How mast cells make decisions

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Mast cells (MCs) are present in various tissues and are responsible for initiating many of the early inflammatory responses to extrinsic challenges. Recent studies have demonstrated that MCs can tailor their responses, depending on the stimulus encountered and the tissue in which they are stimulated. In this issue of the *JCI*, Gaudenzio and colleagues examine the mechanistic differences between MC responses observed after engagement of Fcε receptor I and those seen after MC stimulation via the recently identified G protein–coupled receptor MRGPRX2. By showing that discrete cellular activation patterns affect the phenotype of the MC response in vivo and in vitro, the authors provide important information about how MCs differentially process various stimuli into distinct degranulation programs.

Mast cells: sentinel cells and first responders

Increasing lines of evidence indicate that mast cells (MCs) are key coordinators of both homeostasis and inflammation in a variety of tissues. Conventionally, MCs have largely been considered to be harmful effector cells that indiscriminately release a variety of powerful mediators from stored granules upon activation. Yet recent reports have demonstrated that MCs possess sophisticated information-processing functions and are able to translate various incoming alarm signals into very specific and highly diverse response programs. These responses are dependent on the tissue and the signal encountered.

MC activation occurs in response to IgE-mediated aggregation of Fcε receptor I (FcεRI); however, MCs are also equipped with a wide range of surface receptors, giving them an intrinsic capacity to identify and respond to a number of stimuli. Particularly, as MCs are present in a variety of tissues at the host-environment interface, they are poised to serve as first responders to a variety of extrinsic challenges, including allergens and pathogens (reviewed in ref. 1). MC activation triggered through different receptors, however, can initiate very different responses. For example, MCs exhibit a distinct cytokine profile following exposure to TLR2 and TLR4 ligands. While TLR2 stimulation triggers degranulation, activation of TLR4 does not (2). Similarly, MCs evoke a powerful antiviral type 1 IFN response following viral infection but not in response to bacterial challenge (3).

MC degranulation itself is not a uniform event either, as the composition of the granules themselves is heterogeneous and differs between MCs. Granule formation is the result of targeted membrane fusion processes that are mediated by members of the soluble *N*-ethylmaleimide–sensitive factor attachment protein receptor (SNARE) family. The distribution of SNAREs appears to be highly variable between individual MCs and depend on MC phenotype and granule composition. For example, serotonin-containing, but not histamine-containing, MC granules highly express the vesicle SNARE protein VAMP-8. While VAMP-8-deficient MCs exhibit defects in exocytosis of serotonin- and cathepsin D–containing granules after FcεRI stimulation, histamine and TNF-α

granules appear to be processed normally in the absence of VAMP-8 (4).

Tissue-dependent effects may also contribute to the versatile responses and functions of MCs. Unlike that of most other immune cells, maturation of MCs is determined by the milieu of their respective target tissue. As a consequence, MCs from different body sites display marked differences in sensitivity to various stimuli, granule composition, and tissue-specific receptor and cytokine expression patterns (5, 6). Additionally, protease expression patterns in MCs can be altered by exposure to certain conditioning factors or infection (7–9). MC diversity is further compounded by the fact that MCs are long-lived cells capable of regranulating and replenishing stored pools of inflammatory mediators following activation. MCs' properties are highly influenced by the microenvironment that they are in; therefore, the granule composition after activation and regranulation may be completely different from that in the original MC if granules are replenished in an inflamed microenvironment as opposed to the original steady-state environment.

Receptor-specific differences in MC degranulation responses

Although it is increasingly appreciated that complex regulatory pathways drive MC heterogeneity, there has not been a concerted effort to delineate how MCs differentially process stimulatory signals into distinct effector release programs. In this issue, Gaudenzio and colleagues (10) present an intriguing study that details mechanisms underlying specific MC responses. Specifically, the authors carefully compared degranulation and mediator responses in MCs that had been activated via the classical FcεRI receptor or the recently described G protein–coupled receptor (GPCR) MRGPRX2 (or its murine ortholog MRGPRB2), which binds various cationic ligands, such as substance P (SP), and is almost exclusively found on MCs (11). While activation of either path-

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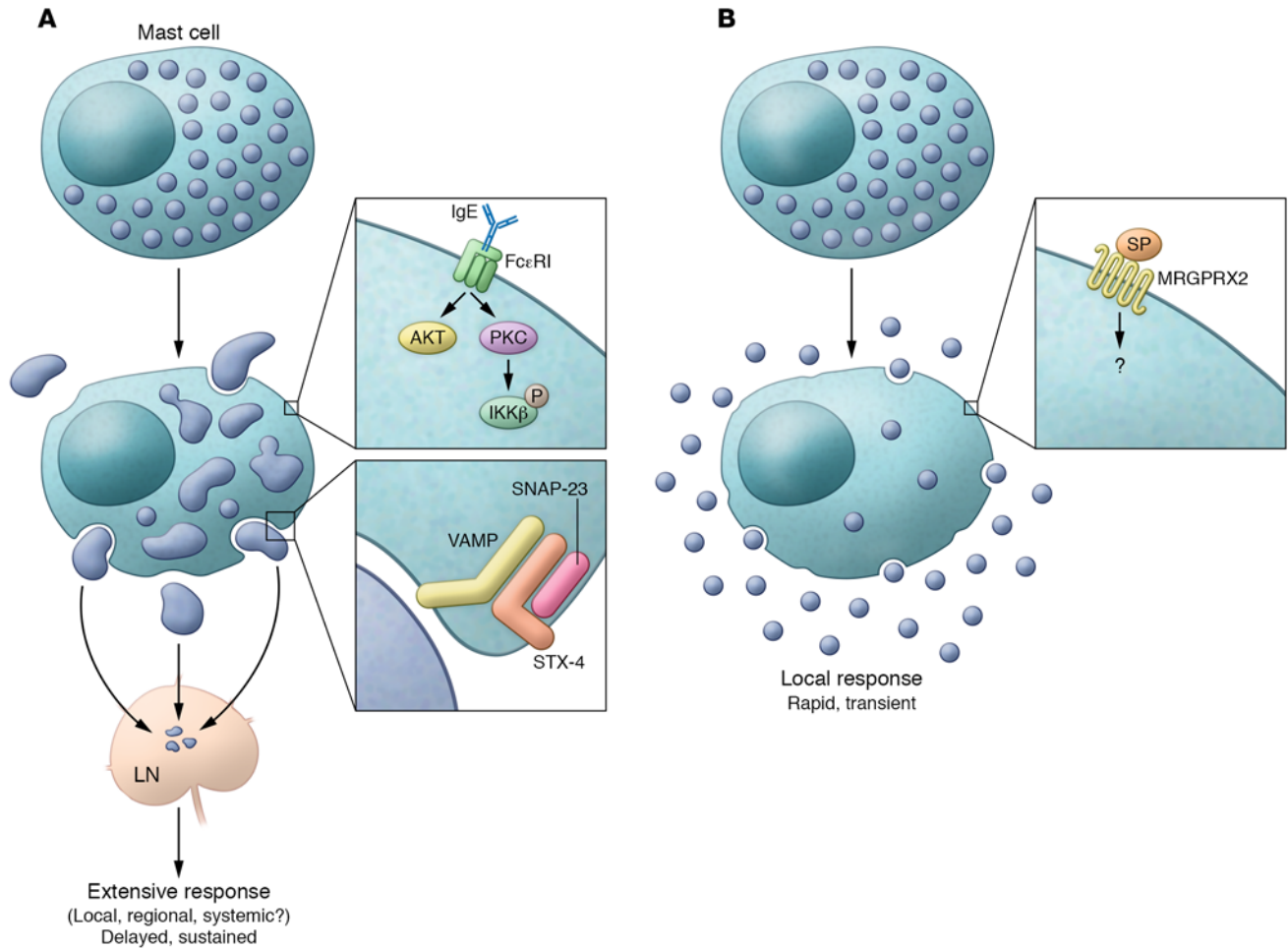
Figure 1. Differential granule processing after FcεRI engagement versus GPCR engagement. Mast cells (MCs) launch very specific response programs depending on the nature of the stimulus. In this issue, Gaudenzio and colleagues show that MC degranulation is mediated by at least two distinct pathways. (**A**) Engagement of FcεRI results in substantial release of proinflammatory mediators. Degranulation is relatively delayed and occurs after granule fusion into large, irregularly shaped granules. Because of their large size, effectors within these granules are slowly released and thereby can mediate immune responses at sites distant from the original site of MC degranulation, such as in regional lymph nodes. The sustained release is associated with AKT and PKC activation, phosphorylation of IKK-β, and the formation of SNAP23-STX4 complexes, which mediate granule exocytosis. (**B**) Engagement of the recently identified GPCR MRGPRX2 by its ligand substance P (SP) does not lead to a notable release of inflammatory mediators. Degranulation here occurs as a rapid but transient release of small, spherical granules, which appear to be relatively unstable and do not seem to carry their cargo to regional lymph nodes.

way elicited a comparable degranulation response, FcεRI stimulation triggered greater release of prostaglandin E$_2$ (PGE$_2$), cytokines, and VEGF. In MRGPRX2-stimulated cells, the PGE$_2$ response was limited and no VEGF was detected. In addition, FcεRI-mediated degranulation was linked to a slower but sustained Ca^{2+} response, whereas MRGPRX2-mediated degranulation was very rapid, with a transient Ca^{2+} response (10). Because Ca^{2+} signaling is critical for MC degranulation, it is not surprising that each receptor evoked distinct degranulation dynamics. Moreover, FcεRI-mediated degranulation involved initial fusion events between granules

followed by the emergence of complex granules that were present as large and nonspherical particles in a limited number of openings. The misshapen appearance of granules in MCs undergoing FcεRI-mediated exocytosis is presumed to be the result of the initial granule fusion events. Conversely, MRGPRX2-triggered degranulation was characterized by direct release of individual, spherical granules from all over the MC surface.

Gaudenzio and colleagues also examined the signaling events downstream of receptor activation in isolated human MCs and found that AKT and PKC signaling pathways were activated and that IKK-β

was phosphorylated in response to FcεRI engagement but not MRGPRX2 activation (10). Furthermore, FcεRI-mediated degranulation required the formation of a complex containing the SNARE proteins synaptosomal-associated protein-23 (SNAP23) and syntaxin-4 (STX4). This complex required IKK-β, as treatment of MCs with an IKK-β antagonist prevented complex formation following FcεRI activation. Interestingly, IKK-β inhibition altered the FcεRI-mediated degranulation phenotype toward the rapid, simple degranulation observed in MRGPRX2-mediated MC degranulation. Together, these results indicate that different signaling pathways regulate MC

degranulation and that these pathways use distinct transport systems for the delivery of granules to the plasma membrane.

Importantly, these distinct MC degranulation profiles were also evident in mice following stimulation of either FcεRI or MRGPRB2. Like in isolated human MCs, FcεRI-mediated degranulation developed slowly but tended to occur over a longer time period, often in excess of 60 minutes, whereas MRGPRB2-mediated degranulation was rapidly induced and concluded relatively quickly, in less than 5 minutes. Moreover, the ensuing pathological responses evoked by each of these MC receptors seemed to mirror characteristics of the degranulation response. For example, following stimulation of FcεRI, vascular leakage, immune cell recruitment, and anaphylactic responses (assessed by a drop in body temperature) developed slowly and were much greater in magnitude and duration compared with the same pathological responses evoked by MRGPRB2, which developed quickly but were transient in nature (10).

It should be noted that activation of MCs by IgG immune complexes resulted in a spatiotemporal degranulation pattern that was very similar to that observed with IgE-mediated MC activation, indicating that other antibody receptors use similar signaling and transport machineries. Moreover, the spatiotemporal patterns of granule release evoked by other known MC activators, including C3a, C5a, and endothelin 1, were remarkably similar to those elicited by SP, even though they bind different GPCRs on the MC surface (12). Together, these observations indicate that there are at least two distinct programs that regulate the MC degranulation process (Figure 1). Although there does not seem to be much overlap between these pathways, it does appear that IKK-β is critical in distinguishing rapid/simple versus delayed/complex granule release.

Conclusions

Together, these findings provide further insights into the long-standing clinical observation that pseudoallergic (MRG-PRX2-mediated) responses are often rapid but transient whereas IgE-triggered events are prolonged and have a decidedly inflammatory component (13, 14). IgE-mediated MC activation is widely thought to be important in parasite defense and in

response to antigenic substances in foods or in the atmosphere (15, 16). IgE-mediated responses are antigen specific; therefore, it is not surprising that FcεRI engagement triggers elaborate and robust inflammatory programs. Activation of MRGPRX2 and other GPCRs, on the other hand, promotes an entirely different response. For these receptors, which are activated by a diverse set of ligands, a sustained and potent MC response could be detrimental. Instead, MC activation here may have a different and not necessarily inflammatory role. Proteases are a major component of the mediators released by MCs and have direct functions in tissues, including the degradation of potentially harmful endogenous proteins, such as VIP (16) and endothelin 1 (17), and exogenous substances, such as venoms (18–20), as well as degradation of signaling molecules, like SP (21); therefore, a rapid, quickly resolved release of proteases may be enough to limit the effect of such substances and restore tissue homeostasis without incurring unnecessary damage.

However, the study by Gaudenzio and colleagues has not only identified differences in the magnitude and timing of FcεRI- and MRGPRX2-mediated MC responses, but, importantly, has demonstrated a link between these differences, including granule size and the number released, and pathological responses in mice (10). It has long been assumed that once exteriorized, MC granules, whose components are held together by powerful electrostatic interactions, promptly disintegrate and release their cargo. Recent in vivo and in vitro studies undertaken by Kunder et al. have revealed that this is not the case (22). Specifically, Kunder and colleagues determined that exteriorized granules released in response to MC activation in vivo not only retain their physical structure in the extracellular microenvironment but also contain powerful immunoregulatory cytokines, such as TNF-α, within the granule matrix. Presumably, these extracellular granules serve as slow-release vehicles for pharmacologically active mediators at sites of MC activation. As soluble mediators are rapidly diluted or degraded in the extracellular milieu, prolonged retention of these bioagents in the granule matrix should extend their biological activity. It is conceivable

that cargo within smaller granules is likely to be released more rapidly into the extracellular milieu than cargo within larger granules, and thereby mediate responses at different rates and intensities. The findings of Gaudenzio et al. further imply that the powerful and sustained pathological responses associated with FcεRI-mediated MC activation can be attributable to the larger-sized exteriorized granules, which persist for longer periods and slowly release their cargo of inflammatory mediators. Interestingly, not all exteriorized MC granules remain in the vicinity of the cellular source in vivo. Kunder and colleagues previously reported that granules released by MCs within the periphery are trafficked via the lymphatic system to the draining lymph node, where mediators within these granules, such as TNF-α, are able to kickstart the development of adaptive immune responses (22). Gaudenzio and colleagues now show that such granule trafficking only occurs after FcεRI-mediated MC degranulation, whereas MC granules were not observed in the draining lymph node following MRGPRB2 stimulation, most likely because these granules dissociate before reaching the lymph node.

MCs located in the skin and mucosal barriers are routinely challenged by a wide array of extrinsic agents, ranging from inanimate particles to highly virulent microorganisms. The nature of the MC response to each of these challenges is critical, as inappropriate or delayed responses can have very harmful consequences. Therefore, MCs precisely regulate both the intensity and the timing of the responses to each challenge encountered. The regulation of MC activity appears to be mediated, at least in part, by the various receptors that decorate the MC surface. Gaudenzio and colleagues (10) have added to the evidence that these receptors are hard-wired to induce distinct signaling pathways and exocytic trafficking systems in MCs; thus, engagement of a specific receptor coordinates a response that results in the discharge of a specific collection of de novo–synthesized mediators and granules of a particular size. As larger granules have the capacity to traffic via the lymphatics to distal draining lymph nodes and persist for longer periods, MC degranulation can have a substantial impact on both the local

innate immune response and the development of the adaptive immune response at distal sites. Together, the results from the study by Gaudenzio et al. provide a limited, but very valuable, glimpse of how MCs perceive and react to different activating agents. As more information regarding the dynamics and scope of the MC decision-making process in response to specific challenges becomes available, it may be possible to discern exactly how MC activities shape disease states and, importantly, how the mediators of these activities may be specifically targeted by novel therapeutics.

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1. Shelburne CP, Abraham SN. The mast cell in innate and adaptive immunity. *Adv Exp Med Biol*. 2011;716:162–185.
2. Supajatura V, et al. Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J Clin Invest*. 2002;109(10):1351–1359.
3. Dietrich N, et al. Mast cells elicit proinflammatory but not type I interferon responses upon activation of TLRs by bacteria. *Proc Natl Acad Sci U S A*. 2010;107(19):8748–8753.
4. Puri N, Roche PA. Mast cells possess distinct secretory granule subsets whose exocytosis is regulated by different SNARE isoforms. *Proc Natl Acad Sci U S A*. 2008;105(7):2580–2585.
5. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev*. 1997;77(4):1033–1079.
6. Xing W, Austen KF, Gurish MF, Jones TG. Protease phenotype of constitutive connective tissue and of induced mucosal mast cells in mice is regulated by the tissue. *Proc Natl Acad Sci U S A*. 2011;108(34):14210–14215.
7. Dayton ET, et al. 3T3 fibroblasts induce cloned interleukin 3-dependent mouse mast cells to resemble connective tissue mast cells in granular constituency. *Proc Natl Acad Sci USA*. 1988;85(2):569–572.
8. Karimi K, Redegeld FA, Heijdra B, Nijkamp FP. Stem cell factor and interleukin-4 induce murine bone marrow cells to develop into mast cells with connective tissue type characteristics in vitro. *Exp Hematol*. 1999;27(4):654–662.
9. Friend DS, et al. Reversible expression of tryptases and chymases in the jejunal mast cells of mice infected with Trichinella spiralis. *J Immunol*. 1998;160(11):5537–5545.
10. Gaudenzio N, et al. Different activation signals induce distinct mast cell degranulation strategies. *J Clin Invest*. 2016;126(10):3981–3998.
11. McNeil BD, et al. Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature*. 2015;519(7542):237–241.
12. Kashem SW, Subramanian H, Collington SJ, Magotti P, Lambris JD, Ali H. G protein coupled receptor specificity for C3a and compound 48/80-induced degranulation in human mast cells: roles of Mas-related genes MrgX1 and MrgX2. *Eur J Pharmacol*. 2011;668(1-2):299–304.
13. Paton WD. Histamine release by compounds of simple chemical structure. *Pharmacol Rev*. 1957;9(2):269–328.
14. Rothschild AM. Mechanism of histamine release by animal venoms. *Mem Inst Butantan*. 1966;33(2):467–475.
15. Palm NW, Rosenstein RK, Medzhitov R. Allergic host defences. *Nature*. 2012;484(7395):465–472.
16. Mukai K, Tsai M, Starkl P, Marichal T, Galli SJ. IgE and mast cells in host defense against parasites and venoms. *Semin Immunopathol*. 2016;38(5):581–603.
17. Maurer M, et al. Mast cells promote homeostasis by limiting endothelin-1-induced toxicity. *Nature*. 2004;432(7016):512–516.
18. Akahoshi M, et al. Mast cell chymase reduces the toxicity of Gila monster venom, scorpion venom, and vasoactive intestinal polypeptide in mice. *J Clin Invest*. 2011;121(10):4180–4191.
19. Higginbotham RD. Mast cells and local resistance to Russell's viper venom. *J Immunol*. 1965;95(5):867–875.
20. Metz M, et al. Mast cells can enhance resistance to snake and honeybee venoms. *Science*. 2006;313(5786):526–530.
21. Caughey GH, Leidig F, Viro NF, Nadel JA. Substance P and vasoactive intestinal peptide degradation by mast cell tryptase and chymase. *J Pharmacol Exp Ther*. 1988;244(1):133–137.
22. Kunder CA, et al. Mast cell-derived particles deliver peripheral signals to remote lymph nodes. *J Exp Med*. 2009;206(11):2455–2467.