

New roles and controls of mast cells

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Mast cells are innate immune cells implicated in immune surveillance and defense. They are filled with secretory granules where a vast array of molecules endowed with multiple biological activities are stored. The process of granule secretion, named degranulation, is a tightly controlled biological phenomenon that allows mast cells to rapidly and efficiently release bioactive mediators in response to extracellular stimuli. MC degranulation allows fighting pathogens, limiting envenomation and contributes to tissue homeostasis. However, it is also a potentially harmful response that plays a key role in the development of allergy and inflammatory diseases. Recent findings revealed that MC degranulation is a complex modular process that can be controlled at multiple levels. First, mast cells can decode different activation stimuli into two main patterns of degranulation that differently impact inflammatory responses. Second, mast cells in contact with antibody-opsonized cells or parasites form antibody-dependent degranulatory synapse for dedicated secretion and defense. Third, IL-33 fine-tunes FcR-mediated degranulation at the single cell level. Together these recent findings show how mast cells adapt their degranulation responses to environmental cues and highlight the remarkable functional plasticity of these cells.

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Introduction

Mast cells (MCs) belong to an unique cellular lineage of the innate immune system [1^{••}]. They are located in virtually all tissues and are noticeably abundant in tissues at the host–environment interface such as skin and mucosa. For instance, MCs represent about 10% of the immune cells in the skin [1^{••}] and about 5% in the intestine [2]. They constitute a tissue resident population

in close vicinity to blood vessels and terminal nerve endings. Being strategically located, MCs are among the first immune cells to encounter antigens or incoming pathogens [3,4,5].

Based on their tissue location and granule content, murine MCs are typically classified as either connective tissue (CTMC) or mucosal-type mast cells (MMC) [6,7]. Their human counterparts are defined according to the proteases they produce: MC_{TC}, expressing trypsin and chymase and MC_T, expressing trypsin only. It should be noted that this classification is simplistic since MC phenotypes and roles are profoundly molded by the tissues environment where they mature. MCs are indeed endowed with a high degree of plasticity [7].

Upon stimulation, MC can release a vast array of powerful biological mediators that can dramatically affect the extracellular milieu. MC cytoplasm is filled with hundreds of secretory lysosomes, named secretory granules (SGs) where a huge panel of mediators is stored (such as histamine, heparin, trypsinases, chymase, carboxypeptidase A3, TNF, *etc.*) [8]. These mediators can be swiftly released upon degranulation allowing MC to impact tissue homeostasis (vascular permeability, vasomotricity, matrix remodeling, *etc.*), to participate in inflammatory processes and to fight pathogens. Several proteases are embedded within SG proteoglycan matrix (trypsinases, chymase, carboxypeptidase A, *etc.*) [8]. Upon degranulation, these proteases are released and can affect the function of a variety of proteins (ex: activation, inhibition or degradation of cytokines, receptor shedding, venom degradation, *etc.*) [8,9]. Upon appropriate stimulation, MC can also release neo-synthesized mediators such as eicosanoids and a large array of cytokines and chemokines [8]. Although MC function and SG release are involved in physiologic tissue homeostasis, excessive MC activation plays a crucial role in the pathogenesis of allergy and of organ-specific inflammatory disorders such as psoriasis or inflammatory bowel diseases [10,11].

MC degranulation has been extensively studied in the context of allergy and is classically seen as a rapid process, triggered by the aggregation of high-affinity IgE receptors by soluble ligands. In this review, we discuss recent results showing that MCs are capable of setting up several degranulation modalities. These findings extend our knowledge on how MC sense signals derived from extracellular milieu and provide adapted responses.

Triggering of secretory granule release: multiple secretory pathways in response to multifaceted stimuli

Secretory pathways

The release of the mediators stored in SGs, is a complex and tightly regulated process that has been extensively studied, but is still incompletely understood [12,13]. In simple terms, SG exocytosis consists in the fusion of SG membrane with cell plasma membrane followed by the release of the SG matrix into the extracellular space. Beyond individual granule exocytosis, other patterns of SG content release have been described. ‘Kiss-and-run’ exocytosis allows partial release of the SG cargo through the formation of a transient fusion pore with the plasma membrane. ‘Piecemeal exocytosis’ is a mechanism by which shuttle vesicles move cargos of SG stored mediators to the plasma membrane. Finally, ‘compound exocytosis’ (also named anaphylactic degranulation) is based on intracellular homotypic SG-SG fusion leading to the formation of degranulation chambers, whereupon these multi-granular structures fuse with the plasma membrane to deliver their content [12,13,14,15]. While compound exocytosis allows MCs to swiftly and massively release their SG content, piecemeal degranulation allows surgical delivery of chosen SG components.

Triggering stimuli

MC degranulation is efficiently triggered by two main classes of receptors: i) Fc receptors (FcRs) that allow MCs

to specifically respond to antibody-targeted antigens and ii) G-protein-coupled receptors (GPCR) that allow MCs to degranulate in response to soluble mediators (Table 1).

Most FcRs are ITAM-containing activating receptors that signal upon aggregation induced by Ig-bound antigens [16]. All MCs express the high affinity IgE receptor (FcεRI) that allows them to participate to IgE-dependent allergic processes. Skin human MCs also constitutively express the low affinity IgG receptor FcγRIIA. Moreover, human MCs can express the IFN-γ inducible high affinity IgG receptor FcγRI (CD64) [17]. Murine MCs express the low affinity IgG receptor FcγRIIA and the inhibitory receptor FcγRIIB that tightly controls their activation [16].

Besides FcR, other receptors can induce MC degranulation (Table 1). We focus here on the newly described Mas-related G-protein-coupled receptor X2 in human (MRGX2 or MGRPRX2) and in rodent (Mrgprb2, the mouse orthologue of MRGPRX2) [18,19**]. These GPCR bind to various basic cationic molecules collectively called basic secretagogues, such as neuropeptides (Substance P and neuropeptide Y) and antimicrobial peptides (LL37 and human β-defensins) [19**,20,21]. Several drugs involved in pseudoallergic reactions, such as morphine, Icatibant or Cetorelix have been identified as MRGPRX2 agonists [19**]. Beside small-diameter neurons in the dorsal-root and trigeminal ganglia, connective tissue MCs are the unique immune cells that

Table 1

Receptors that trigger MC degranulation.

Receptor	Stimulus	References
FcR		
FcεRI	IgE/multivalent antigen	[29]
FcγRIIA(human)/FcγRIIIa(mouse)	IgG immune complexes	[30,31]
FcγRI*	IgG/multivalent antigen	[17]
GPCR		
C5aR (CD88)	C5a	[32,33]
C3aR	C3a	[34]
MRGPRX2(human)/MrgprB2(mouse)	Large array of basic secretagogues: -Neuropeptides: Substance P, VIP, Cortistatin 14 -Compound 48/80 -Opiates: morphine, dynorphin -Several cationic drugs (Cetorelix, Icatibant . . .) -AMPs: LL37, hBD2 -Eosinophil granule proteins (MBP, EPO) - Wasp peptide toxins : mastoparans	[19**,20,21] [18,22,35**] [19**] [21] [21,36] [23,24,37] [22] [19**]
ET-A**	Endothelin-1 Snake venom sarafotoxins	[38,39] [27]
Others		
P2X7R	ATP	[40,41,42]
TLR2	Peptidoglycan	[43]
CD48	FimH 2B4	[44] [45]

AMP, antimicrobial peptide; VIP, Vasoactive intestinal polypeptide; MBP, Major basic protein; EPO, eosinophil peroxidase.

* FcγRI is inducible in human MC but is not expressed on mouse MC.

** ET-A was reported to be expressed on rodent CTMC.

constitutively express MRGPRX2. MRGPRX2 expression on MC surface is upregulated in some pathological conditions such as chronic urticaria [22]. MRGPRX2 signaling is poorly understood although both MRGPRX2-coupled pertussis toxin-sensitive and insensitive signaling pathways have been described [23,24]. Unlike many other GPCR, MRGPRX2 is resistant to ligand-induced receptor desensitization and internalization [23]. Interestingly, proteases such as tryptase and chymase released upon MC degranulation were shown to degrade some MRGPRX2 ligands, thus providing a feedback mechanism for MRGPRX2 activity [20]. Beside MRGPRX2, some MC subsets and *in vitro* expanded MC lines have been reported to also express specific receptors for SP (neurokinin receptor, NK-1R) or VIP (Vasointestinal peptide receptors) [25], nevertheless the real implication of these receptors in controlling degranulation in pathophysiologic contexts remains to be elucidated [26].

Finally, several peptide toxins present in venoms from different species, such as the wasp venom-derived peptide mastoparan, were shown to induce MC degranulation [19^{••},27,28].

MCs adapt the size of exteriorized granules to the stimulus

MRGX2 and FcεRI are the most effective receptors to trigger MC degranulation, nevertheless they exhibit subtle differences in the extent and quality of triggered responses. Gaudenzio et al. compared in detail *in vitro* MC responses elicited by SP or IgE/Ag used at concentrations providing similar amount of β-hexosaminidase release [35^{••}]. In spite of a comparable degranulation magnitude, the amount of released cytokines (TNF, IL-13, VEGF and MCP-1) and produced eicosanoids (PGE₂ and PGD₂) upon SP stimulation was significantly lower when compared to IgE/anti-IgE stimulation.

A thorough analysis of MC degranulation also revealed different modalities in the degranulation process following GPCR and FcR triggering. The analysis was carried out using time-lapse confocal laser scanning microscopy and fluorescent avidin, a probe that, when added in the stimulation buffer, immediately binds to the budding granules, due to its selective affinity for heparin, and allows to monitor degranulation in real time [35^{••},46^{••},47,48,49]. The measured degranulation kinetics induced by FcR aggregation (both FcεRI and FcγRIIA) was slower than the one induced by GPCR triggering (MRGX2 or Complement R) and that the lag time between the early increase in [Ca²⁺]_i and the first exteriorized SG detection was longer upon FcR engagement.

During the FcR-mediated degranulation lag time, SG-SG fusion occurred, leading to the exteriorization of large and irregularly shaped SGs. Conversely, spherical unit SGs were swiftly released following MRGX2 triggering.

Interestingly, IKK2 inhibition switched the FcεRI-mediated degranulation pattern toward a pattern similar to the one induced by MRGPRX2. The role played by IKK2 in MC degranulation is still elusive, but this kinase seems important for N-ethylmaleimide-sensitive factor attachment protein 23 (SNAP23) receptor (tSNARE) and syntaxin-4 (STX4) complex formation. IKK2 might phosphorylate SNAP-23 [50,51] and thus could be involved in the control of SG-SG fusion.

In vivo experiments showed that distinct MC degranulation profiles elicited distinct inflammatory responses. Namely, MC stimulation by SP resulted in short-lived and rapidly developing inflammatory reactions, whereas IgE-dependent reactions took longer to develop, but were more intense and long lasting [35^{••}] (Figure 1). Although measured vascular events described in Gaudenzio et al. are known to be mainly triggered by SG mediators, the above observation cannot rule out the possibility that other mediators, such as eicosanoids produced in larger amount upon FcR triggering, might participate to the observed *in vivo* reactions.

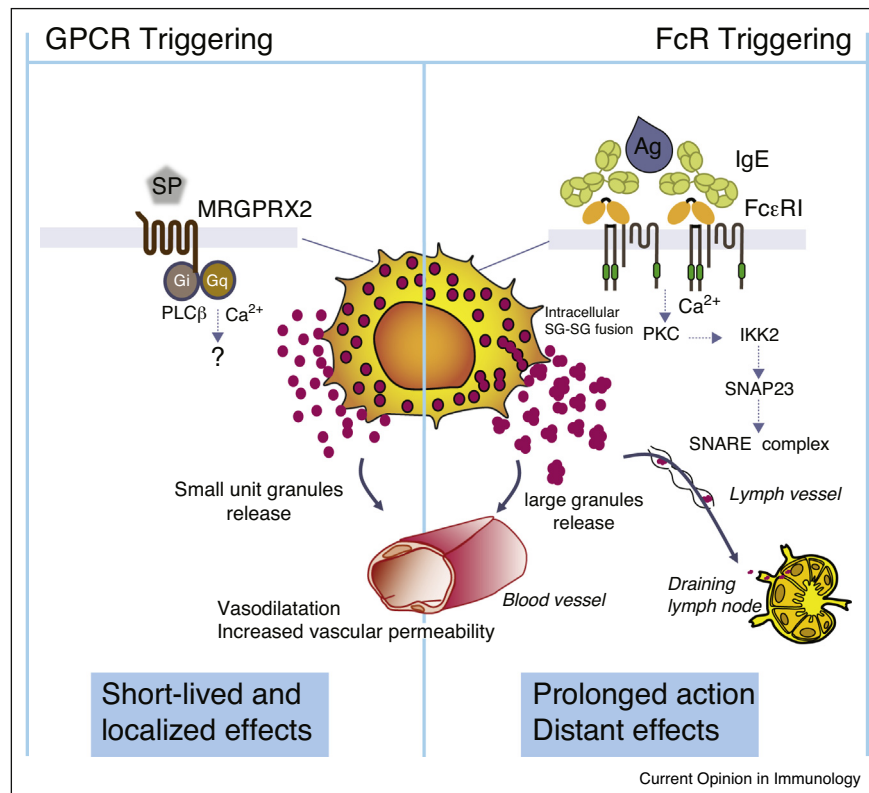
It is tempting to speculate that the observed differences in the size of the exteriorized SG material following different stimuli might be mechanistically linked to the observed different responses *in vivo*. The SG exteriorized remnants might act as biodiffusers that slowly release their cargo of inflammatory mediators. Accordingly, Gaudenzio et al. found the large IgE/Ag triggered SG remnants (and not their SP-triggered counterparts) in draining lymph node showing that these large structures have a longer half-life and can be transported by the lymph. This process might be especially relevant for mediators, such as TNF, chymase and tryptase that are known to remain attached to the SG matrix following exocytosis [52,53,54,55].

Mast cell polarized degranulation: the mast cell degranulatory synapse

Mast cell degranulation is classically studied using soluble stimuli such as secretagogues or antigen/antibody-mediated FcR crosslinking. However, naturally occurring ligands for FcR can also be displayed on the surface of other cells (infected cells targeted by IgG or cells targeted by autoantibodies) or be immobilized within the extracellular matrix network, thus providing localized stimulation. Studies using supported lipid bilayers showed that localized FcεRI stimulation on MC surface leads to receptor clustering and signaling resulting in SG exocytosis [56[•],57,58,59,60].

A detailed spatiotemporal analysis of the dynamics of MC degranulation following stimulation with IgG-targeted or IgE-targeted cell surface antigens revealed that MCs form a unique immunological synapse characterized by molecular clustering and segregation [61]. This specialized

Figure 1



Different degranulation strategies are induced by FcR or GPCR engagement. Engagement of GPCR such as MRGPRX2 and C5aR induce mostly small unit granule release, whereas FcR cross-linking induces preferentially large-sized granule release due to an IKK2-dependent SG-SG fusion. These different degranulation modalities have an important impact on inflammatory response. GPCR triggering induce strong but transient effects. On the contrary FcR triggering induces slower but long-lasting responses. This degranulation modality is accompanied by the release of large-sized granules remnants that are carried to the draining lymph nodes.

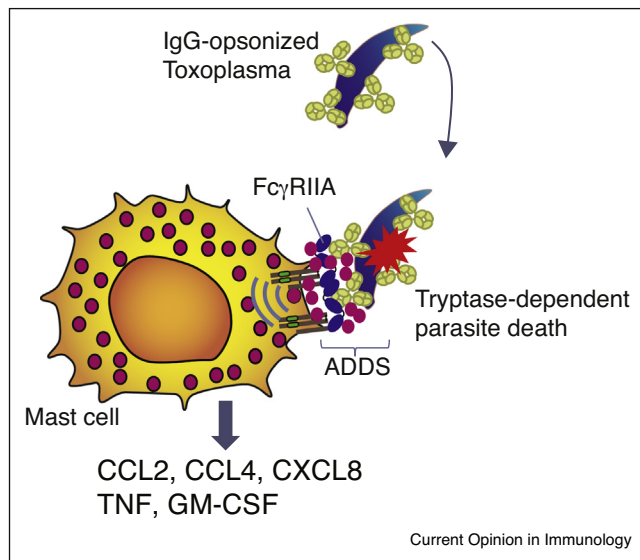
signaling area has been termed ADDS (antibody-dependent degranulatory synapse) and has been shown to occur in MCs previously sensitized with anti-CD20 IgE and conjugated with CD20⁺ EBV-B cells as well as in MC incubated with EBV-B cells previously opsonized with an anti-CD20 IgG [46^{••}]. The ADDS is characterized by a localized degranulation and by the exposure of bioactive SG compounds such as chymase and tryptase at the cell-cell contact site. It is accompanied by FcR clustering, remodeling of the actin cytoskeleton and triggering of the FcR-coupled signaling machinery [46^{••}]. Although exhibiting a polarized degranulation process, MC polarize neither their centrosome nor their Golgi apparatus toward the ADDS. This feature is different from classical immunological synapses formed by T lymphocytes that are known to polarize their secretory machinery [62].

Polarized degranulation was observed in another study in which BMMC transfected with a pHfluorin/VAMP8 fusion protein were employed [63]. Using this cellular tool, the authors monitored degranulation by time-lapse microscopy and observed polarized SG secretion in MC sensitized with

anti-TNP IgE and interacting with TNP-coated beads. In a recent study, Wakefield et al. [56[•]] showed that the induction of local aggregation of FcεRI with micron-scale patterned ligands results in the recruitment of phosphorylated PLCγ1, LAT and SYK to engaged FcεRI in RBL cells. This local area of signaling triggers the secretion of vesicles containing β1- and β3-integrins and fibronectin next to the engaged FcεRI leading to targeted exposure of integrins. It is tempting to speculate that this mechanism might be instrumental to stabilize the ADDS, thus favoring a polarized exocytosis process.

Interestingly, IgG-opsonized *Toxoplasma gondii* tachyzoites trigger ADDS when they attempt to invade MC. The polarized degranulation toward the parasite at the MC surface impedes parasite entry and induces its death *via* a tryptase-dependent mechanism (Figure 2)[46^{••}]. In addition, opsonized parasites stimulate MCs to produce a large amount of inflammatory chemokines and cytokines such as CCL2, CCL4, CXCL8, GM-CSF and TNF. Whether this mechanism could occur with other parasites remains an open question [64,65].

Figure 2



ADDS participate to MC defense against opsonized parasite. IgG opsonized *Toxoplasma gondii* tachyzoites in contact with MC induce FcγR clustering that triggers polarized degranulation toward the parasite. Tryptase containing SGs displayed against the parasite, contribute to parasite death.

The observed polarized SG secretion might have additional functional consequences such as a cytolytic function, reminiscent of antibody dependent cell-mediated cytotoxicity (ADCC). MC SGs have been shown to contain molecules involved in cell death such as TNF, active caspase 3 [66] or granzyme B [67,68]. However, MC do not express perforin, thus do not possess the complete cytolytic machinery necessary for the induction of target cell apoptosis as CTL and NK cells do. Interestingly, Choi et al. recently showed that MCs might indeed kill other cells using an unconventional cytotoxicity pathway. They reported that during cystitis, MC accumulate underneath bladder epithelial cells and release their SGs. SGs are uptaken by the epithelial cells and induce their cytolysis in a chymase-dependent manner [69]. The mechanism triggering MC degranulation and the modalities of this response remain to be elucidated. Moreover, it has been reported that SG proteases could also indirectly participate to cell death or allow MCs to synergize with other cytotoxic cells to kill their targets [70].

Polarized degranulation might also occur in the blood circulation. Indeed, MCs are known to be located close to blood vessels. Two studies showed that they can send protrusions inside the vessel lumen to sample its content [71,72]. This mechanism allows MCs to capture IgE [72] but also to be stimulated by blood allergens [73]. It is tempting to speculate that MCs stimulated *via* FcεRI/ blood allergens undergo ADDS inside vessel lumen, thus exacerbating anaphylaxis.

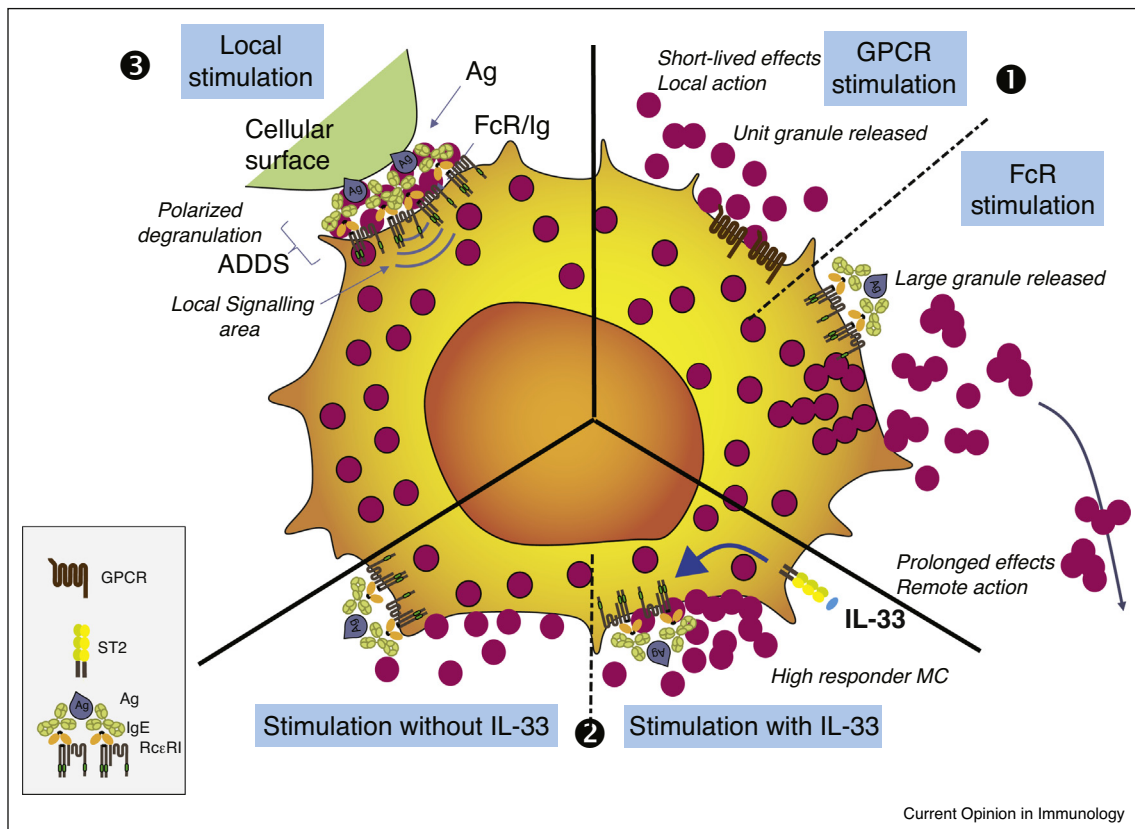
Finally, a polarized degranulation also appears to occur in the proximity of nerve endings. Barbara et al. observed close vicinity between MCs and nerve fibers and localized degranulation in the colonic mucosa of inflammatory bowel syndrome patients [74]. The mechanism underlying this phenomenon and, in particular, whether an ADDS process might take place at the MC/nerve interface remain to be elucidated.

Fine tuning of mast cell degranulation magnitude

The above-described mechanisms of SG secretion highlight the versatility of MC responses to a variety of different soluble and cell-bound stimuli. Interestingly, the receptor-triggered magnitude of MC degranulation is also finely regulated by concomitant engagement of a plethora of receptors [75,76,77]. For instance, SCF and IL-6 were shown to synergize with FcεRI to enhance MC degranulation [78,79]. Moreover, several surface molecules such as TIM3 [80], CD13 [81], TNFSF14 (LIGHT) [82] and tetraspanins [75] exhibit functional cross-talk with FcεRI and influence MC degranulation.

We focus here on the impact of IL-33 on MC degranulation. IL-33 is an alarmin recently shown to be an important booster of MC functions [83]. It is mainly produced by damaged epithelial cells and has been identified as a mediator of type 2 immunity. It is implicated in various inflammatory conditions such as asthma, cardiovascular diseases, and allergy [84,85]. MCs, together with innate lymphoid cell type 2 (ILC2) are the main cell subsets that express the IL-33 specific receptor chain ST2 [86]. IL-33 *per se* triggers cytokine and eicosanoid production by MC, but not degranulation [87,88,89]. In combination with IgE/Ag stimulation, IL-33 was shown to augment degranulation [89]. Nevertheless, most of the studies carried out so far have been based on bulk assays to measure degranulation such as β-hexosaminidase or histamine release assays. These assays measure the amount of SG components released but do not provide any indication about the fraction of MCs that actually degranulate nor about the magnitude of individual cell degranulation. Analysis of degranulation in *ex vivo* mouse peritoneal MC or in primary human MC derived from CD34⁺ precursors, at the single cell level using avidin-based fluorescent probes [47] or CD63 upregulation assays [90] revealed that IL-33 boosts MC degranulation induced by FcεRI crosslinking. In particular Joulia et al. showed that, IL-33 pretreatment increases both the number of degranulating and chemokine-producing MCs and the magnitude of individual MC responses [47]. This single-cell approach revealed that IL-33 can generate high responder MCs indicating that cues derived from the microenvironment can differently modulate individual MC responses to FcεRI cross-linking. In other words, while IgE/Ag function as main a switch of degranulation, IL-33 serves as a rheostat that boosts degranulation magnitude at the single MC level. This potentiation of

Figure 3



Mast cell degranulation strategies and control. 1) GPCRs trigger the degranulation of unit SGs with short lived *in vivo* effects while FcRs trigger the degranulation of large-sized SGs with prolonged action of stored mediators. 2) IL-33 augments the magnitude of MC degranulation and leads to the emergence of high responder MCs. 3) Local engagement of FcRs with Ag-bound cellular surface induces the formation of a degranulatory synapse allowing dedicated secretion.

MC degranulation by IL-33 is expected to be crucial at low antigen concentration, a situation likely to occur *in vivo*.

Interesting, MC proteases (tryptase, chymase, granzyme B) released upon MC degranulation have been shown to generate mature forms of IL-33 that exhibit an increased biological activity, indicating that an activation loop between MC degranulation and IL-33 function exists [91]. On the other hand, prolonged exposure to IL-33 (more than 72 h) decreases MC degranulation by dampening the expression of crucial signaling components [92]. Jung et al. proposed that MC undergo a phenotypic reprogramming upon prolonged IL-33 exposure. It is tempting to hypothesize that an IL-33-based negative regulation loop might be instrumental to avoid MC prolonged activation once the 'alarm signal' has been raised. In line with this hypothesis, it has been reported that MCs chronically exposed to SCF exhibit a significantly reduced capacity to degranulate and to produce cytokines in response to Ag [93].

Conclusion

Being abundantly armed and strategically located outposts of surveillance, MCs have the ability to detect and rapidly react to diverse danger situations. In this review we discussed recent findings showing that MC degranulation is a complex modular process that can be controlled at several levels (Figure 3). First, surface receptors (sensing a plethora of ligands of various biochemical composition and biological origin) can trigger different degranulation processes allowing MCs to adapt their responses to the quality of the sensed stimuli. Second, additional microenvironment-derived signals can fine-tune the magnitude of MC responses. Third, MCs are endowed with the capacity to form degranulatory synapses to react against cell bound ligands and to fight pathogens. The fine molecular mechanisms governing MC functional plasticity at the level of receptor triggering and biological responses are only partially unveiled. The availability of imaging techniques allowing to monitor single MC responses and of RNAseq technologies allowing to determine single cell transcriptomic profiles will help to solve outstanding issues in MC biology.

Conflict of interest statement

The authors declare that they have no competing financial interests.

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