Selected recent advances in understanding the role of human mast cells in health and disease

Francesca Levi-Schaffer, PharmD, PhD,[a] Bernhard F. Gibbs, PhD,[b] Jenny Hallgren, PhD,[c] Carlo Pucillo, MD,[d] Frank Redegeld, PhD,[e] Frank Siebenhaar, MD,[f,g] Joana Vitte, PhD,[h,i] Soraya Mezouar, PhD,[j] Moïse Michel, PharmD,[h,k] Pier Giorgio Puzzovio, MS,[a] and Marcus Maurer, MD[f,g] *Jerusalem, Israel; Oldenburg and Berlin, Germany; Uppsala, Sweden; Udine, Italy; Utrecht, The Netherlands; and Marseille, Montpellier, and Nîmes, France*

Mast cells are highly granular tissue-resident cells and key drivers of inflammation, particularly in allergies as well as in other inflammatory diseases. Most mast cell research was initially conducted in rodents but has increasingly shifted to the human system, with the advancement of research technologies and methodologies. Today we can analyze primary human cells including rare subpopulations, we can produce and maintain mast cells isolated from human tissues, and there are several human mast cell lines. These tools have substantially facilitated our understanding of their role and function in different organs in both health and disease. We can now define more clearly where human mast cells originate from, how they develop, which mediators they store, produce *de novo*, and release, how they are activated and by which receptors, and which neighboring cells they interact with and by which mechanisms. Considerable progress has also been made regarding the potential contribution of mast cells to disease, which, in turn, has led to the development of novel approaches for preventing key pathogenic effects of mast cells, heralding the era of mast cell–targeted therapeutics. In this review, we present and discuss a selection of some of the most significant advancements and remaining gaps in our understanding of human mast cells during the last 25 years, with a focus on clinical relevance. (J Allergy Clin Immunol 2022;149:1833-44.)

Key words: *Allergy, cancer, human mast cells, receptors, signal transduction*

When the biology of human mast cells (MCs) was reviewed 25 years ago, for a contribution to this journal,[1] the review identified many gaps in knowledge and unanswered questions. Since then, MC research has shifted its focus from mouse models to the human system, and the use of robust new scientific methods and approaches has markedly increased our understanding of human MCs. Today we know where human MCs originate from, how they develop, and where they are located throughout the body, as well as which mediators they store, produce *de novo,* and release, which stimuli activate them and how, and which neighboring cells they interact with and by which mechanisms. We have also made considerable progress in our understanding of how MCs contribute to different diseases. This, in turn, has led

From [a]the Pharmacology and Experimental Therapeutics Unit, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem; [b]the Department of Human Medicine, University of Oldenburg, Oldenburg; [c]the Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala; [d]the Laboratory of Immunology, Department of Medicine, University of Udine, Udine; [e]the Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht; [f]the Institute for Allergology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin; [g]Fraunhofer Institute for Translational Medicine and Pharmacology, ITMP Allergology and Immunology, Berlin; [h]Aix-Marseille University, IRD, APHM, MEPHI, Marseille, [i]IDESP, INSERM UA 11, Montpellier; [j]Aix-Marseille University, IRD, MEPHI, Marseille; and [k]Immunology Laboratory, CHU Nîmes, Nîmes.

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Corresponding author: Francesca Levi-Schaffer, PharmD, PhD, Pharmacology and Experimental Therapeutics Unit, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, P.O.B. 12272, Jerusalem 91120, Israel. E-mail: francescal@ekmd.huji.ac.il. Or: Marcus Maurer, MD, Institute for Allergology, Charité – Universitätsmedizin Berlin, Charitéplatz 1, D-10117 Berlin, Germany. E-mail: marcus.maurer@charite.de.

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Abbreviations used

AT: Adipose tissue
BTK: Bruton's tyrosine kinase
DC: Dendritic cell
EMR2: Mucin-like hormone receptor-like
Eos: Eosinophil
ERK: Extracellular-signal-regulated kinase ½
FcεRI: High-affinity IgE receptor
MC: Mast cell
MCp: Mast cell progenitor
MRGPRX2: Mas-Related G Protein–Coupled Receptor-X2
PAR-2: Protease-activated receptor 2
SHR: Systemic hypersensitivity reaction
Siglec: Sialic acid–binding immunoglobulin-type lectins
SYK: Spleen tyrosine kinase
TLR: Toll-like receptor

to the development of novel approaches for preventing key pathogenic effects of MCs, ringing in the era of mast cell–targeted therapeutics. This is not to say that "the riddle of the human mast cell"[2,3] is solved: we are far from that.

This review is meant to provide an overview of our current knowledge of human MCs, taking stock of what we know and do not know. Our focus is on areas of human MC biology with high relevance for clinical application. Specifically, we provide an update on the heterogeneity of human MCs and their drivers and markers, which may serve the development of MC subtype-specific therapies. We review novel activating and inhibitory receptors of human MCs, some of which are already targets for treatments under development for MC-driven diseases. We review the latest insights on human MC signal transduction and its inhibition, a promising mechanism of action of novel oral therapeutics for patients with MC diseases. We also summarize the results of recent studies of how human MCs interact with other cells including the mechanisms involved. Finally, we describe and discuss where we stand regarding human MC biomarkers, concentrating on tryptase and their role in the diagnostic workup and treatment optimization for patients with allergies and other MC-driven diseases. For each of these areas of human MC biology, we provide the current "status quo" of knowledge as well as unanswered questions that need to be addressed by future research.

THE HETEROGENEITY, ORIGIN, AND DEVELOPMENT OF HUMAN MCs

Human MCs are historically divided into subclasses on the basis of their expression pattern of proteases. MCs expressing tryptase and chymase are comparable to the mouse connective tissue–type MCs and dominate in the skin, whereas MCs that express only tryptase resemble mouse mucosal MCs and are common in the airways. However, this division is an oversimplification, as demonstrated by multiple studies over the last decade. Striking differences are found between human MC populations in different and within the same organs, with profound differences in maturation, granule content, and expression of activating and inhibitory receptors. For example, the Mas-Related G Protein–Coupled Receptor-X2 (MRGPRX2) is highly expressed by human skin but not lung MCs,[4] and high-affinity IgE receptor (FcεRI) expression levels vary widely between different MC populations of the human lung.[5]

The Functional ANnoTation Of the Mammalian genome (FANTOM) 5 project identified fundamental differences between human MCs analyzed *ex vivo* and differentiated *in vitro*.[6] The transcriptome of human skin MCs stands out from those of other immune cells by the presence of MC-specific transcripts, the absence of transcripts common in other innate immune cells, and the strong expression of genes weakly expressed by other immune cells. This finding was confirmed by other studies showing that MCs have a strikingly different proteome from other lineages and that their uniqueness (in gene expression) is conserved between mice and men.[7,8] Striking differences are also found between c-kit$^+$ skin cells differentiated to MCs *in vitro* and primary skin MCs.[6] The heterogeneity of the genomic and transcriptional changes that occur in human MCs in response to different triggers such as IgE-mediated *versus* IL-33–induced activation has also been revealed.[9] Moreover, a unique subepithelial MC phenotype induced by type 2 inflammation was recently described in nasal polyps from patients with chronic rhinosinusitis and aspirin-exacerbated respiratory disease.[10] Given that individual MCs are exposed to their local environment of growth and differentiation factors, and over time tuned by many different activating and inhibitory signals, they should all be unique to some extent. However, human MC diversity is rooted in what happens at the very beginning of life.

The earliest immune cells appear in the yolk-sac approximately 2 to 3 weeks postconception, and subsequently, originate in the aorta-gonad-mesonephros before the fetal liver starts to dominate.[11] In line with this, MCs with an immature hypogranular morphology were recently detected in the yolk-sac and fetal liver based on KIT and Tpsab1 expression in single cells.[12] Herein, fetal liver MCs were also associated with high expression of GATA-binding factor 2, Mitf, Hes1, and nuclear receptor 4a3, previously linked to MC development (Fig 1, *A*). Fetal skin and airway MCs were described early by morphological studies.[13] More recently, CD45$^+$ CD117$^+$ cells were demonstrated in fetal skin 9 to 11 weeks postconception. They increased in number and granularity over time, and became chymase immunopositive in the 18- to 24-week-old fetus,[14] suggesting that progenitors, later differentiating into tryptase- and chymase-positive MCs, populate tissues such as the skin and airways during fetal development (Fig 1, *A*). Human fetal skin contains both granulated and immature MCs, some of which are IgE$^+$, indicating MC sensitization *in utero*.[15]

In the adult bone marrow, MCs are known to arise from CD34$^+$ CD117$^+$ cells.[16,17] As in other species, immature human MC progenitors (MCps) exit the bone marrow to populate peripheral tissues via blood circulation (Fig 1, *B*). A rare population of lineage Lin$^-$ CD34^{hi} CD117$^{int/hi}$ FcεRI$^+$ MCps was first discovered in peripheral blood.[18] Circulating MCps are lymphocyte-sized cells with few granules, which express tryptases (Tpsab1/Tpsab2) and carboxypeptidase A3 (Cpa3), but lack chymase (Cma1). This suggests that Cma1 is expressed at a later developmental stage, for example, at specific sites in the periphery, or that MCps preferentially give rise to tryptase-positive cells. Later, a similar but more frequent MCp population was demonstrated in healthy adult bone marrow.[19] Although KIT signaling is critical for the maturation and survival of human MCs, this pathway is dispensable for the development and survival of human MCps, resulting in intact levels in patients treated with the tyrosine kinase inhibitor imatinib.[20]

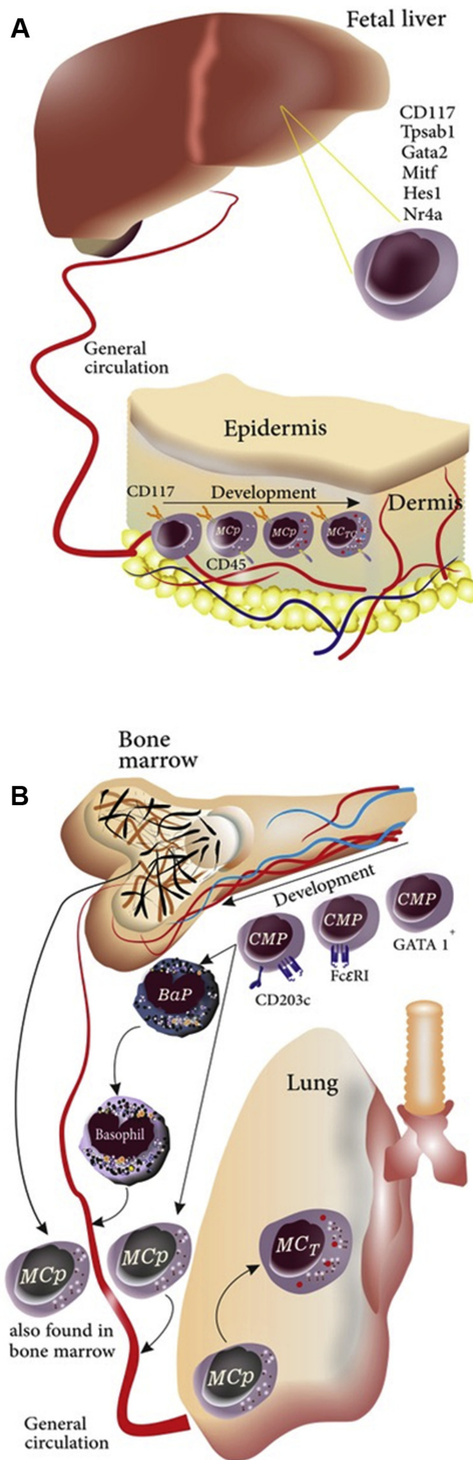

FIG 1. Differences in the origin and development of MCs contribute to the extensive heterogeneity found in humans. **A,** Fetal MCps originating in the yolk-sac or fetal liver populate preferentially the peripheral connective tissues, such as the skin, where they mature into tryptase- and chymase-positive MCs (MC_{TC}). **B,** Later during the fetal development and in adults, the bone marrow becomes the major source of human MCps, which are found within the CMP population. Recent data suggest that human bone marrow–derived MCs originate from CMP, which are GATA1$^+$, possibly differentiate to express FcεRI, and thereafter CD203c from which basophil progenitors (BaPs) and MCps are derived. We speculate that in adults, MCps continuously home, or are being actively recruited to the peripheral tissues due to biological signal substances induced by infections, inflammation, and stress. Within the tissues, MCs likely develop in concert with tissue-specific signals and change continuously due to activating and inhibitory signals due to, for example, inflammation, infection, and aging to yield an enormous diversity of human MCs in the periphery. *CMP*, Common myeloid progenitor.

TABLE I. The top 10 ToDos in human MC research, in the opinion of the authors and not ranked by priority

1. Identify MC-activating mechanisms in MC-driven diseases, eg, urticaria and MC activation syndrome
2. Identify blood markers of human MC numbers and activation status, ideally organ specific
3. Characterize effects of MC-silencing and MC-depleting drugs in disease and on health
4. Identify and characterize drivers of human MC expression of receptors and mediators
5. Investigate and characterize human MC-heterogeneity and underlying mechanisms
6. Identify the physiological and disease-preventing and disease-limiting functions of human MC
7. Explore the mechanisms and relevance of human MC crosstalk with other cells
8. Develop noninvasive imaging methods for assessing human MCs *in vivo*
9. Continue to build an interactive, collaborative, and global scientific community of human MC researchers
10. Increase awareness of MC-driven diseases and advocate for the development of curative treatments

Human MCps lack CD45RA and express the IL-3 receptor (CD123).[15,20] Therefore, these cells are a part of the common myeloid progenitors, defined as Lin$^-$, CD34$^+$, CD38$^+$, CD123$^+$, CD45RA$^-$ cells. Defined subpopulations of common myeloid progenitors give rise to distinct oligopotent progenitors with either MC/basophil/eosinophil or neutrophil/monocyte potential.[21] For the GATA1$^+$ MC/basophil/eosinophil progenitors, this bifurcation precedes their separation from megakaryocyte/erythrocyte potential. In a separate study, MC/basophil and erythroid potential were found among FcεRI$^+$ common myeloid progenitors, indicating that CD203c expression separates cells with MC/basophil potential from cells with the ability to develop into erythrocytes.[22] Altogether, these data suggest that human MCps and basophils codevelop until they commit to their lineage within the bone marrow. We speculate that the bone marrow is the major site of MC origin in adults, and that MCps home or are being actively recruited to the peripheral tissues such as the lung throughout life (Fig 1, *B*).

Although knowledge of the origin and development of MCs with specific phenotypes has markedly expanded over recent years, new questions have arisen. It will be imperative to determine how MC development and heterogeneity are affected by disease onset and aging and to differentiate between these processes. Worth noting is that human MCps become activated by IgE cross-linking and as a result present CD63 and LAMP-1 on their plasma membrane.[23] Future studies should also address the function of MCps, and the consequences of the expression changes that occur in MCs during their and our lives (Table I).

ACTIVATING HUMAN MC RECEPTORS

Many new activating human MC receptors have been identified in the last 25 years. Human MC activation via these receptors

results in mediator release, priming, differentiation, proliferation, and/or migration.[24]

Human MCs express a substantial number of activating receptors, more than any other human cell type. These activating receptors induce the release of mediators in response to a multitude of endogenous and exogenous triggers such as damage-associated molecular pattern and pathogen-associated molecular pattern molecules, purine nucleosides, neuropeptides, complement, endothelins, and lipid mediators (recently reviewed in Redegeld et al[25] and Yu et al[26]).

Of particular interest is MRGPRX2, which is highly expressed on human cutaneous MCs.[27,28] Activation of MRGPRX2 is triggered by a wide variety of cationic compounds such as major basic protein and eosinophil cationic protein, neuropeptides (substance P, neuropeptide Y, somatostatin, cortistatin, vasoactive intestinal peptide), host defense peptides (LL-37 and human β-defensins), peptidergic drugs (icabitant, cetrorelix, leuprolide, ocreotide, sermorelin), small-molecule drugs (nicotine antagonists, nonsteroidal neuromuscular-blocking drugs), opioid drugs, and endogenous prodynorphin peptides.[29] Targeting MRGPRX2 is of therapeutic interest in drug hypersensitivity, neurogenic inflammation, allergic asthma, rheumatoid arthritis, and skin diseases such as chronic urticaria, atopic dermatitis, rosacea, and chronic pruritus.[29,30] No specific inhibitors of MRGPRX2 are presently available for clinical use, though retinoic acid was shown to decrease the expression of MRGPRX2 in human skin MCs[31] (Fig 2, *A*).

Another interesting G-coupled receptor is the adhesion G protein–coupled receptor also known as EGF-like module-containing mucin-like hormone receptor-like (EMR2). Adhesion G protein–coupled receptor is involved in IgE-independent MC activation in vibratory urticaria. Patients with autosomal-dominant vibratory urticaria have a missense substitution in this receptor, leading to a reduced threshold for activation by dermatan sulfate in the skin.[32,33] Of note, alpha/beta tryptase tetramers can cleave and activate adhesion G protein–coupled receptor/EMR2, which is another potential mechanism for vibratory urticaria linked to hereditary alpha-tryptasemia.[34]

It has long been recognized that IgE-dependent MC activation is enhanced by costimulation of KIT receptors with stem cell factor (SCF). Recently, several other cytokines have been shown to act as priming factors that enhance IgE-dependent MC degranulation, including IL-6 and IL-33.[35,36] In contrast, IL-4 does not affect degranulation but was shown to have striking priming effects by shifting human MC cytokine production away from proinflammatory ones (eg, IL-6) to favor the T_H2-type cytokines IL-3, IL-5, and IL-13[37,38] as well as thymic stromal lymphopoietin.[39] Interestingly, in intestinal human MCs, IL-4 acts as a primer together with SCF to differentially increase IgE-dependent chemokine expressions (eg, CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL18, CCL20, CXCL2, CXCL3, CXCL8, and XCL1), while inhibiting the expression of CCL20, CXCL2, and CXCL3[40] (Fig 2, *B*).

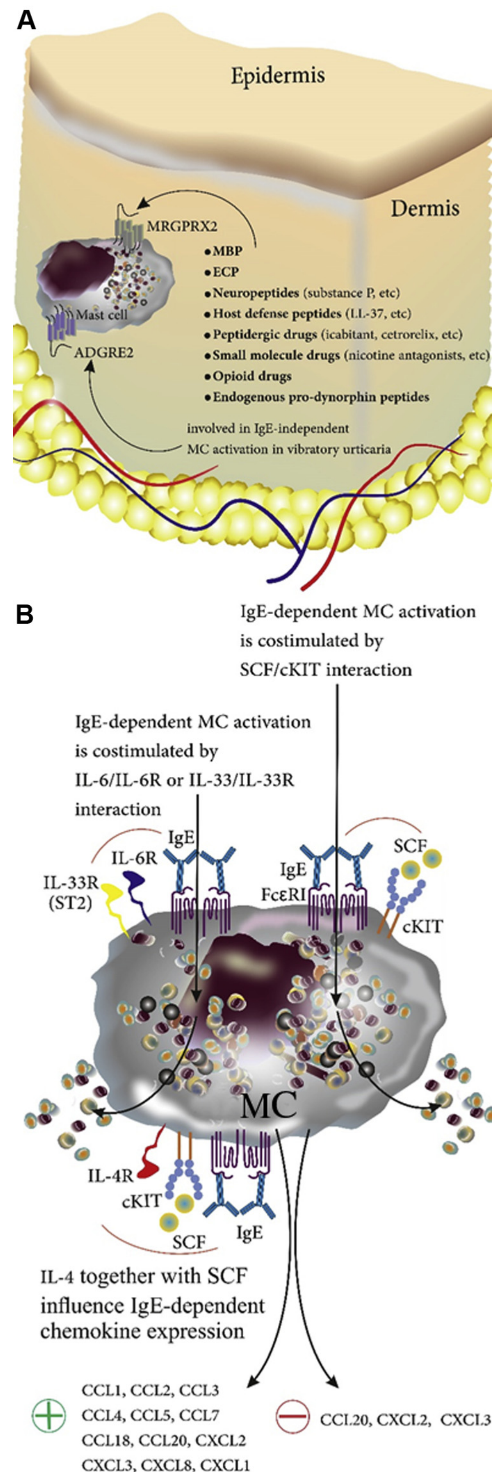

FIG 2. Human IgE-dependent and IgE-independent MC activation. **A,** Non–IgE-dependent MC activation mechanisms in the skin. **B,** IgE-dependent activation of MCs enhanced by IL-6/IL-33 or SCF/KIT.

INHIBITORY HUMAN MC RECEPTORS

Human MCs express various inhibitory receptors on their cell surface such as the inhibitory low-affinity IgG receptor FcγRIIB, members of the CD300 family, and the sialic acid–binding immunoglobulin-type lectins (Siglec) family. CD300a is constitutively expressed on MCs, and co-cross-linking of CD300a and IgE with a bispecific antibody was shown to inhibit human MC activation and the development of IgE-driven anaphylaxis and asthma in preclinical murine models.[41] Of the Siglec family inhibitory receptors, Siglec-6, Siglec-7, and Siglec-8 are expressed at considerable levels on human MCs. Cross-linking of Siglec-6[42] or Siglec-8[43] results in the inhibition of IgE-mediated MC

activation, whereas simultaneous co-cross-linking of Siglec-7 with FcεRI is needed to elicit inhibitory activity.[44] Interestingly, lirentelimab (AK002), a humanized anti–Siglec-8 antibody, showed promising activity in patients with eosinophilic gastritis and duodenitis,[45] and clinical trials in other indications such as eosinophilic esophagitis (NCT04322708), kerato- and perennial allergic conjunctivitis (NCT03379311), antihistamine-resistant chronic urticaria (NCT03436797), and indolent systemic mastocytosis (NCT02808793) are ongoing.

HUMAN MC SIGNAL TRANSDUCTION

In recent years, many studies have contributed to our understanding of human MC signal transduction, especially regarding the role of spleen tyrosine kinase (SYK) and Bruton's tyrosine kinase (BTK). Both SYK and BTK play an essential role in early signaling events following FcεRI-mediated MC activation, by phosphorylating various downstream signaling proteins governing mediator synthesis and release.

The importance of SYK as a potential therapeutic target in blocking human MC activity is supported by reports that the SYK inhibitors R112 and JTE-852 block IgE-dependent histamine release as well as the synthesis of eicosanoids and cytokines in cultured human MCs.[46-48] More recently, Ramirez Molina et al[49] demonstrated that *ex vivo* dermal application of the SYK inhibitor GSK2646264 inhibited the release of histamine from skin MCs. Furthermore, SYK inhibitors have been shown to inhibit IgE-dependent contraction of human precision-cut lung slices as well as the release of histamine and leukotrienes.[50] This highlights the therapeutic potential of targeting SYK in MC-driven diseases such as allergic asthma and chronic urticaria,[51] in line with earlier reports indicating clinical efficacy of the SYK inhibitor R112 in allergic rhinitis.[52,53]

BTK is a member of the TEC family kinases and contains pleckstrin homology domain that binds to PIP3, the stimulatory product of phosphatidylinositol-3-kinase activation, which is crucially involved in FcεRI signaling. BTK has long been known to be involved in murine MC signaling.[54,55] The development and use of specific BTK inhibitors, such as acalabrutinib, have led to important insights into the role and relevance of BTK in human MCs. BTK inhibition blocks IgE-mediated human skin MC degranulation and cytokine release and substantially reduces anti–IgE-induced contractility of human bronchi.[56] Interestingly, acalabrutinib completely prevented IgE-mediated anaphylaxis in a humanized mouse model containing human MCs and basophils.[56] BTK inhibition may have therapeutic potential in IgE-driven MC diseases, although basophil responses are similarly known to be regulated by BTK.[57,58] Fenebrutinib, remibrutinib, and other BTK inhibitors are currently being developed for the treatment of chronic urticaria and other MC-driven diseases.[59] BTK may also play a role in the KIT-independent proliferation of neoplastic MCs,[60] although a later study showed heterogeneous effects of various BTK inhibitors in terms of inhibiting the growth of HMC1 cell lines, even though IgE-dependent basophil activation was blocked by all inhibitors.[57]

Pharmacological evidence also suggests an important role for calcineurin in human MC signal transduction, where cyclosporine and FK506 were shown to inhibit IgE-dependent lung MC degranulation[61] and, in a separate study, pimecrolimus blocked the release of histamine, tryptase, LTC_4, and TNF-α from skin MCs.[62] However, although calcineurin was detected in lung MCs, Harrison et al[61] also observed that inhibition of Ca^{2+}-dependent protein phosphatase activity by various calcineurin inhibitors did not reflect their MC-stabilizing properties. Although calcineurin inhibitors have provided a new therapeutic approach for treating MC-related disorders in the past decades, it is unclear whether their attenuation of MC degranulation is due to the specific targeting of calcineurin. This is because the phosphatase promotes nuclear factor of activated T-cell translocation to the nucleus, subsequently regulating MC cytokine synthesis, but there are as yet no studies that account for calcineurin regulating other signaling pathways leading to acute mediator release from MCs.

Calcineurin-nuclear factor of activated T-cell signaling was also reported to be involved in the IgE-independent production of IL-8 from the LAD2 human MC line stimulated via the Toll-like receptor (TLR)-2.[63] Many TLRs (eg, TLR2, TLR4, and TLR9) are now known to activate human MCs to generate proinflammatory cytokines and, to a lesser extent, eicosanoid synthesis, but they are not generally associated with degranulation (reviewed in Sandig and Bulfone-Paus[64]). This preferential induction of cytokine signaling was shown to be potentiated by simultaneous IgE-receptor triggering of human peripheral blood–derived MCs,[65] although the mechanisms have not been elucidated in primary human MCs.

TLR2 stimulation of LAD2 cells was also shown to be potentiated by substance P priming, enhancing the activation of key mitogen-activated protein kinases including JNK, p38 mitogen-activated kinase, and extracellular-signal-regulated kinase 1/2 (ERK) as well as the nuclear translocation of c-Jun, nuclear factor-kappa B, and activating transcription factor 2.[66] Substance P–mediated priming of TLR2-activated LAD2 cells did not result in degranulation but in enhanced release of LTC_4 and IL-8 as well as mRNA expression for TLR2, TLR4, TLR8, and TLR9.[66] Interestingly, ERK activation was recently shown to play an essential role in the ability of primary human skin MCs to generate proinflammatory cytokines on MRGPRX2 activation by substance P as well as FcεRI-cross-linking,[67] indicating ERK as a therapeutic target for blocking MC-related cytokines in both IgE-dependent and IgE-independent diseases.

ERK was also recently shown to be centrally involved in anaphylatoxin (C3a and C5a)-mediated signaling in human blood–derived MCs and was potentiated by prior treatment of the cells with the alarmin IL-33, a cytokine that exacerbates allergic inflammation by T_H2-type immune cell activation.[68] IL-33 selectively stimulates the release of CCL1, CCL2, IL-5, IL-8, IL-13, and TNF-α in human skin MCs, but, in contrast to the above, this was associated with p38 mitogen-activated protein kinase activation and nuclear factor-kappa B.[69] Short-term priming with IL-33 also potentiated IgE- and MRGPRX2-mediated skin MC activation[69] and was largely p38 mitogen-activated protein kinase–dependent but long-term IL-33 exposure attenuated degranulation.[70,71]

The hypoxic signaling pathway involving the stabilization of hypoxia-inducible factor 1-α (HIF-1α) was also shown to play a crucial role in enabling the LAD2 human MC line to generate the angiogenic cytokine vascular endothelial growth factor as well as TNF-α following TLR2-, TLR4-, or IgE-mediated stimulation.[72] The HIF-1 transcription complex facilitates cellular adaptation to inflammatory stress and low oxygen availability, directly regulating the production of angiogenic factors and glycolysis, which protects cells against ATP depletion. Interestingly, the HIF-1 pathway was shown to be upregulated under normoxic conditions

in both human MCs and basophils following either IgE-mediated or IgE-independent stimulation.[72,73] Furthermore, HIF-1 was shown, at least in IgE-mediated signaling, to be controlled upstream by the mammalian target of rapamycin kinase,[74] indicating a potential therapeutic role for mammalian target of rapamycin inhibitors in preventing MC angiogenic and proinflammatory cytokines generation.

Important unmet needs regarding human MC signaling include the translation of findings on stimulatory and inhibitory signaling pathways from studies with MC lines and cord blood–derived MCs to primary human MCs.

Also, given the substantial functional and pharmacological heterogeneity of MCs across locations and subtypes, there is a crucial unmet need to comprehensively analyze IgE-dependent (and IgE-independent) stimulatory and inhibitory signaling pathways in primary human MCs isolated and purified from various tissues.

MC INTERACTIONS WITH IMMUNE AND NONIMMUNE CELLS

Human MCs interact with a plethora of immune and nonimmune cells, via surface receptors and released cytokines and chemokines, resulting in bidirectional functional modulation. Translating a large body of evidence from mouse work, recent studies of human MCs have significantly extended our understanding of interactions with other cells especially with eosinophils (Eos) as well as T and B cells.[75]

The allergic effector unit, that is, physical and mediator-facilitated crosstalk between MCs and Eos, importantly contributes to allergic and nonallergic inflammation. The human allergic effector unit has mainly proinflammatory outcomes, with increased MC and Eos activation and the release of proinflammatory and chemotactic mediators.[76,77] Physical contact between the 2 cells, for example, in human nasal polyps and asthmatic bronchi, is mediated by MC-expressed CD48 and Eos-expressed 2B4 receptors,[76,77] and is also hypothesized to contribute to sickle cell anemia.[78] Human MCs and Eos also physically interact in the gut lamina propria of patients infected with *Trypanosoma cruzi*, and MCs recruit Eos to the gut via tryptase release and protease-activated receptor 2 (PAR-2) activation on Eos.[79] Human MCs and Eos aggregate in lesions of patients with eosinophilic esophagitis, and esophageal Eos produce and release IL-9, which regulates MC maturation and activation.[80] Interaction of MCs with Eos is also observed in chronic spontaneous urticaria, and it is feasible that MCs are the main driver for Eos infiltration in urticarial skin lesions via their chemotactic mediators.[81]

The proximity between MCs and T cells and/or B cells has been demonstrated in several homeostatic and inflammatory conditions. Human MCs can directly induce T-cell activation either by direct antigen presentation, ultimately modulating T-cell proliferation, cytokine production and release, and T-cell differentiation, or by polarization. IFN-γ–primed human skin–derived MCs can take up and process antigens and act as antigen-presenting cells.[82] Moreover, human MCs can shape immune responses by taking up antigens recognized through FcεRI-bound IgE and then transferring them to professional antigen-presenting cells that can activate T cells.[83]

As demonstrated *in vitro*, human MCs can shuttle antigens as well as surface molecules and cytokines to T cells and B cells via exosomes.[83,84] Moreover, human MCs release exosomes that carry immunologically relevant molecules on their surface, such as MHC class II, CD86, LFA-1, ICAM-1, and OX40L, which regulate T-cell activation.[85-87] These exosomes can induce T-cell proliferation and expression of IL-2 and IFN-γ,[86] and promote T_H2 polarization via OX40L-OX40 interaction.[87] In addition, depending on the activating stimulus, MCs can release different cytokines that influence T-cell polarization. For example, MC-derived IL-1β induces T_H17-cell expansion within the memory $CD4^+$ T-cell population. In addition, MCs can drive T-cell polarization through priming of dendritic cells (DCs), inducing them to release high levels of IFN-γ and IL-17, thereby promoting T_H1 and T_H17 responses.[88]

Less is known regarding the consequences of crosstalk between human MCs and B cells. Human $CD40L^+$ MCs and IL-6 induce B-cell expansion and their differentiation to $CD138^+$ plasma cells as well as regulate isotype switch and secretion of IgA.[89]

In addition to Eos and T and B cells, human MCs importantly interact with DCs, endothelial cells, sensory nerves, fibroblasts, and adipocytes. Human skin MCs and DCs interact via surface integrins, forming synapses, and this interaction is followed by cytoskeletal rearrangements. This results in MC granules being directed toward DCs and allows for IgE-bound allergens to be transferred to DCs for processing and subsequent T-cell activation.[90] Direct contact between human MCs and DCs qualitatively and quantitatively alters their release of mediators, upregulating proinflammatory mediators.[90,91] This is probably due to cross-linking of DC-bound antigens with IgE/FcεRI on MCs. Last but not least recent, evidence has described interactions between MCs and innate immune cells.[75]

Human MCs also importantly interact with endothelial cells. Using the human MC line LUVA, it was recently shown that MCs impair the formation of new blood vessels when cocultured with fetal human pulmonary microvascular endothelial cells. This effect is due to MC-mediated blockade of endothelial cells interactions via chymase release. Interestingly, pulmonary MCs that express only tryptase but not chymase did not significantly affect fetal human pulmonary microvascular endothelial cells tube formation.

Human MCs are known to interact with peripheral nerves in the skin, creating functional units that increase in number during pathologic events.[92,93]

Human peripheral blood–derived MCs were recently demonstrated to increase migration of lung fibroblasts after coculture and IgE-mediated activation.[94] This effect was detected also after coculture with the human MC line LAD-2, though FcεRI/IgE cross-linking did not significantly affect the migration of lung fibroblasts.[94] The increased migration is due to human MC tryptase and PAR-2 expression on fibroblasts.[94] MC-derived tryptase was also found to increase proliferation of fibroblasts via PAR-2.[95]

MCs were found to be resident in adipose tissue (AT) and to accumulate in the AT of obese individuals.[96] This is due to adipokines released by adipocytes, which cause MC degranulation, resulting in an inflammatory state. Thus, MCs are thought to contribute to adipogenesis, obesity, and insulin resistance.[96,97] However, recent evidence showed that, in healthy individuals, adipose tissue–associated MCs promote the transformation of white AT into the beige type, associated with higher energy consumption after exposure to cold.[98] This is due to MC-derived histamine, which induces uncoupling protein 1 expression in the white AT, thus promoting its metabolic reprogramming into beige AT.[98]

Important questions that remain unanswered include how the interactions of human MCs with other cells can be pharmacologically modulated in relevant disease conditions. Further investigations are likely to discover additional interactions of MCs and their cellular neighbors that contribute to homeostasis or the pathogenesis of diseases.

HUMAN MC MEDIATORS: TRYPTASE

MC tryptase has been the most widely used serum biomarker for human MCs since the 1980s, but proteomic approaches may well extend the spectrum of clinically available MC mediators.[99] In parallel, insight was gained into the genetics, function, and pathophysiology of MC peptidases such as tryptase, chymase, and carboxypeptidaseA3. Because of major advances in recent years, this section will focus mainly on tryptase.

Human tryptase isoforms are secreted mainly by MCs and can be measured in the blood (Fig 3). Their assessment is informative of MC burden and activity. Over the last 25 years, the "total tryptase assay" has been adopted worldwide. Established in the late 1990s, this assay differs from earlier ones by its ability to detect and quantitate circulating tryptase not only in anaphylaxis (systemic hypersensitivity reaction, SHR) and mastocytosis but also in healthy individuals, with significant interindividual variations.[100,101] By assessing the involvement of MCs through tryptase measurements and the implementation of a consensus algorithm, taking into account acute and baseline tryptase measurements,[102-104] SHRs can now be addressed by endotype analysis.[101,105] Conversely, persistently increased tryptase levels may be indicative of a clonal MC disorder, with levels greater than or equal to 15 μg/L suggesting systemic forms of mastocytosis[92] and greater than or equal to 20 μg/L being a minor criterion for systemic mastocytosis[93] (Fig 4). SHRs and clonal MC disorders can be life-threatening, although they are rare.

Human tryptase hosts considerable genetic polymorphism (recently reviewed in Sprinzl et al[106]). Considering the secreted human α- and β-tryptase isoforms, there are 3 common genotypes: 0α:4β, 1α:3β, and 2α:2β.[106] Their frequency greatly varies among ethnic groups.[107] The 0α:4β genotype, also known as an α-tryptase deficiency, was reported in 29% of individuals.[108] Nonfunctional β-tryptase alleles exhibit frequency variations according to ethnicity, with people of African descent having comparatively more functional alleles than those of Asian descent.[107] This finding suggests a trade-off between the proinflammatory and defense roles of tryptase and may have implications for therapeutic targeting of tryptase.[107,109] Less frequent but with an already established effect as an anaphylaxis modifier, hereditary α-tryptasemia is an autosomal-dominant trait due to extra gene copies of α-tryptase. The prevalence of hereditary α-tryptasemia in the general population is 4% to 7.5%, associated with baseline tryptase levels of 8 μg/L or higher and an increased risk of SHRs.[94-98] Its association with evocative symptoms is debated.[110] From a pathophysiological viewpoint, excess α-tryptase production supports increased α/β-heterotetramer formation, which exerts distinct effects from those of β-tryptase tetramers.[34] Indeed, α/β-heterotetramers cleave EMR2α, destabilizing its association with the EMR2β subunit and thus facilitating vibration-induced MC degranulation, such as in vibratory urticaria.[34] Another effect restricted to α/β-heterotetramers is their ability to activate PAR-2, which might explain previously described effects on bronchial and intestinal smooth muscle, endothelium, and neurons.[34] Of particular interest was the observation of a dose-response effect related to the number of α-tryptase alleles.[34] Thus, assessing the number of α-tryptase gene copies might not only complement diagnostic tryptase measurements in patients with SHRs[99] but also contribute to fine-tuning of tryptase-targeted therapy. The pathophysiological implication of MCs and their peptidases in asthma endotypes[111,112] has led to intensive therapeutic research.[109,113] MC numbers and mediators are increased in severe asthma independently of the T_H2 response, and the count of functional β-tryptase alleles is inversely

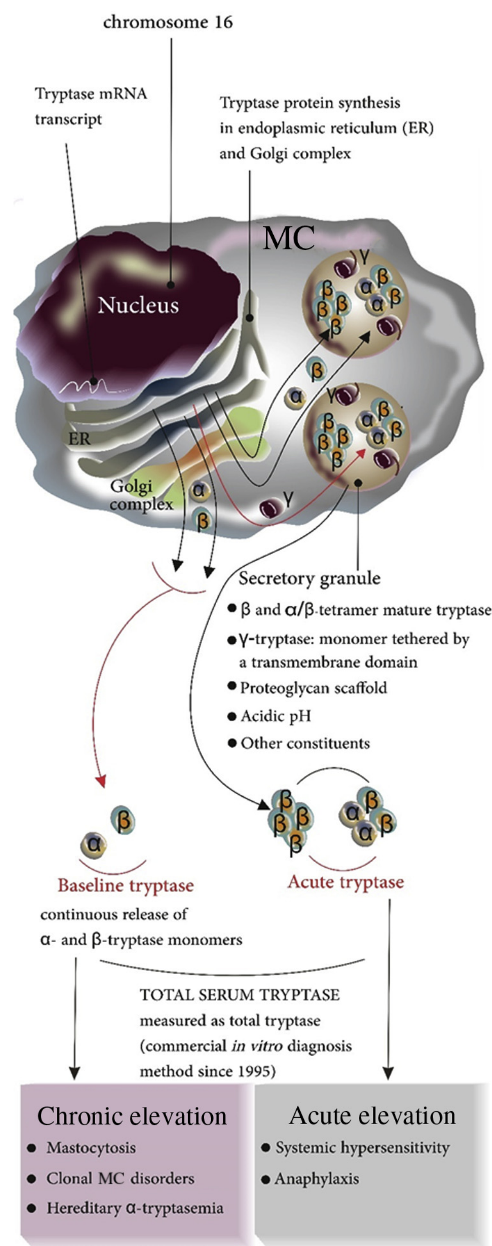

FIG 3. Human tryptases. Of the 4 human tryptase isoforms, only α- and β-tryptase are secreted, either continuously as monomeric protryptases, or following secretory granule exocytosis of tetrameric mature tryptase. The continuous secretion of α- and β-protryptases accounts for the baseline tryptase level, whereas MC activation events result in a transient increase in tryptase levels, known as "acute tryptase" comprising the baseline monomers plus exocytosed tetrameric mature forms.

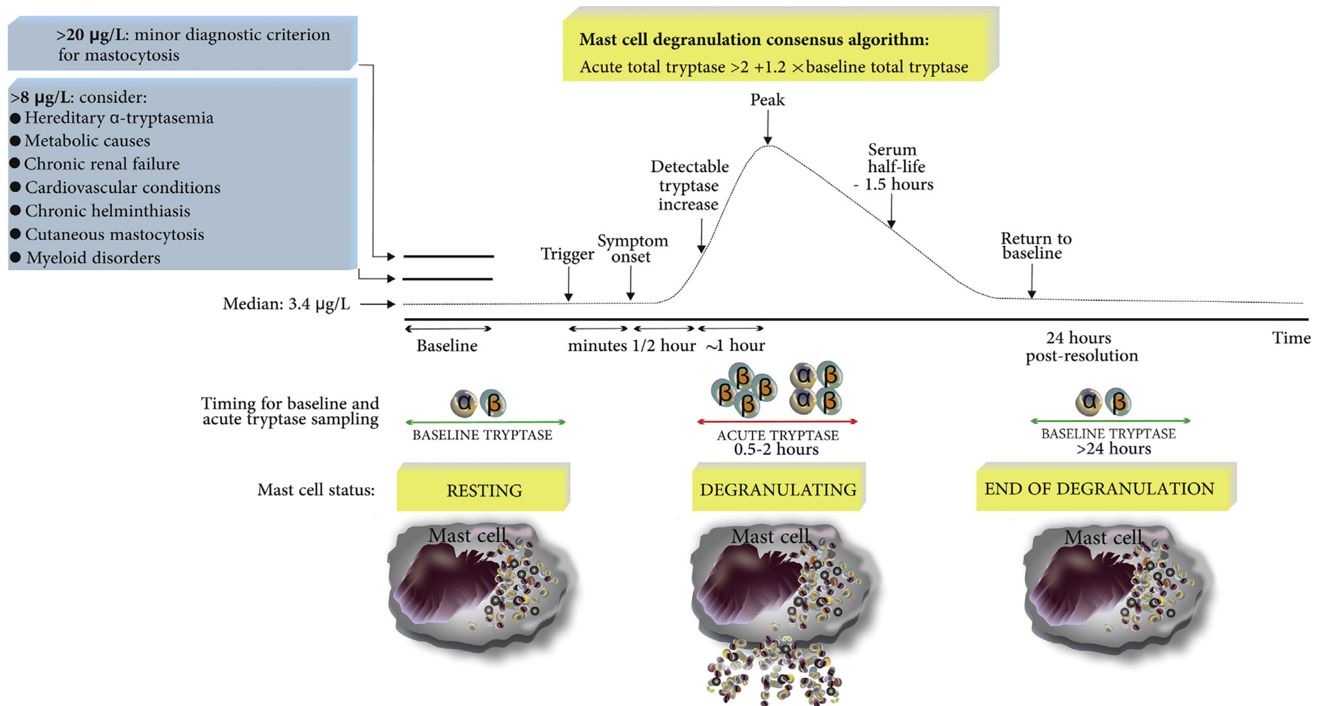

FIG 4. Serum tryptase: kinetics and sampling during MC degranulation. Detection of an acute elevation in tryptase following an MC activation event with systemic reaction is optimal only after 30 minutes following the onset of symptoms, plateauing around 1 hour, then decreasing with an apparent half-life estimated at 1.5 hours, and returning to baseline. Tryptase assessment during an MC activation event requires 2 samples adequately collected: one at 30 minutes to 2 hours after the onset of symptoms ("acute tryptase") and the other either 24 hours after complete resolution of symptoms and signs of MC degranulation, or before the MC activation event. The interpretation requires the use of the consensus algorithm; that is, MC activation is confirmed if sAT > 1.2 sbT + 2 μg/L. *sAT*, Serum acute tryptase; *sBT*, baseline tryptase.

associated with the efficiency of anti-IgE therapeutics, prompting the design of an inhibitory antibody against β-tryptase with promising preclinical efficiency.[113]

Circulating tryptase variations can contribute to defining endotypes of various diseases with MC involvement. Because of space limitations, we will cite only 1 further example, relating to aspirin-exacerbated respiratory disease. In a subset of patients, aspirin-induced MC activation in the lower airways may result in bronchoconstriction, concomitant with a transient increase in circulating tryptase, despite prophylaxis with drugs blocking the type 1 receptor of cysteinyl leukotrienes.[114] These findings underline the role of MC-derived mediators acting on smooth muscle and suggest endotype-based stratification.

RESPONSE OF MCs TO PATHOGENS

When pathogens attack, human MCs importantly modulate innate and adaptive immune responses, owing to (1) their strategic skin and mucosal location in direct relation to the environment, (2) expression of a wide variety of pattern recognition receptors, (3) direct antimicrobial action including phagocytosis, and (4) indirect strategies (Fig 5). The latter include extracellular traps or the release of cytonemes and exosomes, proinflammatory and antimicrobial products including cytokines and chemokines such as TNF, IL-10, IL-12, or IL-13.[115-118] Infections with *Listeria monocytogenes*,[119] *Pseudomonas aeruginosa*,[120,121] *Streptococcus pyogenes*,[122] *Coxiella burnetii*,[123] and *Helicobacter pylori*[124,125] are examples of settings characterized by pleiomorphic antibacterial MC responses relevant for host defense. MC involvement has also been described to be relevant during fungal and parasitic infections, for example, by *Plasmodium* spp, *Leishmania* spp, *Trypanosoma* spp, and *Toxoplasma gondii*,[126] whereas other parasites, such as the nematode *Litomosoides sigmodontis*,[127] escape MC surveillance.[128] Of relevance, MCs contribute to intestinal helminth clearance via interactions with type 2 innate lymphoid cell.[129]

MC granules are rich in antimicrobial peptides and cytokines/chemokines able to fight effectively and quickly against viral infections, while also recruiting further immune cells and reinforcing the cellular antiviral immune response.[130] Under specific circumstances, MC responses can be deleterious, serving as a reservoir for viruses such as HIV,[131] or through uncontrollable inflammatory responses that cause tissue damage. Severe acute respiratory syndrome coronavirus 2 was reported to activate MCs and induce the release of histamine and proinflammatory IL-1, leading to a harmful contribution to lung inflammatory processes.[132] Autopsy findings support a role for MCs in the progression of pulmonary lesions and fibrosis,[133] suggesting a clinically relevant implication of MCs during severe acute respiratory syndrome coronavirus 2 infection. Moreover, patients with mastocytosis or MC activation syndrome might be at a higher risk for developing a severe form of coronavirus disease 2019,[134] whereas

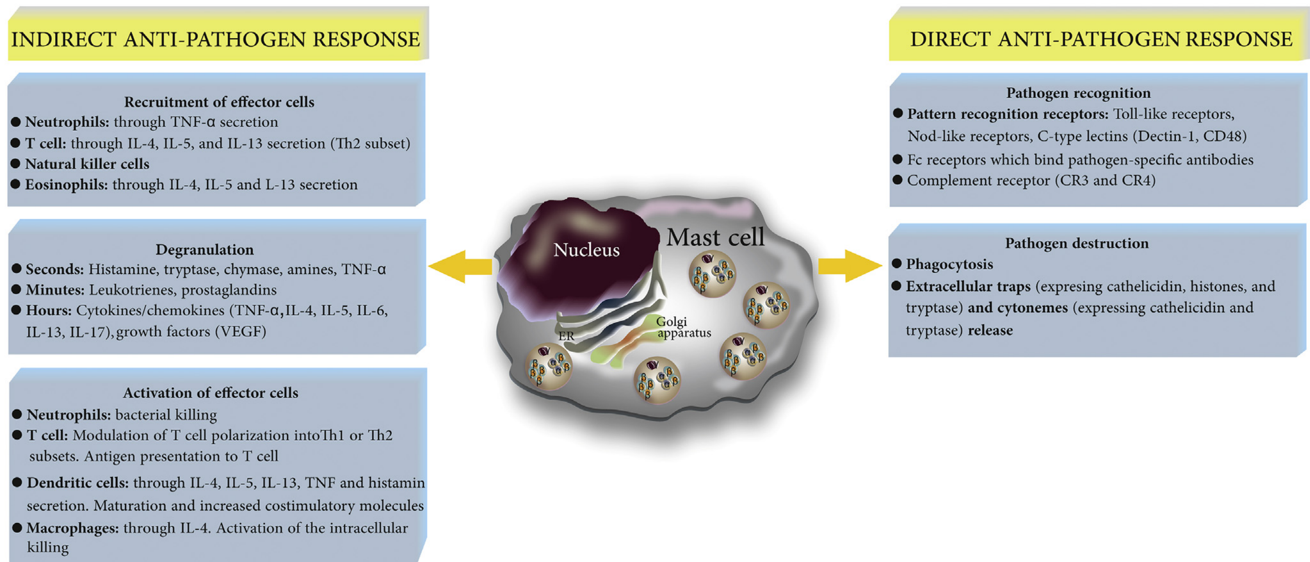

FIG 5. MC response to pathogens and infections. MCs are innate immune cells acting as sentinels at the interface between the host and the environment. As such, they exert direct and indirect antipathogen effects, with the former aiming at the destruction and elimination of pathogens and the latter initiating inflammation and leukocyte recruitment.

the mortality following severe acute respiratory syndrome coronavirus 2 infection in patients with MC disorders including mastocytosis was found to be comparable with that in the general population.[135] Taken together with these reports, human MCs may be key players in anti-infectious responses.

Limitations

The past 25 years have led to many significant advances in our understanding of human MCs not covered by our present review. These include, but are not limited to, novel insights linking asthma and allergic rhinitis endotypes, including clinical response phenotypes such as corticosteroid resistance, to airway MC Cma1 and Cpa3 expression. We also do not cover the important role of MCs in irritable bowel syndrome and eosinophilic esophagitis and the potential role of therapeutic agents targeting MC growth and activation in this condition. These are just 2 examples for dozens of other seminal and ground-breaking developments in human MC biology over the past years that our current article could not cover and that should be reviewed in the future.

CONCLUSIONS AND OUTLOOK

The past 25 years of research on human MCs has led to important insights into their role and relevance and functions in diseases. In this regard, MC research has most certainly benefited from the recent improvements in methodology and technological advancements. Now more than ever we can define the contribution of MCs to human pathology, and indeed MCs were found to intervene in a plethora of pathophysiological processes other than their canonically and worldwide acknowledged involvement in allergic inflammation. Moreover, with the new analytical tools, we can dissect phenotypical and functional differences among MCs from different tissues, investigate the interactions of MCs with immune and structural cells, and use MCs and their mediators as biomarkers for human diseases. These and other potentially relevant physiological functions of human MCs must be kept in mind and further characterized as we move to use MC-targeted treatments that aim to silence, inhibit, and/or deplete them.

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