significant reduction in the number of peritoneal mast cells compared to control mice. In addition to mast cell reduction, a moderate reduction

in the lymphocyte population was observed, although, only when a higher dose of mefloquine had been administered. In contrast, no significant cytotoxic effect on peritoneal macrophages or neutrophils was seen.

In summary, this paper identifies mefloquine as a novel anti-mast cell agent with high efficiency both *in vitro* and *in vivo*. Mefloquine was found to induce mast cell apoptosis through granule permeabilization and induction of oxidative stress.

Paper II

Induction of human lung mast cell apoptosis by granule permeabilization: a novel approach for targeting mast cells

To assess the effect of lysosomotropic agents on human lung cells, specimens were obtained from the non-tumor parts of lung tissue dissected from patients undergoing surgery for lung cancer. Incubation of human lung specimens with either mefloquine or siramesine resulted in a substantial reduction in the number of tryptase$^+$ mast cells in the lung tissue, indicating that these lysosomotropic compounds have cytotoxic effects on human lung mast cells *in situ*. Notably, no sign of tissue destruction was seen in mefloquine- or siramesine-treated samples. To determine the mechanism underlying the mast cell reduction, TUNEL-tryptase double staining was performed on cross sections of the lung biopsies to detect apoptotic mast cells. Treatment of lung tissue biopsies with mefloquine caused a marked decrease in the number of viable mast cells (tryptase$^+$/TUNEL$^-$) and at the same time a significant increase in the number of apoptotic mast cells (tryptase$^+$/TUNEL$^+$). This is in line with findings from **Paper I** and our earlier reports showing that lysosomotropic compounds induce apoptotic cell death in mast cells[201, 202, 231, 235]. As an alternative strategy to verify the apoptotic nature of mefloquine-induced cell death, a flow cytometry-based approach that enables distinction between apoptotic and necrotic cell death was used. Treatment of freshly isolated c-kit$^+$ lung cells, which constituted an enriched population of mature mast cells, with mefloquine predominantly resulted in appearance of apoptotic cells (Annexin V$^+$/DRAQ7$^-$). Similarly, when mixed lung cells were incubated with mefloquine after being extracted from human lung tissue, Lin$^-$ c-kit$^+$ FcεRI$^+$ mast cells were found to undergo apoptosis (Annexin V$^+$/DRAQ7$^-$).

To determine whether mefloquine shows selectivity for mast cells, the extent of mefloquine cytotoxicity on various non-mast cell populations present in the human lung was assessed by flow cytometry. Mefloquine, at concentrations that were cytotoxic for mast cells, did not induce cell death in T/B lymphocytes (CD4$^+$ CD8$^+$ CD19$^+$ cells), monocytes/macrophages (CD14$^+$), epithelial cells (CD45$^-$ CD326$^+$) or primary human airway smooth muscle cells. In

accordance with these findings, microscopic analysis of lung tissue sections that were double stained for tryptase and with TUNEL revealed that non-mast cells were minimally affected by the mefloquine treatment, suggesting that mefloquine shows selectivity for mast cells.

Based on the central role of ROS in mefloquine-induced cell death in mouse cultured mast cells, i.e., BMMCs, we explored the impact of oxidative stress on cell death in human lung mast cells exposed to lysosomotropic challenge. Incubation of these cells with mefloquine enhanced the cellular ROS levels in lung mast cells (c-kit$^+$ lung cells). Furthermore, preincubation of the cells with NAC inhibited the apoptotic cell death of mast cells (Lin$^-$ c-kit$^+$ FcεRI$^+$ cells) in response to mefloquine. Altogether, these results indicate that ROS plays an essential role in the pathway leading to cell death, in both mouse and human mast cells, upon exposure to lysosomotropic compounds.

Lastly, we sought to determine whether induction of mast cell death by lysosomotropic challenge has the potential to suppress the levels of mast cell-expressed pathogenic cytokines, such as VEGF and IL-6. As measured by ELISA, the incubation of human lung tissues with either mefloquine or siramesine significantly reduced the levels of VEGF and IL-6 in supernatants recovered from lung tissue specimens.

In summary, in **Paper II** we extended the studies performed *in vitro* and *in vivo* on mouse mast cells, to explore the possibility of using lysosomotropic agents to interfere with mast cells in a clinically relevant setting. Our results indicate that lysosomotropic agents efficiently and selectively induce apoptosis of human lung mast cells. Moreover, we demonstrate that apoptosis induced by lysosomotropic agents is dependent on the production of ROS, and that the treatment of lung tissue with such compounds causes a decrease in the release of mast cell cytokines that play pathogenic roles in the context of inflammatory lung diseases. Hence, we believe that lysosomotropic agents could be explored as therapeutics for lung diseases in which mast cells contribute to the pathogenesis, e.g., asthma.

Paper III

Lysosomotropic challenge of mast cells causes intra-granular reactive oxygen species production

In **Paper I** we found that the Nonyl-acridine orange (a marker for mitochondrial integrity) fluorescence signal was not decreased upon incubation of mast cells with mefloquine. Moreover, α-tocopherol, an antioxidant that protects mitochondrial membranes from oxidative stress-induced damage, did not rescue ROS-mediated mast cell death in response to mefloquine. Altogether,

these data suggest that it is unlikely that mitochondrial ROS plays a major role, if any, in mast cell apoptosis upon lysosomotropic challenge. Based on these findings and the fact that lysosomotropic agents were shown to efficiently target the secretory granules of mast cells, we hypothesized that the ROS production in response to lysosomotropic challenge could occur within the secretory granules. To investigate this hypothesis, mast cells were incubated with LysoTracker and CellROX to allow visualization of granules and monitoring of the ROS production before and after mefloquine challenge using live confocal imaging. Prior to mefloquine treatment of mast cells, granules were clearly visible and intact, and only low levels of intracellular ROS were detected. After addition of mefloquine, a rapid elevation of ROS production accompanied by a marked reduction in the LysoTracker signal was seen, the latter indicating a damage to the mast cell granules. To determine if the ROS generated in mefloquine-treated mast cells arose from mast cell granules, the colocalization of LysoTracker and CellROX was monitored. At the time of mefloquine addition, a low degree of LysoTracker/CellROX colocalization was detected. However, LysoTracker/CellROX colocalization was significantly enhanced over time, hence strongly implicating mast cell granules as key subcellular sites for ROS production in response to mefloquine. The central role of mast cell secretory granules in the ROS production in response to lysosomotropic agents was further supported by two additional findings. Firstly, ROS production in response to mefloquine was markedly attenuated in mast cells lacking serglycin, a secretory granule-restricted proteoglycan. Secondly, ROS production in response to mefloquine was completely abolished when granule acidification was disrupted by bafilomycin-A1 (BAF), a V-ATPase inhibitor.

Having found that serglycin-deficient ($SG^{-/-}$) mast cells produce less ROS in response to mefloquine, we sought to determine whether the absence of serglycin affects the course of mefloquine-induced cell death. Live confocal imaging revealed that both WT and $SG^{-/-}$ mast cells start to express cell death markers (Annexin V and DRAQ7) after incubation with mefloquine; however, Annexin V/DRAQ7 positivity appeared with a delay in $SG^{-/-}$ mast cells compared to their WT counterparts. Similarly, when cell death was assessed in response to mefloquine by flow cytometry, a higher proportion of viable mast cells were found in cultures of $SG^{-/-}$ mast cells than their WT counterparts at any given time point tested. Altogether, these data indicate that the absence of serglycin causes a delay in mast cell death induced by lysosomotropic challenge.

Given that ROS production in response to lysosomotropic challenge of mast cells was found to take place within granules, the role of ROS in inducing granule membrane damage and the potential impact of granule acidity on this process was explored. In accordance with its previously shown granule-

permeabilizing effect, mefloquine caused a dramatic drop in granule acidification and a substantial increase in the level of granular tryptase (mMCP6) present in the cytosol. Of note, preincubation of mast cells with the potent ROS-inhibitor NAC did not prevent the drop in granule acidity and had a partial inhibiting effect on the release of granular mMCP6 to the cytosolic compartment in response to mefloquine. More strikingly, inhibition of granule acidification by BAF profoundly blocked the translocation of mMCP6 into the cytosol. The inhibitory effect of BAF was not limited to inhibition of granule permeabilization. In fact, pretreatment of mast cells with BAF completely abolished the mefloquine-induced ROS production and also prevented cell death. Collectively, these data show that granule acidification is essential for mediating granule permeabilization, ROS production and cell death in response to lysosomotropic challenge.

To identify the origin(s) of ROS generated in mast cells upon lysosomotropic challenge, lysosomal iron and NADPH oxidase activity were investigated as potential candidate sources of ROS in the present context. Pretreatment of mast cells with an iron chelator (DFO), but not with a NADPH oxidase inhibitor (apocynin), significantly blocked ROS generation in mefloquine-challenged mast cells. This suggests that lysosomal iron is a major source of ROS in mast cells exposed to mefloquine. This finding, and the fact that $SG^{-/-}$ mast cells produced less ROS than WT mast cells in response to mefloquine, prompted us to investigate the possibility of serglycin being involved in the storage of redox active metal ions. In this context, measurement of cellular concentrations of Fe, Cu, Mg and Zn in WT and $SG^{-/-}$ mast cells revealed that a significantly lower concentration of Fe is present in $SG^{-/-}$ mast cells. This observation is in favor of the notion that serglycin may be involved in storage of iron in mast cell granules, likely through electrostatic interactions of negatively charged serglycin proteoglycans with iron cations as previously proposed[236].

To further delineate the downstream signaling events involved in mefloquine-induced oxidative stress, ROS production in mast cells treated with a panel of inhibitors of candidate pathways was evaluated. Preincubation of mast cells with an inhibitor of MEK1/2 (U0126), but not inhibitors of either tryptase (Nafamostat), NFκB, Akt (MK2206), AP-1 (SR11302) or P38 MAP kinase (SB203580), significantly blocked mefloquine-induced ROS production. These data show that the ERK1/2 MAP kinase signaling pathway contributes to ROS production.

Proteomic analysis of WT and $SG^{-/-}$ mast cells before and after treatment with mefloquine revealed that the levels of granzyme B in naïve cells are significantly higher in WT mast cells compared to their $SG^{-/-}$ counterparts. Moreover, upon exposure to mefloquine, the level of granzyme B was downregulated

in WT mast cells, but was unaffected in $SG^{-/-}$ cells. Based on this data and the known pro-apoptotic function of granzyme B[237] we evaluated the potential impact of granzyme B on ROS production in response to lysosomotropic challenge using a specific granzyme B inhibitor. Intriguingly, preincubation of mast cells with the granzyme B inhibitor blocked ROS production under these conditions, demonstrating that granzyme B has a key role in the ROS production following lysosomotropic challenge of mast cells.

Collectively, **Paper III** presents mast cell secretory granules as major sites for ROS production, identifies lysosomal iron as the main source for ROS production and demonstrates a role of ERK1/2 MAP kinase signaling pathway and granzyme B in ROS production in response to lysosomotropic challenge. Importantly, we found that granule acidification plays a central role in ROS generation and in the pro-apoptotic response triggered downstream of secretory granule permeabilization.

Concluding remarks and future perspectives

Mast cells are currently gaining increased attention for their detrimental roles in orchestrating the inflammatory responses in numerous pathological conditions. Therefore, mast cells are emerging as major targets for therapeutic intervention in mast cell-associated diseases, including asthma. The therapeutic options that are available today to counteract the harmful activities of mast cells have certain limitations[65, 107, 116]. For example, they generally target only a limited fraction of all of those mediators that mast cells secrete upon activation, or they are effective mainly when IgE-mediated pathways are the major cause of mast cell activation in a disease setting. However, the total impact of mast cells on any pathological setting most likely represents the sum of the effects of all the individual mediators that are released by mast cells, not only via IgE-mediated pathways, but also via numerous other IgE-independet pathways[3, 5]. Therefore, to be able to effectively counteract mast cell-related activities, the simultaneous blockade of an extensive panel of mast cell-derived mediators, regardless of the pathways mediating the mast cell activation, can be difficult to achieve[107, 116]. A conceivably more efficient strategy to accomplish a full blockade of the harmful events mediated by various mast cell mediators could, therefore, be to locally eliminate harmful mast cell populations altogether by inducing cell death (discussed in [155]). However, induction of mast cell death should preferably be achieved by inducing apoptosis rather than necrosis to avoid the adverse inflammatory effects that accompany necrotic cell death. Furthermore, in order to avoid undesired side effects, it is essential to develop a selective strategy that causes minimal apoptosis in cells other than mast cells.

In this thesis we evaluated a novel concept for selective induction of apoptosis in mast cells through a granule-mediated pathway. More specifically, we demonstrate that mefloquine, an approved antimalarial drug with previously proposed lysosomotropic properties, causes secretory granule permeabilization and induces apoptosis of mast cells through induction of oxidative stress. *Figure 5* illustrates our current understanding of the mechanisms involved in ROS production and cell death induced in mast cells exposed to mefloquine.

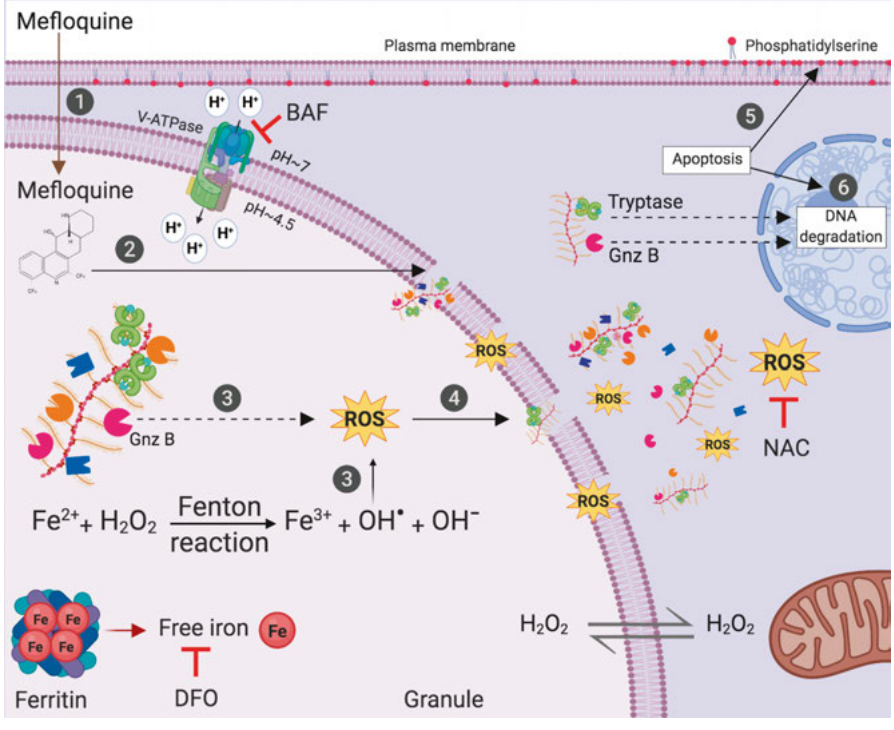

Figure 5. **Overview of a suggested mechanism by which mefloquine induces granule membrane permeabilization and apoptosis in mast cells. (1) Accumulation of mefloquine inside the granules**. Mefloquine, a weakly basic compound, can in its unprotonated form passively diffuse through the mast cell plasma and granule membranes. In the acidic interior of granules, mefloquine becomes protonated and can no longer pass through the membrane, thus accumulating inside the granules. **(2) Granule membrane permeabilization**. When the mefloquine concentration reaches a certain threshold, mefloquine acquires detergent-like properties and induces granule membrane damage and permeabilization. **(3) Induction of oxidative stress within granules**. Hydrogen peroxide (H_2O_2) freely diffuses into the granules. In the granules, the acidic pH and the presence of free iron, possibly caused by the degradation of iron-containing proteins (e.g., ferritin), promote the oxidation of iron and the generation of ROS molecules such as hydroxyl radicals ($HO^{\bullet}$) via Fenton-type reactions. The electrostatic interaction between negatively charged serglycin and cationic iron likely participates in maintaining the iron pool within mast cell granules, thus contributing to the generation of ROS. The generated ROS cause further destabilization of granule membranes leading to the release of many granule components into the cytosol. Granzyme B also participates in induction of ROS production upon exposure to mefloquine. **(4) Release of granule contents into the cytosol**. Due to the granule permeabilization, granule contents (e.g., fully active proteases in complex with serglycin, ROS and iron) enter the cytosol. **(5)** and **(6) Induction of apoptosis**. Mefloquine-induced granule permeabilization and the subsequent translocation of the granule contents to the cytosol induce apoptosis manifested by phosphatidylserine externalization and DNA degradation. The mast cell proteases, such as tryptase and granzyme B, may participate in apoptosis in response to mefloquine. Abbreviations: BAF, bafilomycin-A1; DFO, deferoxamine mesylate; Gnz B, granzyme B; NAC, N-acetylcysteine; ROS, reactive oxygen species.

Although our observations reveal limited mefloquine cytotoxicity toward cell types other than mast cells, we anticipate that this approach can be further optimized in order to introduce an additional level of selectivity toward mast cells. This can be achieved, for example, by developing a liposome-based system for targeted delivery of lysosomotropic agents to mast cells. For this purpose, mefloquine, as a candidate lysosomotropic agent, can be packed within nanosized lipid particles, e.g., liposomes, followed by conjugation of liposomes with the c-kit ligand, i.e., SCF (*Figure 6*). Of note, c-kit expression is largely restricted to mast cells among mature cell populations present in most tissues. Furthermore, when other c-kit-expressing cell types are present in a given tissue, their abundance and surface level of c-kit are often considerably lower than those of mast cells. In line with this notion, a recent study demonstrated that in healthy individuals and patients with COPD, mast cells constitute the majority of c-kit$^+$ cells in the lung, and that the expression level of c-kit is distinctly higher in mast cells in comparison to other less abundant c-kit$^+$ cells found in the lung[238]. Based on the facts described above, targeting through the SCF–c-kit axis has the potential to provide additional selectivity for mast cells.

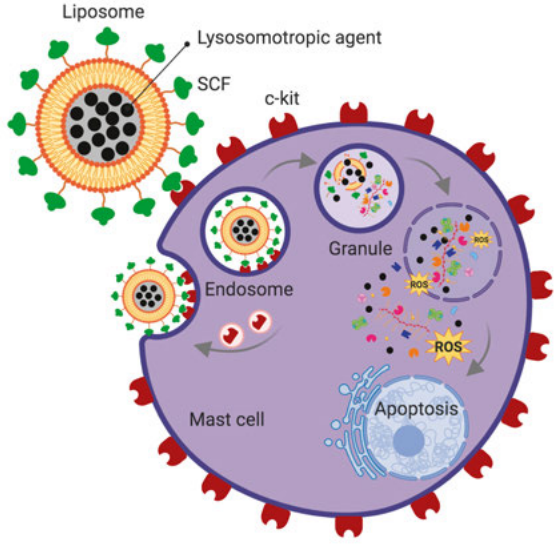

Figure 6. **A potential strategy for targeted delivery of lysosomotropic agents to mast cells**. SCF-conjugated liposomes that are loaded with a candidate lysosomotropic agent (e.g., mefloquine) can be internalized by mast cells through endocytosis. Once inside the mast cells, the lysosomotropic agent will be released (by the action of lysosomal enzymes) and accumulated within the granules. As a result, granule permeabilization will be induced leading to mast cell apoptosis.

Interestingly, with recent advances in the field of nanotechnology and nanomedicine, it has become possible to significantly enhance the selectivity of liposomes for their target cells by employing a dual-receptor targeted approach[239]. Such an approach minimizes non-selective targeting and toxicity because it provides an enhanced cellular uptake of liposomes by cells expressing both target receptors whilst it offers minimal uptake by cells that do not simultaneously express both receptors[239]. In this regard, it is conceivable that development of a dual-receptor targeted liposomes, for example using ligands to c-kit and FcεRI (non-cross-linking), may provide a highly selective approach for delivery of lysosomotropic agents to mast cells.

The findings presented in this thesis introduce the possibility of using lysosomotropic agents to ameliorate mast cell-mediated detrimental effects in the context of asthma. To provide proof-of-principle for this notion, it would be interesting to further evaluate the effect of mefloquine on various asthma features such as AHR, eosinophilic inflammation, increased mucus production and tissue remodeling in a relevant animal model of asthma. For this purpose, rodents such as mice or guinea pigs can be employed as models. Mice are widely used for modelling respiratory inflammatory diseases due to their advantages including relatively low associated costs, well-characterized immune system, as well as availability of reagents and various genetically-modified animals[240]. Despite these advantages, most studies that have demonstrated promising therapeutic outcomes in mouse asthma models have not successfully transferred well to the clinic or to the treatment of asthmatics[241]. This might be explained, at least partly, by physiological and anatomical differences between mouse and human airways and lungs[241]. By contrast, guinea pigs have several key similarities to humans with regard to the airways and lungs, which make them useful in asthma research. These similarities are mainly related to the quantity and localization of mast cells in the airways and lungs, the repertoire of mediators released by mast cells, the airway anatomy and branching, the anatomy and function of airway smooth muscle (i.e., its response to contractile and relaxant agonists) and the presence of early- and late-phase asthmatic airway reactions to allergen, which are attributed largely to the actions of histamine and cysteinyl leukotrienes through their respective receptors[240-244]. These similarities suggest that, compared to mouse, guinea pig might represent a more clinically relevant animal model, to assess the potential anti-mast cell therapeutic effect of mefloquine in the context of asthma.

It is worth emphasizing that the main lysosomotropic agent evaluated in this thesis, i.e., mefloquine, is approved and safe for use in humans. Hence, if mefloquine is found to have anti-mast cell therapeutic effects in a small animal model of asthma, it can be adapted for clinical use in a near future. For the purpose of treating asthma, we envisage that it is preferable to only deplete mast cells locally in the airways and lungs to avoid potential side effects of

depleting mast cells at other tissue locations. Thus, in order to establish mefloquine for use in humans locally in airways, it is necessary to assure that this drug is safe for administration into the airways, for example by examining its safety in large animals such as pigs. Ultimately, if these experiments show that mefloquine is safe for administration into the airways, this will set the stage for initiation of a small-scale clinical trial in humans.

Separate investigations by our group have revealed that other lysosomotropic agents that are cytotoxic for mouse mast cells[201, 202], namely siramesine and LLME, efficiently reduce the number of human skin mast cells *ex vivo* via induction of selective apoptosis of these cells[245, 246]. Thus, we envision that the concept of using lysosomotropic agents to diminish mast cell-mediated proinflammatory impacts can be extended to broader clinical settings other than inflammatory lung diseases. These include, but are not limited to, inflammatory skin diseases such as atopic dermatitis, cutaneous mastocytosis and psoriasis. Lastly, future attempts to find and evaluate other lysosomotropic agents with mast cell cytotoxic effect might lead to the identification of agents with higher killing efficacy and selectivity toward mast cells. Ideally, this would allow minimizing the potential side effects of the candidate lysosomotropic agent and improve its safety for clinical therapeutic use.

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References

1. Kitamura Y, Shimada M, Hatanaka K, Miyano Y. Development of mast cells from grafted bone marrow cells in irradiated mice. *Nature* 1977; **268**(5619)**:** 442-443.
2. Kitamura Y, Go S, Hatanaka K. Decrease of mast cells in W/Wv mice and their increase by bone marrow transplantation. *Blood* 1978; **52**(2)**:** 447-452.
3. da Silva EZ, Jamur MC, Oliver C. Mast cell function: a new vision of an old cell. *J Histochem Cytochem* 2014; **62**(10)**:** 698-738.
4. Gurish MF, Austen KF. Developmental origin and functional specialization of mast cell subsets. *Immunity* 2012; **37**(1)**:** 25-33.
5. Reber LL, Sibilano R, Mukai K, Galli SJ. Potential effector and immunoregulatory functions of mast cells in mucosal immunity. *Mucosal Immunol* 2015; **8**(3)**:** 444-463.
6. Wernersson S, Pejler G. Mast cell secretory granules: armed for battle. *Nat Rev Immunol* 2014; **14**(7)**:** 478-494.
7. Mukai K, Tsai M, Saito H, Galli SJ. Mast cells as sources of cytokines, chemokines, and growth factors. *Immunol Rev* 2018; **282**(1)**:** 121-150.
8. Stevens RL, Fox CC, Lichtenstein LM, Austen KF. Identification of chondroitin sulfate E proteoglycans and heparin proteoglycans in the secretory granules of human lung mast cells. *Proc Natl Acad Sci U S A* 1988; **85**(7)**:** 2284-2287.
9. Mendez-Enriquez E, Hallgren J. Mast Cells and Their Progenitors in Allergic Asthma. *Front Immunol* 2019; **10:** 821.
10. Irani AA, Schechter NM, Craig SS, DeBlois G, Schwartz LB. Two types of human mast cells that have distinct neutral protease compositions. *Proc Natl Acad Sci U S A* 1986; **83**(12)**:** 4464-4468.
11. Grujic M, Braga T, Lukinius A, Eloranta ML, Knight SD, Pejler G *et al.* Serglycin-deficient cytotoxic T lymphocytes display defective secretory granule maturation and granzyme B storage. *J Biol Chem* 2005; **280**(39)**:** 33411-33418.
12. Gri G, Frossi B, D'Inca F, Danelli L, Betto E, Mion F *et al.* Mast cell: an emerging partner in immune interaction. *Front Immunol* 2012; **3:** 120.
13. Beghdadi W, Madjene LC, Benhamou M, Charles N, Gautier G, Launay P *et al.* Mast cells as cellular sensors in inflammation and immunity. *Front Immunol* 2011; **2:** 37.
14. Juremalm M, Nilsson G. Chemokine receptor expression by mast cells. *Chem Immunol Allergy* 2005; **87:** 130-144.
15. Lu L, Kulka M, Unsworth LD. Peptide-mediated mast cell activation: ligand similarities for receptor recognition and protease-induced regulation. *J Leukoc Biol* 2017; **102**(2)**:** 237-251.
16. Okayama Y, Hagaman DD, Woolhiser M, Metcalfe DD. Further characterization of FcgammaRII and FcgammaRIII expression by cultured human mast cells. *Int Arch Allergy Immunol* 2001; **124**(1-3)**:** 155-157.

17. Katz HR. Inhibitory receptors and allergy. *Curr Opin Immunol* 2002; **14**(6)**:** 698-704.
18. Yokoi H, Myers A, Matsumoto K, Crocker PR, Saito H, Bochner BS. Alteration and acquisition of Siglecs during in vitro maturation of CD34+ progenitors into human mast cells. *Allergy* 2006; **61**(6)**:** 769-776.
19. Yokoi H, Choi OH, Hubbard W, Lee HS, Canning BJ, Lee HH *et al.* Inhibition of FcepsilonRI-dependent mediator release and calcium flux from human mast cells by sialic acid-binding immunoglobulin-like lectin 8 engagement. *J Allergy Clin Immunol* 2008; **121**(2)**:** 499-505 e491.
20. Bulfone-Paus S, Nilsson G, Draber P, Blank U, Levi-Schaffer F. Positive and Negative Signals in Mast Cell Activation. *Trends Immunol* 2017; **38**(9)**:** 657-667.
21. Yang Y, Lu JY, Wu X, Summer S, Whoriskey J, Saris C *et al.* G-protein-coupled receptor 35 is a target of the asthma drugs cromolyn disodium and nedocromil sodium. *Pharmacology* 2010; **86**(1)**:** 1-5.
22. Abraham SN, St John AL. Mast cell-orchestrated immunity to pathogens. *Nat Rev Immunol* 2010; **10**(6)**:** 440-452.
23. Urb M, Sheppard DC. The role of mast cells in the defence against pathogens. *PLoS Pathog* 2012; **8**(4)**:** e1002619.
24. Frossi B, De Carli M, Pucillo C. The mast cell: an antenna of the microenvironment that directs the immune response. *J Leukoc Biol* 2004; **75**(4)**:** 579-585.
25. Supajatura V, Ushio H, Nakao A, Akira S, Okumura K, Ra C *et al.* Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J Clin Invest* 2002; **109**(10)**:** 1351-1359.
26. Allakhverdi Z, Smith DE, Comeau MR, Delespesse G. Cutting edge: The ST2 ligand IL-33 potently activates and drives maturation of human mast cells. *J Immunol* 2007; **179**(4)**:** 2051-2054.
27. Allakhverdi Z, Comeau MR, Jessup HK, Yoon BR, Brewer A, Chartier S *et al.* Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. *J Exp Med* 2007; **204**(2)**:** 253-258.
28. Iikura M, Suto H, Kajiwara N, Oboki K, Ohno T, Okayama Y *et al.* IL-33 can promote survival, adhesion and cytokine production in human mast cells. *Lab Invest* 2007; **87**(10)**:** 971-978.
29. Sandig H, Bulfone-Paus S. TLR signaling in mast cells: common and unique features. *Front Immunol* 2012; **3:** 185.
30. Agier J, Pastwinska J, Brzezinska-Blaszczyk E. An overview of mast cell pattern recognition receptors. *Inflamm Res* 2018; **67**(9)**:** 737-746.
31. Migalovich-Sheikhet H, Friedman S, Mankuta D, Levi-Schaffer F. Novel identified receptors on mast cells. *Front Immunol* 2012; **3:** 238.
32. Karra L, Levi-Schaffer F. Down-regulation of mast cell responses through ITIM containing inhibitory receptors. *Adv Exp Med Biol* 2011; **716:** 143-159.
33. Kraft S, Kinet JP. New developments in FcepsilonRI regulation, function and inhibition. *Nat Rev Immunol* 2007; **7**(5)**:** 365-378.
34. Reber LL, Hernandez JD, Galli SJ. The pathophysiology of anaphylaxis. *J Allergy Clin Immunol* 2017; **140**(2)**:** 335-348.
35. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012; **18**(5)**:** 693-704.
36. Mukai K, Tsai M, Starkl P, Marichal T, Galli SJ. IgE and mast cells in host defense against parasites and venoms. *Semin Immunopathol* 2016; **38**(5)**:** 581-603.

37. Varadaradjalou S, Feger F, Thieblemont N, Hamouda NB, Pleau JM, Dy M *et al.* Toll-like receptor 2 (TLR2) and TLR4 differentially activate human mast cells. *Eur J Immunol* 2003; **33**(4)**:** 899-906.
38. Barbosa-Lorenzi VC, Peyda S, Scheynius A, Nilsson G, Lunderius-Andersson C. Curdlan induces selective mast cell degranulation without concomitant release of LTC4, IL-6 or CCL2. *Immunobiology* 2017; **222**(4)**:** 647-650.
39. Saluja R, Delin I, Nilsson GP, Adner M. FcepsilonR1-mediated mast cell reactivity is amplified through prolonged Toll-like receptor-ligand treatment. *PLoS One* 2012; **7**(8)**:** e43547.
40. Robas N, Mead E, Fidock M. MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion. *J Biol Chem* 2003; **278**(45)**:** 44400-44404.
41. Motakis E, Guhl S, Ishizu Y, Itoh M, Kawaji H, de Hoon M *et al.* Redefinition of the human mast cell transcriptome by deep-CAGE sequencing. *Blood* 2014; **123**(17)**:** e58-67.
42. Tatemoto K, Nozaki Y, Tsuda R, Konno S, Tomura K, Furuno M *et al.* Immunoglobulin E-independent activation of mast cell is mediated by Mrg receptors. *Biochem Biophys Res Commun* 2006; **349**(4)**:** 1322-1328.
43. McNeil BD, Pundir P, Meeker S, Han L, Undem BJ, Kulka M *et al.* Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature* 2015; **519**(7542)**:** 237-241.
44. Kashem SW, Subramanian H, Collington SJ, Magotti P, Lambris JD, Ali H. G protein coupled receptor specificity for C3a and compound 48/80-induced degranulation in human mast cells: roles of Mas-related genes MrgX1 and MrgX2. *Eur J Pharmacol* 2011; **668**(1-2)**:** 299-304.
45. Gaudenzio N, Sibilano R, Marichal T, Starkl P, Reber LL, Cenac N *et al.* Different activation signals induce distinct mast cell degranulation strategies. *J Clin Invest* 2016; **126**(10)**:** 3981-3998.
46. Subramanian H, Gupta K, Guo Q, Price R, Ali H. Mas-related gene X2 (MrgX2) is a novel G protein-coupled receptor for the antimicrobial peptide LL-37 in human mast cells: resistance to receptor phosphorylation, desensitization, and internalization. *J Biol Chem* 2011; **286**(52)**:** 44739-44749.
47. Subramanian H, Gupta K, Lee D, Bayir AK, Ahn H, Ali H. beta-Defensins activate human mast cells via Mas-related gene X2. *J Immunol* 2013; **191**(1)**:** 345-352.
48. Maurer M, Church MK. Inflammatory skin responses induced by icatibant injection are mast cell mediated and attenuated by H(1)-antihistamines. *Exp Dermatol* 2012; **21**(2)**:** 154-155.
49. Bochner BS, Charlesworth EN, Lichtenstein LM, Derse CP, Gillis S, Dinarello CA *et al.* Interleukin-1 is released at sites of human cutaneous allergic reactions. *J Allergy Clin Immunol* 1990; **86**(6 Pt 1)**:** 830-839.
50. Yu Y, Blokhuis BR, Garssen J, Redegeld FA. Non-IgE mediated mast cell activation. *Eur J Pharmacol* 2016; **778:** 33-43.
51. Theoharides TC, Alysandratos KD, Angelidou A, Delivanis DA, Sismanopoulos N, Zhang B *et al.* Mast cells and inflammation. *Biochim Biophys Acta* 2012; **1822**(1)**:** 21-33.
52. Joulia R, L'Faqihi FE, Valitutti S, Espinosa E. IL-33 fine tunes mast cell degranulation and chemokine production at the single-cell level. *J Allergy Clin Immunol* 2017; **140**(2)**:** 497-509 e410.
53. Jackson DJ, Sykes A, Mallia P, Johnston SL. Asthma exacerbations: origin, effect, and prevention. *J Allergy Clin Immunol* 2011; **128**(6)**:** 1165-1174.
54. Graham AC, Temple RM, Obar JJ. Mast cells and influenza a virus: association with allergic responses and beyond. *Front Immunol* 2015; **6:** 238.

55. Edwards MR, Bartlett NW, Hussell T, Openshaw P, Johnston SL. The microbiology of asthma. *Nat Rev Microbiol* 2012; **10**(7)**:** 459-471.
56. Rao KN, Brown MA. Mast cells: multifaceted immune cells with diverse roles in health and disease. *Ann N Y Acad Sci* 2008; **1143:** 83-104.
57. Bradding P, Arthur G. Mast cells in asthma--state of the art. *Clin Exp Allergy* 2016; **46**(2)**:** 194-263.
58. Okayama Y, Kawakami T. Development, migration, and survival of mast cells. *Immunol Res* 2006; **34**(2)**:** 97-115.
59. Akahoshi M, Song CH, Piliponsky AM, Metz M, Guzzetta A, Abrink M *et al.* Mast cell chymase reduces the toxicity of Gila monster venom, scorpion venom, and vasoactive intestinal polypeptide in mice. *J Clin Invest* 2011; **121**(10)**:** 4180-4191.
60. Schneider LA, Schlenner SM, Feyerabend TB, Wunderlin M, Rodewald HR. Molecular mechanism of mast cell mediated innate defense against endothelin and snake venom sarafotoxin. *J Exp Med* 2007; **204**(11)**:** 2629-2639.
61. Metz M, Piliponsky AM, Chen CC, Lammel V, Abrink M, Pejler G *et al.* Mast cells can enhance resistance to snake and honeybee venoms. *Science* 2006; **313**(5786)**:** 526-530.
62. Shimokawa C, Kanaya T, Hachisuka M, Ishiwata K, Hisaeda H, Kurashima Y *et al.* Mast Cells Are Crucial for Induction of Group 2 Innate Lymphoid Cells and Clearance of Helminth Infections. *Immunity* 2017; **46**(5)**:** 863-874 e864.
63. Varricchi G, de Paulis A, Marone G, Galli SJ. Future Needs in Mast Cell Biology. *Int J Mol Sci* 2019; **20**(18).
64. Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008; **454**(7203)**:** 445-454.
65. Reber LL, Frossard N. Targeting mast cells in inflammatory diseases. *Pharmacol Ther* 2014; **142**(3)**:** 416-435.
66. Brown MA, Hatfield JK. Mast Cells are Important Modifiers of Autoimmune Disease: With so Much Evidence, Why is There Still Controversy? *Front Immunol* 2012; **3:** 147.
67. Varricchi G, Galdiero MR, Loffredo S, Marone G, Iannone R, Marone G *et al.* Are Mast Cells MASTers in Cancer? *Front Immunol* 2017; **8:** 424.
68. Cookson H, Grattan C. An update on mast cell disorders. *Clin Med (Lond)* 2016; **16**(6)**:** 580-583.
69. Onnes MC, Tanno LK, Elberink JN. Mast Cell Clonal Disorders: Classification, Diagnosis and Management. *Curr Treat Options Allergy* 2016; **3**(4)**:** 453-464.
70. Andersson CK, Mori M, Bjermer L, Lofdahl CG, Erjefalt JS. Alterations in lung mast cell populations in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010; **181**(3)**:** 206-217.
71. Louis RE, Cataldo D, Buckley MG, Sele J, Henket M, Lau LC *et al.* Evidence of mast-cell activation in a subset of patients with eosinophilic chronic obstructive pulmonary disease. *Eur Respir J* 2002; **20**(2)**:** 325-331.
72. Bot I, Shi GP, Kovanen PT. Mast cells as effectors in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2015; **35**(2)**:** 265-271.
73. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002; **346**(22)**:** 1699-1705.
74. Dougherty RH, Sidhu SS, Raman K, Solon M, Solberg OD, Caughey GH *et al.* Accumulation of intraepithelial mast cells with a unique protease phenotype in T(H)2-high asthma. *J Allergy Clin Immunol* 2010; **125**(5)**:** 1046-1053 e1048.

75. Andersson CK, Bergqvist A, Mori M, Mauad T, Bjermer L, Erjefalt JS. Mast cell-associated alveolar inflammation in patients with atopic uncontrolled asthma. *J Allergy Clin Immunol* 2011; **127**(4)**:** 905-912 e901-907.
76. Carroll NG, Mutavdzic S, James AL. Distribution and degranulation of airway mast cells in normal and asthmatic subjects. *Eur Respir J* 2002; **19**(5)**:** 879-885.
77. Amin K, Ludviksdottir D, Janson C, Nettelbladt O, Bjornsson E, Roomans GM *et al.* Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. BHR Group. *Am J Respir Crit Care Med* 2000; **162**(6)**:** 2295-2301.
78. Balzar S, Chu HW, Strand M, Wenzel S. Relationship of small airway chymase-positive mast cells and lung function in severe asthma. *Am J Respir Crit Care Med* 2005; **171**(5)**:** 431-439.
79. Carroll NG, Mutavdzic S, James AL. Increased mast cells and neutrophils in submucosal mucous glands and mucus plugging in patients with asthma. *Thorax* 2002; **57**(8)**:** 677-682.
80. Yu M, Tsai M, Tam SY, Jones C, Zehnder J, Galli SJ. Mast cells can promote the development of multiple features of chronic asthma in mice. *J Clin Invest* 2006; **116**(6)**:** 1633-1641.
81. Williams CM, Galli SJ. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. *J Exp Med* 2000; **192**(3)**:** 455-462.
82. Sawaguchi M, Tanaka S, Nakatani Y, Harada Y, Mukai K, Matsunaga Y *et al.* Role of mast cells and basophils in IgE responses and in allergic airway hyperresponsiveness. *J Immunol* 2012; **188**(4)**:** 1809-1818.
83. Schleimer RP, MacGlashan DW, Jr., Peters SP, Pinckard RN, Adkinson NF, Jr., Lichtenstein LM. Characterization of inflammatory mediator release from purified human lung mast cells. *Am Rev Respir Dis* 1986; **133**(4)**:** 614-617.
84. Casale TB, Wood D, Richerson HB, Zehr B, Zavala D, Hunninghake GW. Direct evidence of a role for mast cells in the pathogenesis of antigen-induced bronchoconstriction. *J Clin Invest* 1987; **80**(5)**:** 1507-1511.
85. Murray JJ, Tonnel AB, Brash AR, Roberts LJ, 2nd, Gosset P, Workman R *et al.* Release of prostaglandin D2 into human airways during acute antigen challenge. *N Engl J Med* 1986; **315**(13)**:** 800-804.
86. Wenzel SE, Fowler AA, 3rd, Schwartz LB. Activation of pulmonary mast cells by bronchoalveolar allergen challenge. In vivo release of histamine and tryptase in atopic subjects with and without asthma. *Am Rev Respir Dis* 1988; **137**(5)**:** 1002-1008.
87. Wenzel SE, Larsen GL, Johnston K, Voelkel NF, Westcott JY. Elevated levels of leukotriene C4 in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. *Am Rev Respir Dis* 1990; **142**(1)**:** 112-119.
88. Wenzel SE, Westcott JY, Larsen GL. Bronchoalveolar lavage fluid mediator levels 5 minutes after allergen challenge in atopic subjects with asthma: relationship to the development of late asthmatic responses. *J Allergy Clin Immunol* 1991; **87**(2)**:** 540-548.
89. Liu MC, Hubbard WC, Proud D, Stealey BA, Galli SJ, Kagey-Sobotka A *et al.* Immediate and late inflammatory responses to ragweed antigen challenge of the peripheral airways in allergic asthmatics. Cellular, mediator, and permeability changes. *Am Rev Respir Dis* 1991; **144**(1)**:** 51-58.

90. Sedgwick JB, Calhoun WJ, Gleich GJ, Kita H, Abrams JS, Schwartz LB *et al.* Immediate and late airway response of allergic rhinitis patients to segmental antigen challenge. Characterization of eosinophil and mast cell mediators. *Am Rev Respir Dis* 1991; **144**(6)**:** 1274-1281.
91. Curzen N, Rafferty P, Holgate ST. Effects of a cyclo-oxygenase inhibitor, flurbiprofen, and an H1 histamine receptor antagonist, terfenadine, alone and in combination on allergen induced immediate bronchoconstriction in man. *Thorax* 1987; **42**(12)**:** 946-952.
92. Rafferty P, Beasley R, Holgate ST. The contribution of histamine to immediate bronchoconstriction provoked by inhaled allergen and adenosine 5' monophosphate in atopic asthma. *Am Rev Respir Dis* 1987; **136**(2)**:** 369-373.
93. Taylor IK, O'Shaughnessy KM, Fuller RW, Dollery CT. Effect of cysteinyl-leukotriene receptor antagonist ICI 204.219 on allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects. *Lancet* 1991; **337**(8743)**:** 690-694.
94. Findlay SR, Barden JM, Easley CB, Glass M. Effect of the oral leukotriene antagonist, ICI 204,219, on antigen-induced bronchoconstriction in subjects with asthma. *J Allergy Clin Immunol* 1992; **89**(5)**:** 1040-1045.
95. Beasley RC, Featherstone RL, Church MK, Rafferty P, Varley JG, Harris A *et al.* Effect of a thromboxane receptor antagonist on PGD2- and allergen-induced bronchoconstriction. *J Appl Physiol (1985)* 1989; **66**(4)**:** 1685-1693.
96. Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J *et al.* The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* 1997; **155**(6)**:** 1828-1834.
97. Flint KC, Leung KB, Hudspith BN, Brostoff J, Pearce FL, Johnson NM. Bronchoalveolar mast cells in extrinsic asthma: a mechanism for the initiation of antigen specific bronchoconstriction. *Br Med J (Clin Res Ed)* 1985; **291**(6500)**:** 923-926.
98. Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. *Am Rev Respir Dis* 1988; **137**(1)**:** 62-69.
99. Casale TB, Wood D, Richerson HB, Trapp S, Metzger WJ, Zavala D *et al.* Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with methacholine bronchial hyperresponsiveness. *J Clin Invest* 1987; **79**(4)**:** 1197-1203.
100. Broide DH, Gleich GJ, Cuomo AJ, Coburn DA, Federman EC, Schwartz LB *et al.* Evidence of ongoing mast cell and eosinophil degranulation in symptomatic asthma airway. *J Allergy Clin Immunol* 1991; **88**(4)**:** 637-648.
101. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R *et al.* Interleukin-4, -5, and -6 and tumor necrosis factor-alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994; **10**(5)**:** 471-480.
102. Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K *et al.* Interleukin 4 is localized to and released by human mast cells. *J Exp Med* 1992; **176**(5)**:** 1381-1386.
103. Shikotra A, Choy DF, Ohri CM, Doran E, Butler C, Hargadon B *et al.* Increased expression of immunoreactive thymic stromal lymphopoietin in patients with severe asthma. *J Allergy Clin Immunol* 2012; **129**(1)**:** 104-111 e101-109.

104. Ying S, Durham SR, Corrigan CJ, Hamid Q, Kay AB. Phenotype of cells expressing mRNA for TH2-type (interleukin 4 and interleukin 5) and TH1-type (interleukin 2 and interferon gamma) cytokines in bronchoalveolar lavage and bronchial biopsies from atopic asthmatic and normal control subjects. *Am J Respir Cell Mol Biol* 1995; **12**(5)**:** 477-487.
105. Ying S, O'Connor B, Ratoff J, Meng Q, Mallett K, Cousins D *et al.* Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J Immunol* 2005; **174**(12)**:** 8183-8190.
106. Okayama Y, Okumura S, Sagara H, Yuki K, Sasaki T, Watanabe N *et al.* FcepsilonRI-mediated thymic stromal lymphopoietin production by interleukin-4-primed human mast cells. *Eur Respir J* 2009; **34**(2)**:** 425-435.
107. Siebenhaar F, Redegeld FA, Bischoff SC, Gibbs BF, Maurer M. Mast Cells as Drivers of Disease and Therapeutic Targets. *Trends Immunol* 2018; **39**(2)**:** 151-162.
108. Takai S, Jin D, Miyazaki M. Targets of chymase inhibitors. *Expert Opin Ther Targets* 2011; **15**(4)**:** 519-527.
109. Maryanoff BE, de Garavilla L, Greco MN, Haertlein BJ, Wells GI, Andrade-Gordon P *et al.* Dual Inhibition of Cathepsin G and Chymase Is Effective in Animal Models of Pulmonary Inflammation. *Am J Resp Crit Care* 2010; **181**(3)**:** 247-253.
110. Sommerhoff CP, Schaschke N. Mast cell tryptase beta as a target in allergic inflammation: An evolving story. *Curr Pharm Design* 2007; **13**(3)**:** 313-332.
111. Krishna MT, Chauhan A, Little L, Sampson K, Hawksworth R, Mant T *et al.* Inhibition of mast cell tryptase by inhaled APC 366 attenuates allergen-induced late-phase airway obstruction in asthma. *J Allergy Clin Immunol* 2001; **107**(6)**:** 1039-1045.
112. Simons FE. Advances in H1-antihistamines. *N Engl J Med* 2004; **351**(21)**:** 2203-2217.
113. Akdis CA, Blaser K. Histamine in the immune regulation of allergic inflammation. *J Allergy Clin Immunol* 2003; **112**(1)**:** 15-22.
114. Polosa R. Critical appraisal of antileukotriene use in asthma management. *Curr Opin Pulm Med* 2007; **13**(1)**:** 24-30.
115. Kao CC, Parulekar AD. Spotlight on fevipiprant and its potential in the treatment of asthma: evidence to date. *J Asthma Allergy* 2019; **12:** 1-5.
116. Harvima IT, Levi-Schaffer F, Draber P, Friedman S, Polakovicova I, Gibbs BF *et al.* Molecular targets on mast cells and basophils for novel therapies. *J Allergy Clin Immunol* 2014; **134**(3)**:** 530-544.
117. Lin AM, Rubin CJ, Khandpur R, Wang JY, Riblett M, Yalavarthi S *et al.* Mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis. *J Immunol* 2011; **187**(1)**:** 490-500.
118. Hueber AJ, Asquith DL, Miller AM, Reilly J, Kerr S, Leipe J *et al.* Mast cells express IL-17A in rheumatoid arthritis synovium. *J Immunol* 2010; **184**(7)**:** 3336-3340.
119. Appel H, Maier R, Wu P, Scheer R, Hempfing A, Kayser R *et al.* Analysis of IL-17(+) cells in facet joints of patients with spondyloarthritis suggests that the innate immune pathway might be of greater relevance than the Th17-mediated adaptive immune response. *Arthritis Res Ther* 2011; **13**(3)**:** R95.
120. Noordenbos T, Yeremenko N, Gofita I, van de Sande M, Tak PP, Canete JD *et al.* Interleukin-17-positive mast cells contribute to synovial inflammation in spondylarthritis. *Arthritis Rheum* 2012; **64**(1)**:** 99-109.

121. Patel DD, Lee DM, Kolbinger F, Antoni C. Effect of IL-17A blockade with secukinumab in autoimmune diseases. *Ann Rheum Dis* 2013; **72 Suppl 2:** ii116-123.
122. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM *et al.* Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998; **282**(5397)**:** 2261-2263.
123. Rankin JA, Picarella DE, Geba GP, Temann UA, Prasad B, DiCosmo B *et al.* Phenotypic and physiologic characterization of transgenic mice expressing interleukin 4 in the lung: lymphocytic and eosinophilic inflammation without airway hyperreactivity. *Proc Natl Acad Sci U S A* 1996; **93**(15)**:** 7821-7825.
124. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL *et al.* Interleukin-13: central mediator of allergic asthma. *Science* 1998; **282**(5397)**:** 2258-2261.
125. Wilson SJ, Shute JK, Holgate ST, Howarth PH, Bradding P. Localization of interleukin (IL) -4 but not IL-5 to human mast cell secretory granules by immunoelectron microscopy. *Clin Exp Allergy* 2000; **30**(4)**:** 493-500.
126. Liu AY, Dwyer DF, Jones TG, Bankova LG, Shen S, Katz HR *et al.* Mast cells recruited to mesenteric lymph nodes during helminth infection remain hypogranular and produce IL-4 and IL-6. *J Immunol* 2013; **190**(4)**:** 1758-1766.
127. Brightling CE, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID, Bradding P. Interleukin-4 and -13 expression is co-localized to mast cells within the airway smooth muscle in asthma. *Clin Exp Allergy* 2003; **33**(12)**:** 1711-1716.
128. Burd PR, Thompson WC, Max EE, Mills FC. Activated mast cells produce interleukin 13. *J Exp Med* 1995; **181**(4)**:** 1373-1380.
129. Lee SA, Fitzgerald SM, Huang SK, Li C, Chi DS, Milhorn DM *et al.* Molecular regulation of interleukin-13 and monocyte chemoattractant protein-1 expression in human mast cells by interleukin-1beta. *Am J Respir Cell Mol Biol* 2004; **31**(3)**:** 283-291.
130. Toru H, Pawankar R, Ra C, Yata J, Nakahata T. Human mast cells produce IL-13 by high-affinity IgE receptor cross-linking: enhanced IL-13 production by IL-4-primed human mast cells. *J Allergy Clin Immunol* 1998; **102**(3)**:** 491-502.
131. Tung HY, Plunkett B, Huang SK, Zhou Y. Murine mast cells secrete and respond to interleukin-33. *J Interferon Cytokine Res* 2014; **34**(3)**:** 141-147.
132. Kaur D, Gomez E, Doe C, Berair R, Woodman L, Saunders R *et al.* IL-33 drives airway hyper-responsiveness through IL-13-mediated mast cell: airway smooth muscle crosstalk. *Allergy* 2015; **70**(5)**:** 556-567.
133. Wenzel S, Castro M, Corren J, Maspero J, Wang L, Zhang B *et al.* Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting beta2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. *Lancet* 2016; **388**(10039)**:** 31-44.
134. Beck LA, Thaci D, Hamilton JD, Graham NM, Bieber T, Rocklin R *et al.* Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. *N Engl J Med* 2014; **371**(2)**:** 130-139.
135. Simpson EL, Bieber T, Guttman-Yassky E, Beck LA, Blauvelt A, Cork MJ *et al.* Two Phase 3 Trials of Dupilumab versus Placebo in Atopic Dermatitis. *N Engl J Med* 2016; **375**(24)**:** 2335-2348.
136. van der Schaft J, Thijs JL, de Bruin-Weller MS, Balak DMW. Dupilumab after the 2017 approval for the treatment of atopic dermatitis: what's new and what's next? *Curr Opin Allergy Clin Immunol* 2019; **19**(4)**:** 341-349.
137. Deeks ED. Dupilumab: A Review in Moderate to Severe Asthma. *Drugs* 2019; **79**(17)**:** 1885-1895.

138. Barnes PJ. New therapies for asthma. *Trends Mol Med* 2006; **12**(11)**:** 515-520.
139. Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. *Lancet* 2018; **391**(10122)**:** 783-800.
140. Busse W, Corren J, Lanier BQ, McAlary M, Fowler-Taylor A, Cioppa GD *et al.* Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *Journal of Allergy & Clinical Immunology* 2001; **108**(2)**:** 184.
141. Milgrom H, Berger W, Nayak A, Gupta N, Pollard S, McAlary M *et al.* Treatment of childhood asthma with anti-immunoglobulin E antibody (omalizumab). *Pediatrics* 2001; **108**(2)**:** E36.
142. Holgate ST, Djukanovic R, Casale T, Bousquet J. Anti-immunoglobulin E treatment with omalizumab in allergic diseases: an update on anti-inflammatory activity and clinical efficacy. *Clin Exp Allergy* 2005; **35**(4)**:** 408-416.
143. Sheinkopf LE, Rafi AW, Do LT, Katz RM, Klaustermeyer WB. Efficacy of omalizumab in the treatment of atopic dermatitis: a pilot study. *Allergy Asthma Proc* 2008; **29**(5)**:** 530-537.
144. Maurer M, Rosen K, Hsieh HJ, Saini S, Grattan C, Gimenez-Arnau A *et al.* Omalizumab for the treatment of chronic idiopathic or spontaneous urticaria. *N Engl J Med* 2013; **368**(10)**:** 924-935.
145. Zhao ZT, Ji CM, Yu WJ, Meng L, Hawro T, Wei JF *et al.* Omalizumab for the treatment of chronic spontaneous urticaria: A meta-analysis of randomized clinical trials. *J Allergy Clin Immunol* 2016; **137**(6)**:** 1742-1750 e1744.
146. Rafi A, Do LT, Katz R, Sheinkopf LE, Simons CW, Klaustermeyer W. Effects of omalizumab in patients with food allergy. *Allergy Asthma Proc* 2010; **31**(1)**:** 76-83.
147. Erjefalt JS. Mast cells in human airways: the culprit? *Eur Respir Rev* 2014; **23**(133)**:** 299-307.
148. Sinniah A, Yazid S, Flower RJ. The Anti-allergic Cromones: Past, Present, and Future. *Front Pharmacol* 2017; **8:** 827.
149. Guevara JP, Ducharme FM, Keren R, Nihtianova S, Zorc J. Inhaled corticosteroids versus sodium cromoglycate in children and adults with asthma. *Cochrane Database Syst Rev* 2006; (2)**:** CD003558.
150. van der Wouden JC, Uijen JH, Bernsen RM, Tasche MJ, de Jongste JC, Ducharme F. Inhaled sodium cromoglycate for asthma in children. *Cochrane Database Syst Rev* 2008; (4)**:** CD002173.
151. Church MK, Hiroi J. Inhibition of IgE-dependent histamine release from human dispersed lung mast cells by anti-allergic drugs and salbutamol. *Br J Pharmacol* 1987; **90**(2)**:** 421-429.
152. Geahlen RL. Getting Syk: spleen tyrosine kinase as a therapeutic target. *Trends Pharmacol Sci* 2014; **35**(8)**:** 414-422.
153. Lee KS, Lee HK, Hayflick JS, Lee YC, Puri KD. Inhibition of phosphoinositide 3-kinase delta attenuates allergic airway inflammation and hyperresponsiveness in murine asthma model. *FASEB J* 2006; **20**(3)**:** 455-465.
154. Randis TM, Puri KD, Zhou H, Diacovo TG. Role of PI3Kdelta and PI3Kgamma in inflammatory arthritis and tissue localization of neutrophils. *Eur J Immunol* 2008; **38**(5)**:** 1215-1224.
155. Karra L, Berent-Maoz B, Ben-Zimra M, Levi-Schaffer F. Are we ready to downregulate mast cells? *Curr Opin Immunol* 2009; **21**(6)**:** 708-714.
156. Ekoff M, Nilsson G. Mast cell apoptosis and survival. *Adv Exp Med Biol* 2011; **716:** 47-60.

157. Linnekin D. Early signaling pathways activated by c-Kit in hematopoietic cells. *Int J Biochem Cell Biol* 1999; **31**(10)**:** 1053-1074.
158. Kitamura Y, Go S. Decreased production of mast cells in S1/S1d anemic mice. *Blood* 1979; **53**(3)**:** 492-497.
159. Grimbaldeston MA, Chen CC, Piliponsky AM, Tsai M, Tam SY, Galli SJ. Mast cell-deficient W-sash c-kit mutant Kit W-sh/W-sh mice as a model for investigating mast cell biology in vivo. *Am J Pathol* 2005; **167**(3)**:** 835-848.
160. Finotto S, Mekori YA, Metcalfe DD. Glucocorticoids decrease tissue mast cell number by reducing the production of the c-kit ligand, stem cell factor, by resident cells: in vitro and in vivo evidence in murine systems. *J Clin Invest* 1997; **99**(7)**:** 1721-1728.
161. Galli SJ, Iemura A, Garlick DS, Gamba-Vitalo C, Zsebo KM, Andrews RG. Reversible expansion of primate mast cell populations in vivo by stem cell factor. *J Clin Invest* 1993; **91**(1)**:** 148-152.
162. Tsai M, Shih LS, Newlands GF, Takeishi T, Langley KE, Zsebo KM *et al.* The rat c-kit ligand, stem cell factor, induces the development of connective tissue-type and mucosal mast cells in vivo. Analysis by anatomical distribution, histochemistry, and protease phenotype. *J Exp Med* 1991; **174**(1)**:** 125-131.
163. Moller C, Alfredsson J, Engstrom M, Wootz H, Xiang Z, Lennartsson J *et al.* Stem cell factor promotes mast cell survival via inactivation of FOXO3a-mediated transcriptional induction and MEK-regulated phosphorylation of the proapoptotic protein Bim. *Blood* 2005; **106**(4)**:** 1330-1336.
164. Vliagoftis H, Worobec AS, Metcalfe DD. The protooncogene c-kit and c-kit ligand in human disease. *J Allergy Clin Immunol* 1997; **100**(4)**:** 435-440.
165. Al-Muhsen SZ, Shablovsky G, Olivenstein R, Mazer B, Hamid Q. The expression of stem cell factor and c-kit receptor in human asthmatic airways. *Clin Exp Allergy* 2004; **34**(6)**:** 911-916.
166. Makowska JS, Cieslak M, Kowalski ML. Stem cell factor and its soluble receptor (c-kit) in serum of asthmatic patients- correlation with disease severity. *BMC Pulm Med* 2009; **9:** 27.
167. Lei Z, Liu G, Huang Q, Lv M, Zu R, Zhang GM *et al.* SCF and IL-31 rather than IL-17 and BAFF are potential indicators in patients with allergic asthma. *Allergy* 2008; **63**(3)**:** 327-332.
168. Kim YK, Nakagawa N, Nakano K, Sulakvelidze I, Dolovich J, Denburg J. Stem cell factor in nasal polyposis and allergic rhinitis: increased expression by structural cells is suppressed by in vivo topical corticosteroids. *J Allergy Clin Immunol* 1997; **100**(3)**:** 389-399.
169. Kanbe T, Soma Y, Kawa Y, Kashima M, Mizoguchi M. Serum levels of soluble stem cell factor and soluble KIT are elevated in patients with atopic dermatitis and correlate with the disease severity. *Br J Dermatol* 2001; **144**(6)**:** 1148-1153.
170. Brown JM, Wilson TM, Metcalfe DD. The mast cell and allergic diseases: role in pathogenesis and implications for therapy. *Clin Exp Allergy* 2008; **38**(1)**:** 4-18.
171. Cahill KN, Katz HR, Cui J, Lai J, Kazani S, Crosby-Thompson A *et al.* KIT Inhibition by Imatinib in Patients with Severe Refractory Asthma. *N Engl J Med* 2017; **376**(20)**:** 1911-1920.
172. Ustun C, DeRemer DL, Akin C. Tyrosine kinase inhibitors in the treatment of systemic mastocytosis. *Leuk Res* 2011; **35**(9)**:** 1143-1152.

173. Alvarez-Twose I, Matito A, Morgado JM, Sanchez-Munoz L, Jara-Acevedo M, Garcia-Montero A *et al.* Imatinib in systemic mastocytosis: a phase IV clinical trial in patients lacking exon 17 KIT mutations and review of the literature. *Oncotarget* 2017; **8**(40)**:** 68950-68963.
174. Edinger AL, Thompson CB. Death by design: apoptosis, necrosis and autophagy. *Curr Opin Cell Biol* 2004; **16**(6)**:** 663-669.
175. Fuchs Y, Steller H. Programmed cell death in animal development and disease. *Cell* 2011; **147**(4)**:** 742-758.
176. Martinon F, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* 2004; **117**(5)**:** 561-574.
177. Hengartner MO. The biochemistry of apoptosis. *Nature* 2000; **407**(6805)**:** 770-776.
178. Zamzami N, Kroemer G. Condensed matter in cell death. *Nature* 1999; **401**(6749)**:** 127-128.
179. Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Bio* 2008; **9**(3)**:** 231-241.
180. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol* 2007; **35**(4)**:** 495-516.
181. Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. *Nat Rev Cancer* 2002; **2**(9)**:** 647-656.
182. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P *et al.* Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ* 2018; **25**(3)**:** 486-541.
183. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: The calcium-apoptosis link. *Nat Rev Mol Cell Bio* 2003; **4**(7)**:** 552-565.
184. Tait SWG, Green DR. Mitochondria and cell death: outer membrane permeabilization and beyond. *Nat Rev Mol Cell Bio* 2010; **11**(9)**:** 621-632.
185. Ashkenazi A, Dixit VM. Death receptors: Signaling and modulation. *Science* 1998; **281**(5381)**:** 1305-1308.
186. von Karstedt S, Montinaro A, Walczak H. Exploring the TRAILs less travelled: TRAIL in cancer biology and therapy. *Nat Rev Cancer* 2017; **17**(6)**:** 352-366.
187. Berent-Maoz B, Piliponsky AM, Daigle I, Simon HU, Levi-Schaffer F. Human mast cells undergo TRAIL-induced apoptosis. *Journal of Immunology* 2006; **176**(4)**:** 2272-2278.
188. Golstein P. Cell death: TRAIL and its receptors. *Curr Biol* 1997; **7**(12)**:** R750-R753.
189. Daniels RA, Turley H, Kimberley FC, Liu XS, Mongkolsapaya J, Ch'en P *et al.* Expression of TRAIL and TRAIL receptors in normal and malignant tissues. *Cell Res* 2005; **15**(6)**:** 430-438.
190. Karlberg M, Ekoff M, Huang DCS, Mustonen P, Harvima IT, Nilsson G. The BH3-Mimetic ABT-737 Induces Mast Cell Apoptosis In Vitro and In Vivo: Potential for Therapeutics. *Journal of Immunology* 2010; **185**(4)**:** 2555-2562.
191. Reinhart R, Rohner L, Wicki S, Fux M, Kaufmann T. BH3 mimetics efficiently induce apoptosis in mouse basophils and mast cells. *Cell Death Differ* 2017.
192. Peter B, Cerny-Reiterer S, Hadzijusufovic E, Schuch K, Stefanzl G, Eisenwort G *et al.* The pan-Bcl-2 blocker obatoclax promotes the expression of Puma, Noxa, and Bim mRNA and induces apoptosis in neoplastic mast cells. *J Leukoc Biol* 2014; **95**(1)**:** 95-104.
193. Stauffer SR. Small molecule inhibition of the Bcl-X-L-BH3 protein-protein interaction: Proof-of-concept of an in vivo chemopotentiator ABT-737. *Curr Top Med Chem* 2007; **7**(10)**:** 961-965.

194. Young KW, Pinon LGP, Dhiraj D, Twiddy D, MacFarlane M, Hickman J *et al.* Mitochondrial fragmentation and neuronal cell death in response to the Bcl-2/Bcl-x(L)/Bcl-w antagonist ABT-737. *Neuropharmacology* 2010; **58**(8)**:** 1258-1267.
195. Boya P, Kroemer G. Lysosomal membrane permeabilization in cell death. *Oncogene* 2008; **27**(50)**:** 6434-6451.
196. Appelqvist H, Johansson AC, Linderoth E, Johansson U, Antonsson B, Steinfeld R *et al.* Lysosome-Mediated Apoptosis is Associated with Cathepsin D-Specific Processing of Bid at Phe24,Trp48, and Phe183. *Ann Clin Lab Sci* 2012; **42**(3)**:** 231-242.
197. Cirman T, Oresic K, Mazovec GD, Turk V, Reed JC, Myers RM *et al.* Selective disruption of lysosomes in HeLa cells triggers apoptosis mediated by cleavage of bid by multiple papain-like lysosomal cathepsins. *Journal of Biological Chemistry* 2004; **279**(5)**:** 3578-3587.
198. Droga-Mazovec G, Bojic L, Petelin A, Ivanova S, Romih R, Repnik U *et al.* Cysteine cathepsins trigger caspase-dependent cell death through cleavage of Bid and antiapoptotic Bcl-2 homologues. *Journal of Biological Chemistry* 2008; **283**(27)**:** 19140-19150.
199. Blott EJ, Griffiths GM. Secretory lysosomes. *Nat Rev Mol Cell Biol* 2002; **3**(2)**:** 122-131.
200. Dragonetti A, Baldassarre M, Castino R, Demoz M, Luini A, Buccione R *et al.* The lysosomal protease cathepsin D is efficiently sorted to and secreted from regulated secretory compartments in the rat basophilic/mast cell line RBL. *J Cell Sci* 2000; **113 (Pt 18):** 3289-3298.
201. Melo FR, Waern I, Ronnberg E, Abrink M, Lee DM, Schlenner SM *et al.* A role for serglycin proteoglycan in mast cell apoptosis induced by a secretory granule-mediated pathway. *J Biol Chem* 2011; **286**(7)**:** 5423-5433.
202. Spirkoski J, Melo FR, Grujic M, Calounova G, Lundequist A, Wernersson S *et al.* Mast cell apoptosis induced by siramesine, a sigma-2 receptor agonist. *Biochem Pharmacol* 2012; **84**(12)**:** 1671-1680.
203. de Duve C. Lysosomes revisited. *Eur J Biochem* 1983; **137**(3)**:** 391-397.
204. Appelmans F, Wattiaux R, De Duve C. Tissue fractionation studies. 5. The association of acid phosphatase with a special class of cytoplasmic granules in rat liver. *Biochem J* 1955; **59**(3)**:** 438-445.
205. Serrano-Puebla A, Boya P. Lysosomal membrane permeabilization in cell death: new evidence and implications for health and disease. *Ann N Y Acad Sci* 2016; **1371**(1)**:** 30-44.
206. Bainton DF. The discovery of lysosomes. *J Cell Biol* 1981; **91**(3 Pt 2)**:** 66s-76s.
207. Saftig P, Klumperman J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. *Nat Rev Mol Cell Biol* 2009; **10**(9)**:** 623-635.
208. Luzio JP, Pryor PR, Bright NA. Lysosomes: fusion and function. *Nat Rev Mol Cell Biol* 2007; **8**(8)**:** 622-632.
209. Eskelinen EL. Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy. *Mol Aspects Med* 2006; **27**(5-6)**:** 495-502.
210. Aits S, Jaattela M. Lysosomal cell death at a glance. *J Cell Sci* 2013; **126**(Pt 9)**:** 1905-1912.
211. Wang F, Gomez-Sintes R, Boya P. Lysosomal membrane permeabilization and cell death. *Traffic* 2018; **19**(12)**:** 918-931.

212. Johansson AC, Appelqvist H, Nilsson C, Kagedal K, Roberg K, Ollinger K. Regulation of apoptosis-associated lysosomal membrane permeabilization. *Apoptosis* 2010; **15**(5)**:** 527-540.
213. Domagala A, Fidyt K, Bobrowicz M, Stachura J, Szczygiel K, Firczuk M. Typical and Atypical Inducers of Lysosomal Cell Death: A Promising Anticancer Strategy. *Int J Mol Sci* 2018; **19**(8).
214. Repnik U, Hafner Cesen M, Turk B. Lysosomal membrane permeabilization in cell death: concepts and challenges. *Mitochondrion* 2014; **19 Pt A:** 49-57.
215. Deng D, Jiang N, Hao SJ, Sun H, Zhang GJ. Loss of membrane cholesterol influences lysosomal permeability to potassium ions and protons. *Biochim Biophys Acta* 2009; **1788**(2)**:** 470-476.
216. Yu Z, Persson HL, Eaton JW, Brunk UT. Intralysosomal iron: a major determinant of oxidant-induced cell death. *Free Radic Biol Med* 2003; **34**(10)**:** 1243-1252.
217. Kurz T, Leake A, Von Zglinicki T, Brunk UT. Relocalized redox-active lysosomal iron is an important mediator of oxidative-stress-induced DNA damage. *Biochem J* 2004; **378**(Pt 3)**:** 1039-1045.
218. Kurz T, Terman A, Gustafsson B, Brunk UT. Lysosomes in iron metabolism, ageing and apoptosis. *Histochem Cell Biol* 2008; **129**(4)**:** 389-406.
219. Cable H, Lloyd JB. Cellular uptake and release of two contrasting iron chelators. *J Pharm Pharmacol* 1999; **51**(2)**:** 131-134.
220. Firestone RA, Pisano JM, Bailey PJ, Sturm A, Bonney RJ, Wightman P *et al.* Lysosomotropic agents. 4. Carbobenzoxyglycylphenylalanyl, a new protease-sensitive masking group for introduction into cells. *J Med Chem* 1982; **25**(5)**:** 539-544.
221. Miller DK, Griffiths E, Lenard J, Firestone RA. Cell killing by lysosomotropic detergents. *J Cell Biol* 1983; **97**(6)**:** 1841-1851.
222. Ghosh M, Carlsson F, Laskar A, Yuan XM, Li W. Lysosomal membrane permeabilization causes oxidative stress and ferritin induction in macrophages. *FEBS Lett* 2011; **585**(4)**:** 623-629.
223. Kavcic N, Pegan K, Turk B. Lysosomes in programmed cell death pathways: from initiators to amplifiers. *Biol Chem* 2017; **398**(3)**:** 289-301.
224. Villamil Giraldo AM, Appelqvist H, Ederth T, Ollinger K. Lysosomotropic agents: impact on lysosomal membrane permeabilization and cell death. *Biochem Soc Trans* 2014; **42**(5)**:** 1460-1464.
225. Boya P, Andreau K, Poncet D, Zamzami N, Perfettini JL, Metivier D *et al.* Lysosomal membrane permeabilization induces cell death in a mitochondrion-dependent fashion. *J Exp Med* 2003; **197**(10)**:** 1323-1334.
226. Glaumann H, Motakefi AM, Jansson H. Intracellular distribution and effect of the antimalarial drug mefloquine on lysosomes of rat liver. *Liver* 1992; **12**(4 Pt 1)**:** 183-190.
227. Tang D, Kang R, Berghe TV, Vandenabeele P, Kroemer G. The molecular machinery of regulated cell death. *Cell Res* 2019; **29**(5)**:** 347-364.
228. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. *Nat Rev Microbiol* 2009; **7**(2)**:** 99-109.
229. Malbec O, Roget K, Schiffer C, Iannascoli B, Dumas AR, Arock M *et al.* Peritoneal cell-derived mast cells: an in vitro model of mature serosal-type mouse mast cells. *J Immunol* 2007; **178**(10)**:** 6465-6475.
230. Guhl S, Babina M, Neou A, Zuberbier T, Artuc M. Mast cell lines HMC-1 and LAD2 in comparison with mature human skin mast cells - drastically reduced levels of tryptase and chymase in mast cell lines. *Exp Dermatol* 2010; **19**(9)**:** 845-847.

231. Melo FR, Grujic M, Spirkoski J, Calounova G, Pejler G. Serglycin proteoglycan promotes apoptotic versus necrotic cell death in mast cells. *J Biol Chem* 2012; **287**(22)**:** 18142-18152.
232. Turk B, Turk V. Lysosomes as "Suicide Bags" in Cell Death: Myth or Reality? *Journal of Biological Chemistry* 2009; **284**(33)**:** 21783-21787.
233. Mates JM, Segura JA, Alonso FJ, Marquez J. Oxidative stress in apoptosis and cancer: an update. *Arch Toxicol* 2012; **86**(11)**:** 1649-1665.
234. Sinha K, Das J, Pal PB, Sil PC. Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis. *Arch Toxicol* 2013; **87**(7)**:** 1157-1180.
235. Paivandy A, Calounova G, Zarnegar B, Ohrvik H, Melo FR, Pejler G. Mefloquine, an anti-malaria agent, causes reactive oxygen species-dependent cell death in mast cells via a secretory granule-mediated pathway. *Pharmacol Res Perspect* 2014; **2**(6)**:** e00066.
236. Simson JV, Spicer SS. Ferritin particles in macrophages and in associated mast cells. *J Cell Biol* 1972; **52**(3)**:** 536-541.
237. Voskoboinik I, Whisstock JC, Trapani JA. Perforin and granzymes: function, dysfunction and human pathology. *Nat Rev Immunol* 2015; **15**(6)**:** 388-400.
238. Lopez-Giraldo A, Cruz T, Molins L, Guirao A, Saco A, Cuerpo S *et al.* Characterization, localization and comparison of c-Kit+ lung cells in never smokers and smokers with and without COPD. *BMC Pulm Med* 2018; **18**(1)**:** 123.
239. Stefanick JF, Omstead DT, Kiziltepe T, Bilgicer B. Dual-receptor targeted strategy in nanoparticle design achieves tumor cell selectivity through cooperativity. *Nanoscale* 2019; **11**(10)**:** 4414-4427.
240. Mercer PF, Abbott-Banner K, Adcock IM, Knowles RG. Translational models of lung disease. *Clin Sci (Lond)* 2015; **128**(4)**:** 235-256.
241. Lei Y, Gregory JA, Nilsson GP, Adner M. Insights into mast cell functions in asthma using mouse models. *Pulm Pharmacol Ther* 2013; **26**(5)**:** 532-539.
242. Canning BJ, Chou Y. Using guinea pigs in studies relevant to asthma and COPD. *Pulm Pharmacol Ther* 2008; **21**(5)**:** 702-720.
243. Schreider JP, Hutchens JO. Morphology of the guinea pig respiratory tract. *Anat Rec* 1980; **196**(3)**:** 313-321.
244. Hyde DM, Hamid Q, Irvin CG. Anatomy, pathology, and physiology of the tracheobronchial tree: emphasis on the distal airways. *J Allergy Clin Immunol* 2009; **124**(6 Suppl)**:** S72-77.
245. Hagforsen E, Paivandy A, Lampinen M, Westrom S, Calounova G, Melo FR *et al.* Ablation of human skin mast cells in situ by lysosomotropic agents. *Exp Dermatol* 2015; **24**(7)**:** 516-521.
246. Hagforsen E, Lampinen M, Paivandy A, Westrom S, Velin H, Oberg S *et al.* Siramesine causes preferential apoptosis of mast cells in skin biopsies from psoriatic lesions. *Br J Dermatol* 2017; **177**(1)**:** 179-187.

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