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Michael Huber, PhD, Andrew C.B. Cato, PhD, George K. Ainooson, PhD, Marc Freichel, MD, Volodymyr Tsvilovskyy, PhD, Rolf Jessberger, PhD, Eva Riedlinger, cand. med., Christian P. Sommerhoff, MD, Stephan C. Bischoff, MD



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Regulation of the pleiotropic effects of tissue resident mast cells

Michael Huber, PhD,^{a*} Andrew C. B. Cato, PhD,^b George K. Ainooson, PhD,^b Marc Freichel, MD,^c Volodymyr Tsvilovsky, PhD,^c Rolf Jessberger, PhD,^d Eva Riedlinger, cand. med.,^e Christian P. Sommerhoff, MD,^e and Stephan C. Bischoff, MD^{f*}

^a Institute of Biochemistry and Molecular Immunology, Medical Faculty, RWTH Aachen University, Pauwelsstr. 30, D-52074 Aachen, Germany

^b Karlsruhe Institute of Technology, Institute of Toxicology and Genetics, Hermann-von-Helmholtz-Platz 1, D-76344 Eggenstein-Leopoldshafen, Germany

^c Institute of Pharmacology, Heidelberg University, Medical Faculty, Im Neuenheimer Feld 366, D-69120 Heidelberg, Germany

^d Institute for Physiological Chemistry, Medical Faculty Carl Gustav Carus, Technische Universität Dresden, Fetscherstr. 74, D-01307 Dresden, Germany

^e Institute of Laboratory Medicine, University Hospital, LMU Munich, Nussbaumstr. 20, D-80336 Munich, Germany

^f Institute of Clinical Nutrition, University of Hohenheim, Fruwirthstr. 12, D-70599 Stuttgart, Germany

* Corresponding authors

Michael Huber, PhD; Institute of Biochemistry and Molecular Immunology, RWTH Aachen University, Pauwelsstr. 30, D-52074 Aachen, Germany; ++49-241-80 88830 (phone); ++49-241-80 82428 (fax); mhuber@ukaachen.de (e-mail)

Stephan C. Bischoff, MD; Institute of Clinical Nutrition, University of Hohenheim, Fruwirthstr. 12, D-70599 Stuttgart, Germany; ++49-711-459 24101 (phone); ++49-711-459 24343 (fax); bischoff.stephan@uni-hohenheim.de (e-mail)

Email addresses: MH: mhuber@ukaachen.de
ACBC: andrew.cato@kit.edu
GKA: gkainooson@knust.edu.gh
MF: marc.freichel@pharma.uni-heidelberg.de
VT: volodymyr.tsvilovskyy@pharma.uni-heidelberg.de
RJ: rolf.jessberger@tu-dresden.de
ER: eva.riedlinger@med.uni-muenchen.de
CPS: sommerhoff@med.uni-muenchen.de
SCB: bischoff.stephan@uni-hohenheim.de

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Abstract

Mast cells, best known for their detrimental role in allergic diseases, act in a diverse array of physiological and pathological functions, made possible by the plurality of mast cell types. Their various developmental avenues and their distinct sensitivity to (micro-) environmental conditions convey extensive heterogeneity, resulting in diverse functions. We briefly summarize this heterogeneity, elaborate on molecular determinants that allow mast cells to communicate with their environment to fulfill their tasks, discuss their protease repertoire stored in secretory lysosomes, and consider different aspects of mast cell signaling. Further, we describe key mast cell governance mechanisms, i.e. the high-affinity receptor for IgE (FcεRI), the stem cell factor receptor KIT, the IL-4 system, and Ca²⁺-dependent as well as phosphatase-dependent mechanisms. Finally, we focus on distinct physiological functions such as chemotaxis, phagocytosis, host defense, and the regulation of mast cell functions at the mucosal barriers of the lung, the gastrointestinal tract, and the skin. A deeper knowledge of the pleiotropic functions of mast cell mediators as well as the molecular processes of mast cell regulation and communication should enable us to promote beneficial mast cell traits in physiology and suppress detrimental mast cell functions in disease.

Key words: FcεRI, stem cell factor, interleukin 4, calcium signaling, phosphatases, chemotaxis, tryptase, chymase, integrins, Toll-like receptors, ion channels

Abbreviations used:

BM:	bone marrow
CD45:	cluster of differentiation 45
CLP:	caecum ligation and puncture
CPA3:	carboxypeptidase A3
CTMC:	connective tissue mast cell
CXCR2:	C-X-C motif chemokine receptor 2

86	CX3CR1:	fractalkine receptor
87	FcεRI:	high-affinity receptor for IgE
88	FcγR:	IgG receptor
89	GASPDs:	granule-associated serine proteases of immune defense
90	GCs:	glucocorticoids
91	GI:	gastrointestinal
92	HLMC:	human lung mast cell
93	HSC:	hematopoietic stem cell
94	IP ₃ :	inositol-1,4,5-trisphosphate
95	ITAM:	immunoreceptor tyrosine-based activation motif
96	MAdCAM-1:	mucosal vascular addressin cell adhesion molecule 1
97	MAPK:	mitogen-activated protein kinase
98	MC:	mast cell
99	MC _C :	mast cell expressing only chymase
100	MCps:	mast cell precursors
101	MC _T :	mast cell expressing only tryptase
102	MC _{TC} :	mast cell expressing tryptase and chymase
103	MMC:	mucosal mast cell
104	PEP:	PEST domain-enriched tyrosine phosphatase
105	PTEN:	phosphatase and tensin homolog
106	PTPα:	protein tyrosine phosphatase alpha
107	PTPε:	protein tyrosine phosphatase epsilon
108	SCF:	stem cell factor
109	SHIP1:	SH2-domain-containing inositol-5'-phosphatase 1
110	SHIP2:	SH2-domain-containing inositol-5'-phosphatase 2
111	SHP-1:	SH2-domain-containing phosphotyrosine phosphatase 1
112	SHP-2:	SH2-domain-containing phosphotyrosine phosphatase 2
113	TLR:	Toll-like receptor
114	TRP:	transient receptor potential
115	TULA-2:	T-cell ubiquitin ligand-2
116	VCAM-1:	vascular cell adhesion molecule 1

Main Text

1. Heterogeneity of mast cells

The term “mast cells (MCs)” describes a highly heterogeneous cell population. Numerous MC subtypes have now been characterized, both in humans and in animals, based on differences in cell morphology, histochemical properties, protease content in granules, piecemeal or anaphylactic type of degranulation, receptor expression, and function ^{1, 2}. A certain subtype plasticity has been recognized depending on the tissue environment, and *in vitro* MCs change their phenotype depending on the culture conditions ³⁻⁵.

In addition to intraspecies heterogeneity, interspecies heterogeneity has been discovered ⁶. This is of relevance, because a large part of the scientific literature on MCs is based on animal work, in particular mouse studies. Data on human MCs are scarce, as they are difficult to collect from tissue sources. Many laboratories therefore use genetically transformed human MC lines instead of primary MCs from tissue, or immature MCs derived from bone marrow or peripheral blood that are partially matured under particular culture conditions. Although, it is well-known that such MCs differ functionally from primary tissue MCs, they have nonetheless provided useful insights into the regulation and function of human MCs.

MC subtypes in humans - Human MCs can be characterized according to the type of proteases they contain. For example, MC_{TC} contain tryptase and chymase, MC_T contain only tryptase or the rare MC_C containing only chymase. MC_{TC} are located at non-mucosal sites (skin, submucosa, adjacent to blood vessels, peritoneum in mice), whereas MC_T are primarily found at mucosal sites (nose, lung, intestine). Further division of these subtypes into site-specific populations have been proposed to reflect the microenvironment of the anatomical compartments where they are localized. For example, the lung MC_T – to reflect its prevalence in bronchi, bronchioles, and the alveolar parenchyma while MC_{TC} – to denote its abundance

in pulmonary vessels and the pleura. Within each MC_{TC} and MC_T population, there are also distinct localization-specific expression patterns of receptors, enzymes and growth factors which can be used for further characterization. For instance, bronchial MC_T consistently express more histidine decarboxylase than alveolar MC_T while for both MC_{TC} and MC_T, the high-affinity receptor for IgE (FcεRI) is highly expressed in conducting airways but virtually absent in alveolar parenchyma⁷. It is not only the tissue site, but also the disease status that influences MC heterogeneity. In poorly controlled, severe, Th2-associated asthma, an altered subtype which could play a role in the pathophysiology of this disease⁸ has been described to express tryptase, chymase, and/or carboxypeptidase A3 (CPA3). Genetic analyses have revealed even more heterogeneity among MCs from different sites within one tissue, although their clinical implications are yet unclear⁹.

MC subtypes in rodents - In mice and rats, MCs are traditionally subtyped into connective tissue (CTMC) and mucosal MCs (MMC) based on their histochemical properties, mediator content and functional properties¹⁰. CTMC can be stained with both alcian blue and safranin, while MMC can only be stained with alcian blue. In terms of their mediator content, heparin proteoglycans predominate in CTMC while it is chondroitin sulfate in MMC. In their functional properties, CTMC typically contain rodent tryptases, chymases, and CPA3, whereas MMC contain chymases only and thus differ fundamentally from their human counterparts in mucosal tissues that contain tryptase only. The subtyping of murine MCs into CTMC and MMC might be an oversimplification for the mouse intestine and peritoneum but not for the lung¹¹.

2. Regulation of MC development

Development of MCs can be subdivided in three major steps: differentiation of hematopoietic stem cells (HSCs) to MC precursors (MCps) in the bone marrow (BM), distribution of MCps

via blood and transendothelial migration into target tissues, and eventually phenotypic maturation in different tissues. Although there are still certain discrepancies concerning our understanding of MCp development in the BM, it is justified to suggest that committed MCps stem from bipotent progenitors, endowed with the capacity to differentiate into both MCs and basophils, as has been studied in mice (reviewed in ¹²).

The expression of the receptor tyrosine kinase KIT for MC development is mandatory. Likewise, the expression of the KIT ligand, stem cell factor (SCF), is obligatory for MC development *in vivo*. Mature MCs are usually characterized by their expression of KIT and FcεRI as well as distinct metachromatic cytoplasmic granules. MCps do not necessarily express the FcεRI and are typically not identifiable by metachromatic staining, indicating that commitment to the MC lineage occurs prior to FcεRI expression. Indeed, early committed MCps lack FcεRI expression but show KIT expression ¹³. Hence, MCps ready to leave the BM can be either FcεRI-positive or –negative. Less is known about committed MCps in humans although an immature human MCp population, similar to the mouse MCp in terms of amount and developmental state, has recently been identified in human blood. These have been reported to be lineage-negative CD34^{hi} KIT^{int/hi} FcεRI⁺ and to give rise only to granulated tryptase-positive KIT⁺ FcεRI⁺ MCs ¹⁴. Interestingly, the frequency of development of such MCs starting from a lineage-negative CD34^{hi} KIT^{int/hi} FcεRI⁺ cell subset was reduced by more than a factor of ten, indicating the importance of FcεRI expression for MC commitment.

After leaving the BM, MCps are distributed via the blood to their target tissues. This requires productive interaction between surface molecules of MCps and endothelial cells for transendothelial migration. Homing of MCps to the small intestine requires the expression of the α4β7 integrin on the MCps ¹⁵ and, MAdCAM-1 and VCAM-1 as counterligands on the intestinal endothelial cells for interaction ^{15, 16}. Integrin activation requires chemokine

receptor-mediated inside-out signaling by CXCR2 expressed on MCps¹⁶. While $\beta 7$ integrin-deficient mice showed impaired MCp homing to the intestine, homing to the lung was still possible, albeit reduced. Using a model of ovalbumin-induced allergic airway inflammation, pulmonary recruitment of MCps was shown to depend on both $\alpha 4\beta 7$ and $\alpha 4\beta 1$ integrins on the MCps, and on VCAM-1 on the endothelial side¹⁷. The same transmigration mechanism was recently demonstrated in Influenza A-infected mice¹⁸. Homing of MCps to the small intestine occurs in a constitutive manner in naïve mice¹⁵, although it can be amplified by inflammatory conditions. In contrast, in the lung, the numbers of MCps that home constitutively is strongly exceeded by the numbers recruited upon inflammation in a T cell-dependent manner¹⁹. In addition to the intestine and the lung, the skin is an important MC-containing tissue. However, less is known about MCp homing to the skin, though a promoting role for the fractalkine receptor CX3CR1 on MCps has been suggested²⁰.

After migration to their respective tissues, MCps differentiate into two major subclasses of mature tissue MCs, MC_{TC} and MC_T (for detection of and discrimination between various MC types of different species, see chapter 1). Finally, maturation of MCs, *in vitro* as well as in different tissues, is associated with the downregulation of cell surface adhesion molecules and chemokine receptors. Exemplary, primary peritoneal and pulmonary MCps from naïve mice express considerably higher levels of $\beta 7$ integrin than the respective mature MCs²¹. Moreover, analysis of MCps and mature MCs differentiated *in vitro* from human cord blood revealed expression of four different chemokine receptors (CXCR2, CCR3, CXCR4, and CCR5) on MCps, whereas only CCR3 was retained on mature MCs²². Generally, expression of plasma membrane proteins important for migration changes during MC maturation.

Very recently, with the aid of a new hematopoietic fate mapping model, murine MCs were demonstrated to have dual developmental origin arising via both primitive (yolk sac-derived) and definitive (HSC-derived) hematopoiesis²³. MC maintenance in adult tissues was found to occur largely independent of the bone marrow, probably via proliferation of tissue-resident

MCps differentiated from HSCs during embryogenesis²³. This intriguing study proves that much more remains to be discovered in MC development and migration to target tissues, and it would be exciting to learn about the respective processes in humans.

3. Regulation and function of MC proteases

Proteases, particularly the tryptases, chymases, and CPA3 whose expression is largely restricted to MCs, contribute importantly to the pleiotropic effector functions of these cells. Proteases are the major proteins of MC secretory granules that also contain histamine, serotonin, and proteoglycans. After release by degranulation, these proteases primarily exert pro-inflammatory functions and have been implicated in the pathogenesis of MC-related disorders, but they also contribute to tissue homeostasis and host defense (for reviews see e.g.²⁴⁻²⁶).

Human MCs express four tryptases, α , β , γ and δ , two proteases with chymotrypsin-like activity, chymase and cathepsin G (also expressed in neutrophils), and CPA3. Tryptase β , which forms non-covalent tetramers with limited trypsin-like activity, is considered the prototypical and biological active "tryptase"^{27, 28}. Tryptase α can assemble to virtually identical tetramers but is inactive²⁹. Tryptase δ lacks the C-terminal 40 amino acid residues³⁰, and from a structural point of view its folding and activity are enigmatic. Tryptase γ is less closely related to the other tryptases and it is likely membrane-bound in a monomeric form³¹. While chymase and CPA3 are exclusive to MCs, chymase, cathepsin G and CPA3 are usually expressed only in the MC_{TC} subset of MCs. MCs with distinct protease expression patterns such as chymase-only MC_C³² and, more recently, CPA3 positive MC_T have been described in eosinophilic allergic inflammatory disorders^{33, 34}.

Comparative genomic analysis has shown that tryptases and chymases are poorly

conserved between man and rodents and even between man and chimpanzee³⁵. The mouse genome encodes four tryptases, mMCP-6, -7, -11, and tryptase γ /transmembrane tryptase. mMCP-6 is most likely the functional counterpart of human tryptase β , whereas mMCP-7 apparently is dispensable and not expressed in some mouse strains such as C57BL/6³⁶. The chymase locus has expanded considerably and encodes 7 chymases, mMCP-1, -2, -4, -5, -8, -9 and -10. mMCP-4 appears to be the functional counterpart of human chymase, whereas its rodent orthologs mMCP-5/rMCP-5 have altered elastase-like activity^{37, 38}. Instead of being expressed in MCs, mMCP-11 and -8 are preferentially expressed in basophils^{39, 40}, which are virtually devoid of tryptases and chymases in humans. In contrast to the heterogeneity of tryptases and chymases, a single CPA3 gene is expressed in man and rodents.

Considering their role in host defense vs. their pro-inflammatory potential, it is not surprising that the expression and activity of MC proteases is extensively regulated. On the genomic level, the variation of α - and β -tryptases is complex: Of the four tryptase genes (*TPSAB1*, *TPSB2*, *TPSG1*, *TPSD1*), *TPSAB1* and *TPSB2* can harbor alleles encoding enzymatically active β , inactive α or deficiency alleles⁴¹. All individuals initially surveyed have two to four β alleles, suggesting that β has essential functions, whereas the enzymatically inactive α is dispensable⁴¹. Recently, individuals with duplications or triplications of the α -encoding *TPSAB1* gene, elevated basal serum tryptase levels and multisystem complaints have been identified, a trait now classified as hereditary α -tryptasemia syndrome⁴².

On the level of gene regulation, the expression of human tryptase, its mouse counterpart mMCP-6 and several mouse chymases is driven by the microphthalmia (mi)-associated transcription factor, which is also central to the development of MCs^{43, 44}. Post-transcriptional and -translational mechanisms contribute to the regulation and result in an increased number of tryptase species. For example, alternative splicing of human α and β tryptases has been reported, which likely affects the tetrameric architecture, stability and enzymatic activity of

these proteins⁴⁵. Posttranslational processing further results in several forms of the proteins with slight differences in glycosylation, charge, and enzymatic activity⁴⁶.

In contrast to most other proteases, tryptases and chymases are not regulated on the level of zymogen activation. Rather, they are activated by cathepsin C and potentially by other cathepsins before storage in granules, a feature shared with other 'granule associated serine proteases of immune defense' (GASPIDs^{47, 48}). Within the granules, the activity of tryptases and chymases is likely controlled by compartmentalization, its acidic pH, and its close packaging. After degranulation, binding to high molecular weight proteoglycans continues to modulate diffusion, activity, and substrate specificity. In particular, the active β tryptase tetramer requires stabilization by bound proteoglycans that protect it from dissociation into (almost) inactive monomers. Enzymatically active monomers with altered substrate and inhibition profile can be formed *in vitro*⁴⁹ but these remain to be demonstrated *in vivo*. Tetramerization is likely the main mechanism regulating tryptase β activity and selectivity *in vivo*, because it restricts the access of large substrates to the active sites located within the central pore and sterically blocks inhibition by most protease inhibitors^{26, 28, 50}. Monomeric chymases are more prone to inhibition by tissue- and plasma-derived inhibitors, but they can evade inhibition by inactivating serpins and they can retain some activity when in circulation in an α 2 macroglobulin complex⁵¹.

4. Mast cell receptors and signaling

4.1 Immunoglobulin receptor signaling and function

MCs express high-affinity receptor for IgE, Fc ϵ RI, and several IgG receptors, Fc γ R on their surface (for reviews see^{52, 53}). Fc ϵ RI is a tetramer consisting of an α -chain that binds IgE, a membrane transversing tetraspanin β -chain, and a homodimeric disulfide-linked γ -chain^{54, 55}.

IgE is bound at a 1:1 stoichiometry at very high affinity ($K_a \geq 10^{10} \text{ M}^{-1}$). Binding of low doses of monomeric IgE to the FcεRI provides a MC survival signal⁵⁶ and IgE levels affect surface expression of FcεRI^{57, 58}. Low binding of IgE or antigen (Ag) to the FcεRI triggers expression and secretion of chemokines and other mediators, while optimal binding triggers degranulation⁵⁹. Supra-optimal crosslinking can suppress MC activation⁶⁰. Human MCs express FcγRIIA and/or FcγRI. Both receptors can stimulate degranulation and cytokine gene expression. In mice, the FcγRIIB, expressed also on human MCs, acts inhibitory and the FcγRIII stimulatory on degranulation.

After cross-linking by Ag, the IgE-prebound FcεRI is phosphorylated by the kinase LYN at tyrosine residues in the ITAMs (immunoreceptor tyrosine-based activation motifs) of β and γ chains. This is followed by activation of SYK and other kinases. The core signaling pathways have been extensively reviewed elsewhere^{53, 55, 61}. The relationships among the two classes of enzymes, other kinases and phosphatases that control MC function, are complex, sometimes hierarchical. Much has been learned from “knockout” or “knockdown” models, which, however, needs to be interpreted with caution as compensatory mechanisms might be turned on. The balance of specific signaling pathways emanating from the FcεRI depends on the type of MC, on their site of residence, on their FcεRI-independent activation status, and other biological parameters.

Cross-linking of the FcεRI by IgE/Ag complexes causes it to translocate into lipid rafts (for reviews see^{62, 63}). LYN is tethered to the cytoplasmic membrane through acylation and co-assembles in lipid rafts, which promote high local concentration of receptor and kinase, resulting in efficient phosphorylation of the FcεRI. Other components of the FcεRI signalosome are embedded in these membrane domains as well like LAT and NTAL. Not only are some proteins included in lipid rafts when the FcεRI becomes crosslinked, but others

such as certain phosphatases are excluded from lipid rafts⁶⁴. Thus, formation of these lipid-protein domains provides a versatile mechanism for the regulation of FcεRI signaling. Other signals that modulate FcεRI-mediated degranulation likely include cell-cell contact-dependent signals from adhesion receptors (RJ; own unpublished results). Embedding MCs in appropriate tissue niches and thereby controlling MC activation may be an important mechanism to keep degranulation under finely balanced control. Many other factors that regulate the final outcome of FcεRI signals are incompletely understood, e.g. non-coding RNAs, membrane biophysics, intracellular liquid phase separation, and physical interactions with other cells including immune and non-immune cells.

A large range of compounds from plant molecules to bacterial substances and snake venoms, but also complement, Toll-like receptor (TLR) ligands, and other stimuli such as pressure or heat may cause MC exocytosis⁶⁵. Thus, a plethora of receptors can trigger MC degranulation independently of or in conjunction with the FcεRI. MC FcεRI-mediated degranulation may also be facilitated by other pathways. Examples such as SCF (see 4.2), prostaglandins, nucleotides, interleukins, and chemokines illustrate the wide spectrum of ligands that modulate MC activity (reviewed in⁶⁶).

MCs, at least BMMC, can be desensitized by various pathways to keep FcεRI signaling and thus degranulation under control^{67, 68}. Low-dosage exposure to monomeric IgE, IgE-bound Ag, or to anti-Fc receptor antibodies reduces FcεRI sensitivity^{69, 70}. Activation of the single-chain FcγRIIB by crosslinking it to FcεRI via immune complexes made of Ig Fc fragments Fcγ and Fcε blocks PI3K-mediated FcεRI signaling⁷¹. The FcεRI may be down-regulated by internalization followed by either degradation or recycling to the cell surface^{61, 72, 73}. Ubiquitination and neddylation are key signals for these endocytic processes and/or the

potential subsequent lysosome- or proteasome-mediated degradation of the Fc receptor complexes⁶³. Further, signaling through cross-linked FcγRs can induce BMMC apoptosis⁷⁴.

4.2. Cytokine receptor signaling and function

SCF, IL-4 and IL-33 are major cytokines regulating MC mediator release. SCF acts not only as a MC growth, survival, and chemotaxis factor (see chapter 2), but also as a regulator of mediator release by either enhancing IgE-dependent responses (degranulation as well as production of leukotrienes and cytokines) or by directly inducing cytokine production and release^{75, 76}. The mechanisms of activation of the SCF receptor KIT in MCs are quite well understood and have been reviewed elsewhere^{77, 78}. In contrast to the acute effects of SCF stimulation, chronic SCF treatment results in suppression of FcεRI-mediated MC activation caused by a significant attenuation of cytoskeletal reorganizations⁷⁹.

In a comparable manner, acute IL-33 stimulation of MCs augments Ag-triggered cytokine production, as studied in BMMCs and human umbilical cord blood-derived MCs^{80, 81}. Interestingly, KIT has been shown to interact constitutively with the β-chain of the IL-33 receptor, IL-1RAcP, and it appears to be required for the full IL-33 response in BMMCs and human mastocytosis MC lines⁸². In opposition to an acute stimulation with IL-33, a chronic treatment of BMMCs and primary human MCs with IL-33 (>72h) resulted in a hyporesponsive secretory phenotype, demonstrating fundamental proteomic and regulatory changes induced by long-term IL-33 stimulation⁸³.

Besides SCF and IL-33, IL-4 is another important human MC regulator. In contrast to SCF, IL-4 does not affect matured MCs by itself, but acts synergistically with SCF on MC survival, proliferation and IgE-dependent mediator release³. Moreover, IL-4 alters the cytokine profile released by human intestinal and skin MCs (Figure 1). The cytokines produced by MCs,

either constitutively or upon activation, vary in different species, depending on the maturity of the MCs, on tissue environmental factors, MC subtypes, and types of stimuli that trigger MCs signaling. Below is a summary of data from primary human MCs, either mucosal MCs from the gut^{84, 85} or skin^{86, 87}. Mucosal MCs from the gut express constitutively small amounts of TNF α and IL-6. Upon Fc ϵ RI-crosslinking, expression of TNF α and IL-6 is enhanced and additional expression of Th2 cytokines such as IL-3, IL-5, and IL-13 occurs. If the mucosal MCs are exposed to an IL-4 environment, IL-6 expression is blocked while IL-3, IL-5 and IL-13 expression is markedly enhanced. Similar variations in cytokine expression can be observed in human skin MCs depending on Fc ϵ RI-crosslinking and IL-4 treatment. However, no Th2 cytokine expression has been described in this cell type (Figure 1C). Human mucosal MCs are also a rich source of several chemokines, the expression of which is again regulated by Fc ϵ RI-crosslinking and IL-4 (Figure 1B). Human skin MCs produce chemokines too, namely CCL2, CCL3 and CCL4, e.g. upon activation by CD30, but their regulation has been less extensively studied⁸⁷. The priming effect of IL-4 is likely not restricted to intestinal or skin MCs, but has been observed also in lung MCs, LAD2 MCs, and other human MCs⁸⁸⁻⁹¹.

IL-4 is a key mediator of allergic inflammation considering that it also induces the development of Th2 cells and IgE switching in B cells³. SCF and IL-4 may thus be considered as primary, co-stimulatory mediators since they do not only enhance Fc ϵ RI-mediated signals but also induce the upregulation of secondary stimulatory receptors, e.g. the substance P (NK-1) receptor. Such co-stimulation draws comparisons between MC and T cell signaling since both require cooperation of two signals for optimal activation: an Ag-dependent signal such as from the TCR or the IgE-bound Fc ϵ RI, and a co-stimulatory molecule, e.g., CD80 in T cells or SCF/IL-4 in MCs.

The priming effect of IL-4 in human MCs is possibly not restricted to Fc ϵ RI-mediated stimulation. In human cord blood-derived MCs, IL-4 pretreatment enhances TNF production

following stimulation with the TLR2 agonist peptidoglycan, and enables MCs to produce TNF in response to LPS ⁹². In the LAD2 human MC line, IL-4 pretreatment enhances IL-31 production following stimulation with IL-33 ⁹¹. These two studies are probably insufficient for the generalization of IL-4 priming effects in IgE-independent signaling in human MCs, but demonstrate that the priming effects are not restricted to the FcεRI-mediated stimulation.

The intracellular mechanisms connecting the SCF and IL-4 signaling pathway have been analyzed in human MC_T isolated from gut mucosa ⁹³. Activation of MC_T by FcεRI cross-linking alone results in phosphorylation of ERK and MAPK p38, but not of AKT. Stimulation with SCF alone also induces phosphorylation of ERK and MAPK p38, and additionally of AKT. As oppose to human MC_T, in murine BMMCs both KIT- and FcεRI-mediated stimulation results in ERK, MAPK p38, and AKT activation ⁷⁶. IL-4 priming of human intestinal MC_T enhanced activation of ERK, but blocked activation of MAPK p38. As activation of MAPK p38 is required for IL-6 production, the reported negative effect of IL-4 on MAPK p38 explains the inhibitory effect of IL-4 on IL-6 expression in human MCs. Moreover, IL-4 priming that antecedes FcεRI cross-linking induces activation of AKT. The combined treatment of MCs with IL-4, SCF and FcεRI cross-linking substantially upregulates activation of AKT, whereas blocking of AKT inhibited the pronounced production and release of cytokines and chemokines in response to the three MC agonists ⁹³.

4.3. Innate immune receptors

The particular localization of MCs makes their expression of receptors recognizing microbial constituents meaningful and mandatory. MCs are equipped with different Toll-like receptors (TLRs), which can productively interact with various pathogen-associated molecular patterns (PAMPs) present on bacteria, viruses, and fungi. Differential types of MCs of different species have been reported to express various TLRs (investigated mainly at the mRNA level).

TLR1-9 have been found in murine and human MCs (see Ref. ⁹⁴⁻⁹⁶), whereas TLR10 can only be detected in human lung MCs ⁹⁷. By far most studies in the field of TLR function and signaling have been published for TLR4 and its ligand lipopolysaccharide (LPS). In macrophages, LPS is bound by mCD14 and eventually transferred to the TLR4-MD-2 complex, hence activating the MYD88-dependent and the TRIF-dependent pathway. The former induces production of pro-inflammatory cytokines, the latter the generation of type-I interferons ⁹⁸. Intriguingly, BMMCs and human intestinal MCs lack mCD14, with the consequence they recognize R-chemotypes of LPS, but not S-chemotypes ^{99, 100}. In correlation, macrophages deficient in CD14 show a LPS-recognition phenotype comparable to MCs ¹⁰¹. Moreover, both murine BMMCs and murine peritoneal MCs (PMCs) were found to lack the adaptor protein TRAM resulting in their inability to activate the TRIF pathway and hence, to produce type-I interferons upon LPS stimulation ^{102, 103}. With respect to MYD88-dependent, pro-inflammatory TLR4 signaling, BCL3, a member of the inhibitor of NF- κ B family, has been shown to play a suppressive role in PMCs, which was not obvious in BMMCs ¹⁰⁴. Most likely, on the level of signal transduction as well, many more differences between diverse MC types are to be found.

C-type lectin receptors have also been found to be involved in MC signaling activated by certain bacteria. MCs differentiated from murine BM or peritoneal cells express mannose receptor and macrophage galactose-type lectin whose roles in the recognition of *Bordetella pertussis* have been demonstrated ¹⁰⁵. In addition, expression of the C-type lectin receptor Dectin-1, which participates in anti-fungal immunity, has been reported in different types of MCs such as BMMCs ¹⁰⁶, human MCs generated from peripheral blood CD34⁺ progenitors ¹⁰⁷, and cord blood-derived MCs ¹⁰⁸. Thus, MCs are well-endowed with different features for pathogen recognition.

4.4. Regulation of Ca²⁺-dependent MC activation

MC activation by numerous stimuli relies on Ca^{2+} entry. Numerous stimuli including Ag acting on the IgE-bound $\text{Fc}\epsilon\text{RI}$, adenosine, endothelin-1 or compound 48/80, for which the receptor in MCs (i.e. MRGPRB2 in mice and MRGPRX2 in humans) was identified only three years ago¹⁰⁹, trigger a marked increase in free cytosolic Ca^{2+} concentration to evoke MC activation. Degranulation achieved by regulated exocytosis of secretory vesicles requires a rise in free cytoplasmic Ca^{2+} concentration, similar to the production of leukotrienes or the activation of several transcription factors, which drive the synthesis of cytokines. Before degranulation can occur, the vesicles loaded with, for example, inflammatory mediators are transported to the plasma membrane where they fuse with the plasma membrane. This process of regulated exocytosis is initiated by a rise in the Ca^{2+} concentration in the vicinity of the membrane which depends on Ca^{2+} -influx through Ca^{2+} permeable channels in the plasma membrane^{110, 111}. A major downstream target of $\text{Fc}\epsilon\text{RI}$ stimulation is phospholipase C (PLC) $\gamma 1$, which leads to generation of inositol-1,4,5-trisphosphate (IP_3). IP_3 -mediated Ca^{2+} release from intracellular stores is subsequently followed by an influx of Ca^{2+} from the extracellular space, a process named store-operated Ca^{2+} entry (SOCE)¹¹². SOCE has been described in MCs^{111, 113} and ionic currents mediating this Ca^{2+} influx were first characterized as Ca^{2+} release-activated Ca^{2+} (CRAC) channels¹¹⁴. Molecular constituents of this Ca^{2+} entry pathway include proteins of the ORAI family. Depletion of intracellular Ca^{2+} stores through IP_3 generation can be achieved by several agonists mentioned above that act on G protein-coupled receptors (GPCR) leading to stimulation of $\text{PLC}\beta$. These signaling pathways are also shown to activate TRP channels.

Ca^{2+} entry into MCs via channels consisting of ORAI proteins. The ORAI family of cation channels consist of three members, ORAI1, ORAI2, and ORAI3, which are all sufficient to build SOCE channels (see refs. in¹¹⁵) (Table 1). In murine BMMCs in which the *Orai1* gene has been targeted by a gene trap approach¹¹⁶, CRAC currents (I_{CRAC}) were reduced

by 66%, and FcεRI-mediated Ca^{2+} entry and release of inflammatory mediators were reduced to a similar extent. In contrast, in *Orai2*^{-/-} mice, Ag-evoked Ca^{2+} rise and degranulation of PMCs as well as passive systemic anaphylaxis were increased¹¹⁵. This finding was initially surprising since overexpression of the *Orai2* or *Orai1* cDNA leads to a pronounced enhancement of both SOCE and I_{CRAC} (see refs. in¹¹⁵). An increase in SOCE was also observed in *Orai2*^{-/-} T cells and macrophages¹¹⁷, which was explained by a role of ORAI2 proteins in fine-tuning the magnitude of SOCE mediated in ORAI1:ORAI2 heteromeric channels¹¹⁷. Ca^{2+} rise triggered by compound 48/80 is increased in *Orai2*^{-/-} PMCs suggesting that Ca^{2+} entry channels triggered by MRGPRB2 receptors contain ORAI2 proteins¹¹⁵. In human lung mast cells (HLMCs), all three *ORAI* genes (also named *CRACM* in some studies) are expressed. Adenovirus-mediated knockdown of *ORAI1* in HLMCs resulted in a significant reduction of ~50% in Ca^{2+} influx and in FcεRI-dependent release of β-hexosaminidase and LTC_4 , whereas *ORAI2* knockdown had only marginal effects¹¹⁸. However, it is still unknown whether any of the three ORAI proteins contribute to Ca^{2+} entry and MC activation triggered by other agonists e.g. those acting via GPCRs.

TRP channels determine Ca^{2+} entry into MCs. Twenty-eight mammalian TRP (transient receptor potential) proteins are classified into six subfamilies according to structural homology: TRPC, TRPV, TRPM, TRPA, TRPML, and TRPP (see refs. in¹¹⁰) (Table 1). TRP channels do not only contribute to Ca^{2+} entry across the plasma membrane, but they also play an important role in electrogenesis regulating the driving force for Ca^{2+} entry via other Ca^{2+} -permeable channels, e.g. CRAC or other SOC channels. In BMMCs, indirect regulation, as a limiting factor of SOCE, was found to be a characteristic feature of TRPM4 channels, which acts as Ca^{2+} -activated cation channels primarily conducting Na^+ . TRPM4 channels depolarize the membrane following FcεRI stimulation, and, thereby, critically decreasing Ca^{2+} influx via CRAC channels. Accordingly, TRPM4 deletion results in an increased Ca^{2+} entry and excessive release of histamine, leukotrienes and $\text{TNF}\alpha$, and aggravates acute cutaneous anaphylaxis¹¹⁹. A similar role has recently been described in

PMCs¹²⁰, in a study that demonstrated that TRPM4 proteins localize to the plasma membrane in response to FcεRI stimulation suggesting that increased translocation of TRPM4 to the plasma membrane is part of the mechanism that limits MC activation.

The other TRP channels that contribute to MC activation do so by directly mediating Ca²⁺ entry. First evidence of the involvement of TRPC in MC activation came from knockdown experiments of TRPC1 and TRPC3 in RBL-2H3 cells, which resulted in decreased sensitivity to Ag stimulation¹²¹. Also in BMMCs, deficits in FcεRI-triggered MC activation were observed following TRPC1 downregulation¹²². However, contrary to expectations, analysis of *Trpc1*^{-/-} mice showed a delayed recovery in passive systemic anaphylaxis and *Trpc1*^{-/-} BMMCs responded to Ag stimulation with an enhanced elevation of intracellular Ca²⁺ compared to wild-type controls. Intriguingly, Ca²⁺ entry as well as degranulation triggered by Ag stimulation was unchanged¹²³. Concerning the TRPC5 channel, Ma et al. found that TRPC5 downregulation in RBL-2H3 cells reduced SOCE and proposed an association of TRPC5 with STIM1 and ORAI1¹²⁴.

Within the TRPV subfamily, several studies have described expression of TRPV1, TRPV2 and TRPV4 in different types of mouse, rat, and human MCs¹¹⁰, including mouse PMCs¹²⁵. However, Ca²⁺ rise triggered by either Ag, endothelin-1 or compound 48/80 was unchanged in PMCs from TRPV1-, TRPV2- and TRPV4-deficient mice, respectively. In a similar manner degranulation triggered by Ag or compound 48/80 was unchanged¹²⁵. While degranulation induced by physical stimuli was found to involve TRPV2 activation in HMC-1 MCs¹²⁶, heat-evoked Ca²⁺ rise and degranulation were unchanged in *TRPV2*^{-/-} PMCs¹²⁵.

Within the TRPM subfamily, expression of TRPM2, TRPM4, TRPM7 and TRPM8 have been reported in different types of mouse, rat, and human MCs¹¹⁰. In *Trpm2*^{-/-} BMMCs, a heat-evoked cation current was lacking, and Ag-evoked Ca²⁺ rise and degranulation were reduced

¹²⁷. Interestingly, SOCE was also reduced in *Trpm2*^{-/-} BMMCs, and the authors raised the concept that TRPM2 can act as SOC channel independently of ORAI1. TRPM7, like TRPM6, serves as a bifunctional protein with the protein kinase domain fused to an ion channel. TRPM7 is permeable to divalent cations including Mg²⁺ and Ca²⁺. TRPM7 currents and histamine release evoked by either MIP-1-α or Substance P are significantly reduced in PMCs from mice lacking the TRPM7 kinase domain ¹²⁸. TRPM8 can be activated by cold and cooling compounds such as menthol. *Trpm8*^{-/-} mice showed enhanced passive systemic anaphylaxis, which could not be explained by alterations in Ag-evoked Ca²⁺ rise or degranulation measured in BMMCs, suggesting that TRPM8 expressed on non-MCs may contribute to the protective role of TRPM8 during anaphylaxis ¹²⁹.

Taken together, it is commonly accepted now that intracellular [Ca²⁺] elevation is indispensable for MC activation and numerous studies demonstrate that proteins of the ORAI and TRP families are essential constituents or modulators of Ag-induced Ca²⁺-entry, however, Ca²⁺-entry pathways evoked by stimulation of GPCRs in MCs remain unclear and will be the subject of upcoming studies.

4.5. Phosphatase regulators of MC signaling

MC effector functions are controlled by regulatory systems which act to counter-regulate excessive activation that would otherwise lead to disease. One of these regulatory systems is phosphatases that inhibit the action of the kinases whose activity is triggered by the activation of the different receptors on MCs.

The role of phosphatases in different MC models have been reviewed in recent years. While most of them have predominantly negative regulatory activities by removing differential phosphorylation in the MC signaling cascade (e.g. PTPα ¹³⁰, PTPε ¹³¹, SHIP1 ^{132, 133}, SHIP2

¹³⁴, PTEN ¹³⁵, LIPIN-1 ¹³⁶, TULA-2 ¹³⁷), others exert positive activities (e.g. DUSP2 ¹³⁸) or some even possess both regulatory properties (positive and negative) (e.g. CD45 ^{139, 140}, SHP-1 ¹⁴¹⁻¹⁴⁴, SHP-2 ^{145, 146}). Almost all the studies on the role of phosphatases in MC function were performed on phosphatase-deficient mice or BMMCs, which are mucosal-like MCs (Table 2). As reports have been made on subtle differences in phosphatase function in different types of tissue resident MCs ¹³³, future studies will be needed to compare the role of phosphatases in different MC types.

A number of the phosphatases are transcriptionally regulated by glucocorticoids (GCs) that are famous therapeutically for their anti-allergic and anti-inflammatory actions. GCs in general are known to exert their anti-allergic actions in MCs by binding to an intracellular receptor (the glucocorticoid receptor) that then interacts with a number of pro-inflammatory transcription factors such as AP-1 or NF- κ B to downregulate their activity (for a review see ¹⁴⁷). However, GCs also acting through the glucocorticoid receptor activate the expression of certain phosphatases as an indirect way of downregulating MC action. Dual specificity phosphatase 1 (also known as MAP kinase phosphatase 1) (DUSP1/MKP1) was the first glucocorticoid-inducible phosphatase gene that was reported to inhibit MC action through the dephosphorylation of the MAP kinases ERK1/2 in RBL-2H3 rat basophilic leukemia cells that have been widely used as rat mucosal MCs ^{148 149}. Later studies carried out in BMMCs showed a more complex regulatory effect of GCs. GC-induced activation of DUSP1 was found to be important for the dephosphorylation of the MAPK p38 at early time points (4-8 h), while it did not alter the phosphorylation of ERK1/2 or JNK. This shows a difference in the regulatory action of DUSP1 in BMMCs compared to RBL-2H3 cells that have been used repeatedly in many MC signaling studies. The results of the GC-induced DUSP1 expression also showed that only a subset of MAPKs was regulated by DUSP1 in BMMCs ¹⁵⁰. It was therefore not surprising that GC-mediated inhibition of pro-inflammatory cytokine and chemokine gene expression (*Ccl2*, *Il6*, *Tnf*) as well as degranulation were unaltered in BMMCs from *Dusp1*^{-/-} mice ¹⁵⁰. A search for other mechanisms that may accompany GC

regulation of MC function showed that several other phosphatases were transcriptionally upregulated by GCs in MCs. Intriguingly, in the *Dusp1*^{-/-} BMMCs, GC upregulated the expression of phosphatases such as DUSP2, DUSP4, DUSP9, and PEST domain-enriched tyrosine phosphatase (PEP)¹⁵⁰. Of these, GC-regulated phosphatases, PEP is the most studied. Unlike the other GC-regulated phosphatases, PEP is exclusively expressed in hematopoietic cells¹⁵¹. Besides, disease associated studies showed that alterations in *LYP*, the gene that codes for the human homolog of PEP is a risk factor for human diseases including inflammatory conditions such as rheumatoid arthritis¹⁵² for which GC therapy is employed¹⁵³.

PEP is a potent negative regulator of TCR signaling that acts on receptor coupled protein tyrosine kinases in T cells^{154, 155}. However, in BMMCs, *Pep* gene deletion resulted in reduced Ag-mediated MC responses such as serum histamine release, and a decreased number of degranulated MCs in the skin of the knockout compared to the wild-type mice, suggesting a positive regulatory function of this phosphatase¹⁵⁶. Glucocorticoid-mediated inhibition of JNK1/2 and PLCγ1 phosphorylation and Ca²⁺ mobilization following FcεRI cross-linking was abolished in *Pep*^{-/-} BMMCs¹⁵⁶. These studies show that although PEP expression is positively regulated by GC, PEP is not a negative regulator of MC action, an apparent discrepancy in the putative role of this phosphatase in GC action.

Thus, whole-genome expression profiling has recently been carried out using RNA-seq analysis on *Pep*^{+/+} and *Pep*^{-/-} BMMCs, to find a mechanistic explanation for the action of PEP in the regulation of MC activity and GC action (see GSE108972). These results identify PEP as a positive and a negative regulator of MC functions. In its absence, cytokine and chemokine gene expression (e.g. *Tnf*, *Il13*, *Csf2*) was downregulated, while others (e.g. *Il33*, *Ccr1*, *Il1r1*, *Tnfrsf12a*) were upregulated in response to Ag (GSE108972;¹⁵⁷). Furthermore PEP was needed for the anti-allergic action of GCs in MCs, as GC-induced negative regulation of Ag-mediated *Cox2* gene expression was attenuated in *Pep*^{-/-} BMMCs

(GSE108972;¹⁵⁷), showing that PEP is a promising target in anti-allergic therapy. In this light, some attempts have already been made to inhibit PEP activity using small molecular weight compounds. The first attempt was with an Au(I)-phosphine complex but as this was less specific¹⁵⁶, a more selective inhibitor, L75NO4 has since been described¹⁵⁸. Further studies will be needed to determine whether PEP/LYP is a valid target for future therapeutic approaches for MCs in humans.

5. Physiological functions of MCs

5.1 Chemotaxis and phagocytosis

Generally, MCs are thought of as long-lived tissue resident cells with little turn-over. The traditional thought is that there is a continuous stream of MCps from the bone marrow, circulating in the blood and replenishing peripheral sites where final maturation happens. This view has very recently been challenged by lineage-tracing experiments that show very little MC replenishment of peripheral tissues from the bone marrow²³. In many inflammatory settings, however, MC numbers strongly increase in target tissues. Local cell divisions alone – if mature, terminally differentiated MCs would proliferate at all – could not account for the massive increase in MC numbers¹⁷. Thus, MC migration remains an important and not sufficiently understood aspect of MC biology (for reviews see^{6, 12, 159-161 162}).

MC chemotaxis is often assayed *in vitro* using transwell assays or similar approaches; less often *in vivo* by injection of labeled MCs through the tail vein and analysis of their arrival at certain anatomical locations including skin, the peritoneal fluid, and the small intestine. However, many of these experiments used mouse models carrying deficiencies in the KIT/KITL system that have multiple effects and thus are problematic. Depletion of specific

MC populations such as through MC-specific diphtheria toxin expression followed by their reconstitution from bone marrow transfer has proved an important tool in many studies¹⁶³⁻¹⁶⁵. Yet, under non-challenged conditions, reconstitution was slow and seemed to occur from endogenous MCps not affected by the toxin²³.

The most prominent receptors described above, FcεRI and KIT, were also found to act in MC migration and thus IgE and SCF are potent inducers of MC migration. Ag-mediated migration, i.e. IgE/Ag-triggered FcεRI activation that causes MCs to migrate, was observed, for example, after sensitizing mice and challenging their lung airways with Ag aerosol. KIT-triggered pathways are involved not only in pro-inflammatory MC activation but also in migration. Key to migratory behavior and the required F-actin- and tubulin-dependent cytoskeletal dynamics (reviewed in¹⁶²) are RHO GTPases, particularly RAC, RHOA and CDC42, and their regulatory factors (e.g.^{166, 167}). Chemotaxis towards SCF, expressed by endothelial cells and fibroblasts, has been demonstrated numerous times *in vitro* for mouse and human cultured MCs^{168, 169}. Since some MCps populations express only low levels of KIT, in such cases, SCF may support chemotaxis that is mainly triggered by chemokines like CCR2¹⁷⁰.

The chemotactic landscape even for the same tissue is highly complex and depends on a plethora of parameters including species, strain, age, activation status, cell types involved, sex, and thus generalized predictions are very difficult as is the assignment of direct effects of chemoattractant to a specific MC type. MC chemotaxis is also induced by many other agents as diverse as lipid mediators like sphingosine-1-phosphate (S1P), leukotrienes (LTB4), prostaglandins (PGE2), and by CC or CXC chemokines^{171 172, 173}. The respective receptors are found on the surface of MCs, including two S1P receptors (S1P1, S1P2) and chemokine receptors such as CCR3, CCR5, CXCR2, CXCR4. For example, in CXCR2-deficient mice MCs fail to home efficiently to their intestine^{10, 16, 160}. Cytokine signaling may result in stimulatory or inhibitory effects on migration, e.g. the anti-inflammatory IL-10 inhibits

TNF- α or NGF-induced MC migration¹⁷⁴. Migration is affected by the environment through which MCs migrate, like extracellular matrix and/or other cell types. Indeed, some immune cells substantially support reconstitution of MC-depleted tissues. Dendritic cells, for example, but not lymphocytes promote MCps trafficking to the intestine¹⁷⁵.

When immature MCs reach a site of inflammation or mature resident MCs become locally activated near that site, these MCs (potentially further matured) do not only rapidly degranulate and express further mediators but they may also become actively involved in phagocytosis. MCs are strategically located in skin, airways, gut and other surface-exposed places to defend attacking microbes. In the skin, MCs are estimated at a density of 3,000 to 7,000 cells per mm³ and thus are prone to contact and attack invading pathogens¹⁷⁶.

Studies using BMMCs indicate that to combat pathogens, the cells can phagocytose using complement and Fc γ Rs, as well as CD48 and TLRs and thus are involved in opsonin-dependent and -independent means of defense, respectively (reviewed in¹⁷⁷). Bacteria like *E. coli*, *K. pneumoniae*, *S. faecium* are phagocytosed, using mouse BMMCs or human cord blood-derived MCs. The endosome-lysosome axis in MCs was reported to internalize and kill pathogens by either reactive oxygen species (ROS) or by non-oxidative means¹⁷⁸. These conclusions are debatable as other authors did not find intracellular destruction of bacteria, but rather extracellular attachments. Furthermore, some bacterial strains may enter MCs and thereby protect themselves from immune attack although at least some of the bacteria, e.g. *S. aureus*, will be destroyed intracellularly^{179, 180}. The pathogenic fungus *Candida albicans* is found on mucosa and thus at a site prominently populated by MCs. The contribution of MCs to defense against *C. albicans* is controversial as some authors suggest that MCs primarily fight the fungi by extracellular means¹⁸¹, while others describe phagocytotic killing of the fungus. After recognition through TLR2 and Dectin-1 receptors, *C. albicans* was reported to be phagocytosed by BMMCs and be killed by a NO-dependent mechanism¹⁸². Following

phagocytosis, MCs display pathogenic peptides on their surface thus supporting the adaptive immune response through this APC activity. A further twist of the defense strategies MCs use – although suicidal – is the formation of extracellular traps (reviewed in ¹⁸³). Here, MCs release DNA to trap microbes that stick to the viscous material. The MCs die but this process is not accidental but rather a programmed mechanism that apparently involves high ROS production.

5.2 Host defense against pathogens

MC_T are important immune cells that fight off selected species of intestinal nematodes (e.g. *Trichinella spiralis*, *Strongyloides venezuelensis*) and worm expulsion is closely associated with intestinal mastocytosis. Different kinetics of resolution of infection correlate with the ability of different mouse strains to mount intestinal MC_T responses ¹⁸⁴. Immune elimination of primary nematode infection in rat correlated with secretion of the neutral protease, rat MC protease II from mucosal MCs ¹⁸⁵. Moreover, glycosaminoglycans, such as chondroitin sulfate-A and heparin, stored in secretory lysosomes of MCs have been found to inhibit attachment and invasion of adult worms into intestinal epithelium, hence promoting worm expulsion ¹⁸⁶.

About 20 years ago, a critical protective role of serosal-type MCs was described using a murine model of acute septic peritonitis, caecum ligation and puncture (CLP) ^{187, 188}. MC-secreted TNF- α upon bacterial recognition acted as chemotactic ligand for neutrophils necessary for the eradication of bacteria. These data were collected comparing WT and *Kit*-mutant, MC-deficient *Kit*^{W^W} mice, which are known to exert additional hematopoietic abnormalities, such as neutrophilia and deficiency of peritoneal macrophages ^{189, 190}. Piliponsky et al. advised caution in formulating general statements, since they found the effect of MC deficiency to depend on mouse strain background, the nature of the mutation

causing MC deficiency, and type as well as severity of infection¹⁹⁰. Particularly, MC engraftment of MC-deficient C57BL/6-*Kit*^{W-Sh/W-Sh} mice even resulted in increased mortality during severe CLP or upon intraperitoneal inoculation of *Salmonella typhimurium*¹⁹⁰. Moreover, using a *Kit* mutant-independent mouse model allowing the conditional ablation of MCs, MCs were found to aggravate severe septic peritonitis by secreting IL-4, which in turn acted on peritoneal macrophages attenuating their phagocytosis of bacteria¹⁶⁵. Nevertheless, though the role of MCs in infection and immunity obviously is multifaceted, it is without doubt that MCs are important actors in infectious and immunological scenarios by secreting various mediators and/or regulating responses of myeloid and lymphoid immune cells.

5.3 Protection and regulation of tissue barriers

MCs are favorably located at boundaries where the host and environment meet such as the skin or the mucosa of the lung and the gastrointestinal (GI) tract. The intestinal mucosa is the largest boundary of the human body (estimated 200-400 m²), and a most challenging one because the intestine hosts a large number of bacteria that need to be controlled to prevent invasion of the host. The second largest boundary is the respiratory mucosa (estimated 100-200 m²), whereas other mucosal sites (< 1 m²) and the skin (2 m²) are rather small. Such boundaries are equipped not only with MCs but also with numerous cells of the innate and the adaptive immune system suggesting a tight communication between these cells as well as with tissue cells including epithelial cells, endothelial cells, fibroblasts, and keratinocytes. The boundaries form unique functional barriers to protect the host against toxins, pathogens and other harmful agents. MCs contribute to such barrier functions. In the intestine, MCs regulate blood flow, smooth-muscle contraction and peristalsis, mucosal secretion, and innate and adaptive immune responses^{191, 192}. This explains why MCs are involved in so many different types of GI diseases not only allergic disorders, but also GI infections and chronic inflammatory disorders, colon cancer, and other malignancies¹⁹³.

780

781 The most effective stimulus for MMC is the crosslinking of cell surface-bound IgE, either by
 782 allergen in sensitized individuals, or by parasitic Ags. However, this pathway is likely of no
 783 importance in healthy, non-allergic individuals. It is not yet very clear which stimuli might play
 784 a role in MC activation in healthy people. Little is known about IgE-independent triggers of
 785 human MMCs of the lung or the GI tract. They may differ from triggers for human skin MCs or
 786 MCs from other species^{91, 92}. Important progress has been made in understanding MC
 787 regulation by the discovery of several inhibitory mechanisms that might balance the agonistic
 788 activities of mediators discussed before¹⁹⁴. Of particular relevance to the GI tract are the
 789 anti-inflammatory cytokines TGF β 1 and IL-10, which are highly expressed in the healthy
 790 intestine. TGF β 1 inhibits the SCF-dependent growth of human GI MCs *in vitro*, and
 791 modulates the mediator profile released upon Fc ϵ RI aggregation by reducing pro-
 792 inflammatory mediator release, except for prostaglandin D2, the production of which is
 793 enhanced by TGF β 1¹⁹⁵.

794

795 Multiple studies in rodents, and to some extent also in humans, have shown that MCs have a
 796 central role in host defense against bacteria, viruses and parasites (see chapter 5.2). More
 797 recently, it was shown that GI MCs interact also with commensal bacteria in the intestine. For
 798 example, intestinal commensal bacteria regulate the migration of murine MCs into the
 799 intestine through the induction of CXCR2 ligands from intestinal epithelial cells in a TLR-
 800 dependent manner. Germ-free mice have lower MC densities in the small intestine than
 801 normal mice¹⁹⁶. On the other hand, commensal bacteria, such as *Enterococcus faecalis*,
 802 suppress degranulation of MCs, at least *in vitro*, in a MyD88-independent manner, for
 803 example, through the partial inhibition of Ca²⁺ signaling upon Fc ϵ RI cross-linking¹⁹⁷. It is
 804 tempting to speculate that MC-commensal bacteria interactions play a role in the GI barrier
 805 and in protection against barrier-related diseases. Using *in vitro* models it could be
 806 demonstrated that MC proteases are directly responsible for the increase of epithelial
 807 paracellular permeability¹⁹⁸. The MC-dependent modulation of intestinal permeability was

confirmed in humans exposed to stress¹⁹⁹. The fact that GI MCs are in intimate contact with the epithelium and nerves further suggests that MCs are involved in regulating mucosal permeability and intestinal barrier function.

Most of the physiological functions of MCs described for the gut also apply to the lung. However, a comprehensive study of MC functions in normal lung physiology is lacking. Most work focusses on MC functions in the pathogenesis of asthma and other pulmonary diseases. It was only recently pointed out that MCs and their activation contribute to lung health via innate and adaptive immune responses to respiratory pathogens.

Human skin MCs are quite distinct in their development, functions and biological properties compared to human MMCs. Skin MC are sensitive to stimulation by substance P, compound 48/80, and other basic non-immunological stimuli. Moreover, also skin MCs are important players in protective immune responses against pathogens. The fact that MCs are largely found at interfaces between the environment and the internal milieu predestines them to be involved in wound healing and tissue remodeling. Wound healing is a complex process of lysis and reconstitution controlled by a series of cell signaling proteins. MCs have been shown to play a significant role in the early inflammatory stage of wound healing and also to influence proliferation and tissue remodeling in skin²⁰⁰. In the skin, but also in the lung and intestinal mucosa, MCs are located around small vessels that are involved in vasodilation. MCs intimately communicate with endothelial cells providing MC growth factors, and in consequence of this communication MCs are involved in the regulation of extravasation of blood-derived immune cells²⁰¹. In summary, MCs regulate a large number of physiological tissue functions, namely maintenance of tissue barriers.

6. Conclusions

The mechanisms underlying the pleiotropic MC functions to support host's homeostasis have been unraveled to a large extent within the last few decades. MCs vary considerably depending on their grade of maturation, on growth and trigger factors, on tissue environmental conditions, and on the presence or absence of pathogens. MCs express a large variety of receptors allowing them to respond specifically to particular conditions. Their major effector functions, whose physiological importance has still to be elucidated in many cases, comprise the generation and release of proteases, amines and cytokines, as well as chemotaxis and phagocytosis. These functions are regulated by SCF, IL-4 and other cytokines and chemokines, by IgE- and IgG-receptor-dependent mechanisms, by innate immune receptors and other mechanisms and depend on intracellular Ca^{2+} rise that is mediated or modulated by TRP and ORAI channels in many cases. Such mechanisms explain how MCs, both in humans and in different animal species, can exert a number of physiological functions, including host defense against pathogens and regulation of mucosal and skin functions. The understanding of such mechanisms not only explains particular body functions, but also offers novel opportunities for pharmacological interventions.

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Figure Legend

Figure 1. Expression of cytokines (A, C) and chemokines (B) in human MCs. Intestinal MCs were isolated from gut (A, B), skin MCs from foreskin and breast skin (C). Cytokine/chemokine mRNA expression without stimulation (upper left quarter), after stimulation with IgE receptor-crosslinking (rcl, right quarters), and after priming with IL-4 (lower quarters) are shown. Cytokine/chemokine mRNA expressions in bold letters in the upper right quadrants were enhanced by IgE receptor crosslinking (IgErcI), while cytokine/chemokine mRNA expressions in bold letters in the lower right quadrants were enhanced by IL-4. (Source: ⁸⁴⁻⁸⁶)

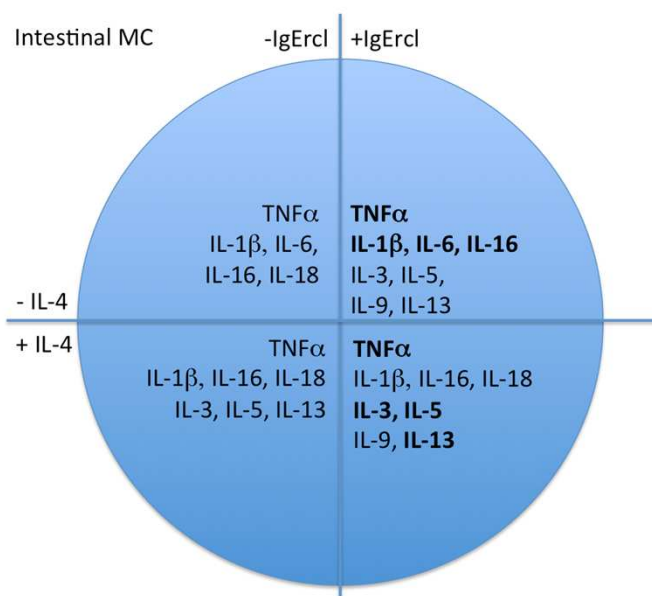
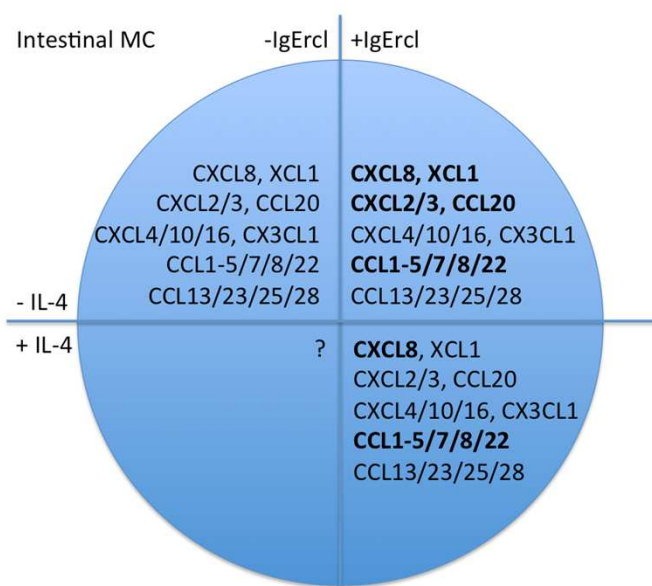
Table 1. Summary of data concerning cation channel functional importance in primary mast cell models obtained using Knock-Out and Knock-Down approaches.

Channel	Consequence of Knock-Out or Knock-Down on		<i>In vivo</i> function in channel-deficient mice	References
	Ca ²⁺ -entry	inflammatory mediator release		
Orai1	decrease	decrease	regulation of passive cutaneous anaphylactic reaction	116, 118
Orai2	increase	increase	regulation of passive systemic anaphylaxis	115
TRPC1	decrease; decreased frequency of Ca ²⁺ wave initiation	decrease	regulation of recovery in passive systemic anaphylaxis	121, 122, 123
TRPC3	decreased frequency of Ca ²⁺ wave initiation	n.d.	n.d.	121
TRPC5	decrease	decrease	n.d.	124
TRPV1	no effect	no effect	n.d.	125
TRPV2	no effect in PMCs	decrease	n.d.	125, 126
TRPV4	no effect	no effect	n.d.	125
TRPM2	decrease	decrease	n.d.	127
TRPM4	increase	increase	regulation of acute cutaneous anaphylaxis	119, 120
TRPM7	n.d.	decrease	n.d.	128
TRPM8	no effect	decrease	regulation of passive systemic anaphylaxis	129

n.d.: not detected

Table II. Phosphatase regulators of mast cell functions

Phosphatase	Function in BMMC	Function in mice	References
Negative regulators			
PTPα	Fc ϵ RI-mediated degranulation	Fc ϵ RI-mediated anaphylaxis	130
PTPϵ	Degranulation and cytokine production	Fc ϵ RI-mediated passive systemic anaphylactic reaction	131
SHIP1	Degranulation and Fc ϵ RI-mediated IL-6, TNF, and IL-5 production	Allergic inflammation and MC hyperplasia	132, 133
SHIP2	Fc ϵ RI-mediated degranulation and cytokine (IL-4 and IL-13) gene expression	-	134
PTEN	Fc ϵ RI-mediated degranulation and cytokine (IL-3 and IL-6) production and survival	Antigen and SCF-induced allergic response	135
LIPIN1	Fc ϵ RI-mediated degranulation and prostaglandin D2 release	Fc ϵ RI-mediated passive systemic anaphylaxis	136
TULA-2	Fc ϵ RI-mediated degranulation	-	137
Positive regulators			
DUSP2	Fc ϵ RI-mediated IL-6 and TNF expression	Promotes the 'K/BxN' model of inflammatory arthritis that depends on MCs and macrophages	138
Dual positive and negative regulators			
CD45	Negative: Degranulation and IL-3-dependent proliferation Positive: Degranulation and cytokine secretion	Positive: Fc ϵ RI-mediated systemic anaphylaxis	139, 140
SHP-1	Negative: IL-4, IL-13, IL-6, and TNF expression upon H ₂ O ₂ , LPS, and Fc ϵ RI cross-linking Positive: IL-3-dependent proliferation	Negative: allergic response	141, 142, 143, 144
SHP-2	Negative: Ca ²⁺ mobilization Positive: Fc ϵ RI-mediated TNF secretion	-	146

A**B****C**