

Controversies

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What is the physiological function of mast cells?

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Abstract: Under physiological conditions, skin mast cells preferentially localize around nerves, blood vessels and hair follicles. This observation, which dates back to Paul Ehrlich, intuitively suggests that these enigmatic, multifaceted protagonists of natural immunity are functionally relevant to many more aspects of tissue physiology than just to the generation of inflammatory and vasodilatory responses to IgE-dependent environmental antigens. And yet, for decades, mainstream-mast cell research has been dominated by a focus on the – undisputedly prominent and important – mast cell functions in type I immune responses and in the pathogenesis and management of allergic diseases. Certainly, it is hard to believe that the very large and rather selectively distributed number of mast cells in normal, uninflamed, non-infected, non-traumatized mammalian skin or mucosal tissue is simply hanging around there lazily day and night, just to wait for the odd allergen or parasite-associated antigen to come by so the mast cell can finally swing into action. Indeed, the past decade has witnessed a renaissance of mast cell research ‘beyond allergy’, along with a more systematic exploration of the surprisingly wide range of physiological functions that mast cells may be involved in. The current debate sketches many of the exciting new horizons that have recently come into our vision during this intriguing, ongoing search.

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Introduction

Mast cells have various functions. However, not only mast cells themselves but also we scientists do not know what is the physiological and what is the pathological function. A given mast cell function plays a physiological role in some situations, but the same function may play a pathological role in other situations. In a sense, the mast cell might be compared with an excellent actor: the actor may beautifully play the role of either a good or evil character.

Probably, the most well-defined function of mast cells is that of an effector of IgE-dependent immediate hypersensitivity reactions. This plays a physiological role in the rejections of a certain species of ticks which infect skin (1). However, the same reaction against tick antigens may play a pathological role in infantile asthma and atopic dermatitis. In most industrial countries, dermal tick infestation is rare in humans, whereas infantile asthma and atopic dermatitis are common. Therefore, the role of mast cells in the IgE-dependent hypersensitivity reaction against tick antigens is considered to be pathological by most people living in industrial countries. If dermal infestation by such a

kind of ticks is prevalent, the role of mast cells might be considered to be physiological.

Taken together, the specific physiological or pathological roles of mast cells appear to be influenced and defined by the individual possessing mast cells and the environment that the individual lives in.

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Viewpoint 1

No doubt man, as well as every other animal, presents structures, which seem to our limited knowledge, not to be now of any service to him.

Charles Darwin, Descent of man, 1872

Why has evolution chosen to provide us with a cell that appears not only to be of no service to us, but which can be most annoying (e.g. itching, wheezing, running nose, ...) and sometimes harmful (anaphylactic shock) as the key effector cell in allergic reactions? This so-called 'riddle of the mast cell' (1) has fascinated scientists ever since Paul Ehrlich's first detailed description of mast cells (MCs) in 1878 (2). Yet it took more than a hundred years before we could actually be sure that MCs are good for something and before we could start to characterize such physiological functions of MCs.

The problem was not a lack of smart people thinking about the problem or the absence of valuable hypotheses to be tested. On the contrary, many renowned scientists have added their view on what MCs are good for to the long list started by Ehrlich himself, who proposed that MCs may modulate the growth of solid tumors, arguing that MCs were particularly abundant in the vicinity of 'neoplastic foci' (3). Other interesting hypotheses originated from the observations that tissue MCs are unique because they are fairly long-lived cells capable of undergoing multiple cycles of de- and regranulation, and that MCs are preferentially located at the host–environment interface such as the skin or the gut. Yet other authors have suggested physiological functions of MCs based on the findings that MCs produce, store, and release an abundant array of mediators, some of which have nothing to do with the initiation of allergic reactions, and that MCs can be activated by many various signals independent of immunoglobulin E (IgE) (Table 1).

Coming up with theory after theory on the true and original function of MCs must have been fun and exciting (and pretty safe), yet frustrating, because there was no way of disproving or proving any of them, which was precisely the problem. What MC aficionados lacked for more than one hundred years were not visionary hypotheses but a model to test them. Such a model, under the best of circumstances, would permit the investigation of hypothetical MC functions *in vivo* using animals that differed solely in lacking, or containing, MC populations, thus facilitating the study of a supposedly MC-dependent condition both in the absence and in the presence of MCs. Ideally, having found that the condition in question is impaired in the absence of MCs, such a model would then allow for attempts to repair this defect by adoptively transferring MCs to 'MC-empty' animals.

Such a model was reported by Galli, Kitamura, and coworkers in 1985 (4). Mice with mutations at *c-kit*, such as *Kit^W/Kit^{W-v}* mice, virtually lack tissue MCs but otherwise have largely normal hematopoiesis, essentially normal B- and T-cell functions, hemostasis, and leukocyte populations (5,6). Also, genetically MC-deficient *Kit^W/Kit^{W-v}* mice can be repaired selectively and both systemically or locally of their MC deficiency by reconstitution with MCs obtained from congenic *Kit^{+/+}* mice (5). Today, anyone who is interested in what MCs are good for can turn to this model (or MC-deficient rats) and find out. In an extension of the original model described above, we and others have recently shown that MC-deficient mice may also be used to pinpoint the contribution of single MC products in MC-mediated reactions (7,8). MC-deficient *Kit^W/Kit^{W-v}* mice may be reconstituted with MCs derived from mice deficient for a certain MC product, i.e. tumor necrosis factor- α (TNF α), to then be compared for differences in MC-mediated reactions with *Kit^W/Kit^{W-v}* mice that have

received adoptive transfer of normal MCs. Because such mice differ only in having MCs that can, or cannot, produce and release TNF α , any differences in MC-mediated reactions found in these mice must be attributed to this MC mediator (Fig. 3).

MC-deficient mice have been extensively used to characterize the role of MCs in numerous pathological conditions, including various models of allergic diseases and other inflammatory disorders (9), while much less use has been made of this model to identify the functions of MCs under physiological conditions. However, some hypotheses of physiological MC functions have been put to the test, and MCs are now known to be beneficial to their host (at least if that host is a mouse) in at least than two settings: 1) host defense and 2) tissue repair and remodeling.

Mast cells – pivotal players in host defense

MCs meet all basic requirements needed to be important players in acquired immunity: they are capable of phagocytizing, processing, and presenting antigens to T cells and of modulating T- and B-cell responses such as lymphocyte growth, recruitment, and production of Igs (10). Also, MCs have been studied in great detail with respect to mechanisms of activation, in particular via Fc ϵ RI, the high-affinity receptor for IgE (11). Because IgE production is substantially up-regulated during immune responses to infections with intestinal parasites, where MC populations undergo considerable expansion, the question of whether MCs are involved in acquired immunity to parasites was one of the first issues investigated in MC-deficient mice. In 1985, Nawa and coworkers (12) reported that *Kit^W/Kit^{W-v}* mice infected with parasitic nematodes show greater and more persistent peak larval counts and slower worm expulsion than normal $\pm$ litter mates. In addition, the reconstitution of connective tissue and mucosal MC populations in *Kit^W/Kit^{W-v}* mice effectively restored protective antiparasite immune responses in these mice (12,13). Using MC-deficient mice and IgE-deficient mice, Watanabe et al. (14) were later able

Table 1. Selected hypotheses of physiological mast cell functions

Hypothetical mast cell function	Author	Year
Protection from cancer	Ehrlich	1877
Phagocytosis of pathogens	Metchnikoff	1892
Endocrine function	Cajal	1896
Lipid metabolism	Ciaccio	1913
Vitamin metabolism	Tuma	1928
Calcium metabolism	Pautrier	1931
Tissue growth and cell proliferation	Syden	1941
Blood clotting and coagulation	Baeckeland	1950
Hair growth	Montagna	1951
Hemopoiesis	Messerschmitt	1955
Local tissue detoxification	Higginbotham	1956
Regulation of blood pressure	Keller	1957
pH regulation	Caselli	1958
Temperature regulation	LeBlanc	1959
Aging	Spicer	1960
Response to stress	West	1962
Fixation of blood-borne particles	Selye	1963
Sweat secretion	Szabo	1964
Peripheral 'memory bank'	Padawer	1978

to show that both MCs and IgE participate in worm expulsion from the intestine.

MCs are not only beneficial in acquired immunity to parasites of the gut but also of the skin: MC-deficient *Kit^W/Kit^{W-v}* mice reportedly show impaired resistance against infestations with larval *Haemaphysalis longicornis* ticks, which are associated with increases in serum IgE levels of more than 100-fold (15). In a series of elegant experiments, Matsuda et al. (16) were able to adoptively transfer protective acquired immunity to *Kit^W/Kit^{W-v}* mice by treatment with immune sera obtained from infected mice, but only when such *Kit^W/Kit^{W-v}* mice had received intracutaneous injections of *Kit^{+/+}* mouse-derived MCs prior to infection. Thus, one of the physiological functions of MCs (and IgE) is to provide resistance and acquired immunity to parasites by mounting protective immediate hypersensitivity reactions against parasitic antigens (10).

If MC-induced inflammation is a good thing when trying to rid the host of worms in the context of acquired immune responses, why should it not also help in dealing with infections by other pathogens, like bacteria, which are mainly controlled by innate immune responses (17)? Recent studies by Malaviya et al. (18) and by Echtenacher et al. (19) indicate that MCs not only promote host defense responses to enterobacteria, but are also necessary for mice to survive infections by such pathogens. In MC-deficient mice, intraperitoneal reconstitution with MCs substantially reduced death and morbidity from cecal ligation and puncture (CLP, a model of acute septic peritonitis), and this protection by adoptive transfer of MC was wiped out by the application of neutralizing anti-TNF α antibodies (19). In collaboration with Michael Carroll and his colleagues, we have recently found that mice deficient in complement components 3 (*C3^{-/-}* mice) or 4 (*C4^{-/-}* mice) – compared to wild-type mice – were much more sensitive to the lethal effects of CLP (20). *C3^{-/-}* mice subjected to CLP also exhibited diminished degranulation of peritoneal MC, reduced intraperitoneal TNF α levels, less neutrophil recruitment, and impaired clearance of bacteria. All of these defects, as well as the increased mortality of *C3^{-/-}* mice after CLP, were improved upon treatment of *C3^{-/-}* mice with purified C3 protein (20). In subsequent experiments involving complement receptor-deficient mice (*CD21/CD35^{-/-}* mice, deficient in complement receptors 1 and 2) and *CD19^{-/-}* mice, we were able to show that the engagement of CD21/CD35 along with CD19 on the MC surface by C3 fragments contribute to MC activation in the CLP model (21). Interestingly, our work done in collaboration with Tanya Mayadas and associates showed that complement receptor 3-deficient mice (*CD11b/CD18^{-/-}* mice) also exhibit markedly reduced survival following CLP. However, this increased susceptibility of *CD11b/CD18^{-/-}* mice to bacterial infections may be the result of reduced numbers of MCs in these mice (22).

To test whether it could actually be beneficial to have increased numbers of MCs when dealing with bacterial infections, we have analyzed whether repetitive administrations of stem cell factor (SCF), a potent growth factor for MCs (6), could influence the survival of mice subjected to CLP (23). We found that such treatment not only increased the number of peritoneal MCs in C57BL/6 mice but also significantly improved the ability of these mice to survive CLP: the more SCF treatment increased MC populations, the more it improved survival. Experiments on MC-reconstituted *Kit^W/Kit^{W-v}* mice confirmed that the protective effect of SCF treatment reflected, at least in part, the actions of SCF on MCs. Moreover, we found that SCF-treated mice did not appear to be at a substantially increased risk for death when IgE-dependent systemic anaphylaxis was induced by intraperitoneal challenge with specific antigen (23). That MCs are important, indeed essential, to the initiation and orchestration of innate and acquired immune responses were striking findings: not only did MCs appear to finally be good for something, but in some instances their presence or absence was a matter of life or death. To no surprise have

these observations sparked interest in investigating the role of MCs in infections by various other pathogens, including viruses (24) and intracellular protozoan parasites such as *Toxoplasma gondii* and *Leishmania* spp. (8,25), studies that will surely expand our understanding of MCs as players in host defense.

Mast cells – key effector cells in tissue repair and remodeling

Turning to *Kit^W/Kit^{W-v}* mice for answers to whether MCs are required under physiological conditions or not for the maintenance of tissue homeostasis, revealed no detectable gross deficits in MC-deficient mice, even at sites which host large MC populations in congenic *+/+* mice. Organs that undergo substantial and continued structural remodeling, namely hair follicles and bones, may be exceptions to that rule. MC-deficient mice have hair of normal length and texture, and hair follicles in these mice appear largely normal when studied by histomorphometry. However, hair follicle cycling, the continued progression of hair follicles through periods of rest and growth, which is associated with tremendous architectural changes of the skin, including proteolysis, angiogenesis, and nerve supply rearrangement, is significantly impaired in the absence of MCs: *Kit^W/Kit^{W-v}* mice exhibit markedly reduced hair follicle growth after the hair follicle's switch from rest to hair production, and hair follicle cycling is also significantly retarded when the hair-producing follicle regresses into the resting stage of its cycle (26,27).

Along the same line, we found that bones of MC-deficient mice are no different from bones in wild-type mice when assessed for bone density or microstructure (28). However, femurs of *Kit^W/Kit^{W-v}* mice differ from those of wild-type mice in bone mass and geometry, in that they are lighter and of thinner shape, resulting in increased fragility (28). When subjected to a cycle of bone remodeling, *Kit^W/Kit^{W-v}* mice exhibited (a) delayed onset of remodeling, (b) decreased duration and extent of the active formation phase, and (c) diminished synthesis of new bone matrix (29). These findings indicate that while MCs are not required in maintaining homeostasis in quiescence, they do play an important role in processes that require tissue remodeling. This view is supported by reports that indicate a pivotal role of MCs in protection from injuries induced by various exogenous agents including acute and chronic damage induced by ultraviolet radiation (30,31), carcinogenous compounds (32), toxic substances (33), foreign bodies (8), or mechanical wounding (34).

If we are asked then (as we are) what MCs are good for under physiological conditions, we would have to say: MCs appear to be good for keeping conditions physiological (Fig. 1). That is, they protect the host from trauma not only in the context of infection, but also in many instances where tissue remodeling and repair is required as a response to changes induced by endogenous or exogenous mechanisms.

Fishing for functions... What to look for next

Let us finally, fully in line with mastocytophiliacs' tradition of (unwarranted) speculation, point out what we believe MC-deficient mice should and will be used for next in exploring physiological functions of MCs, namely to pinpoint their role as partners of sensory nerves (SNs).

As of yet, MCs remain widely under-appreciated by the rapidly emerging field of neuroimmunology (35). At first sight, this appears understandable given that MCs and nerve cells are derived from different lineages and are localized, for the most part, in different organs. However, many arguments suggest that MCs and SNs may be viewed as a functional unit: 1) MCs and nerves share a number of activating signals, for some of which both cells express receptors [e.g. vanilloids (36)]. 2) Both MCs and SNs respond to stimulation by exteriorizing granules loaded

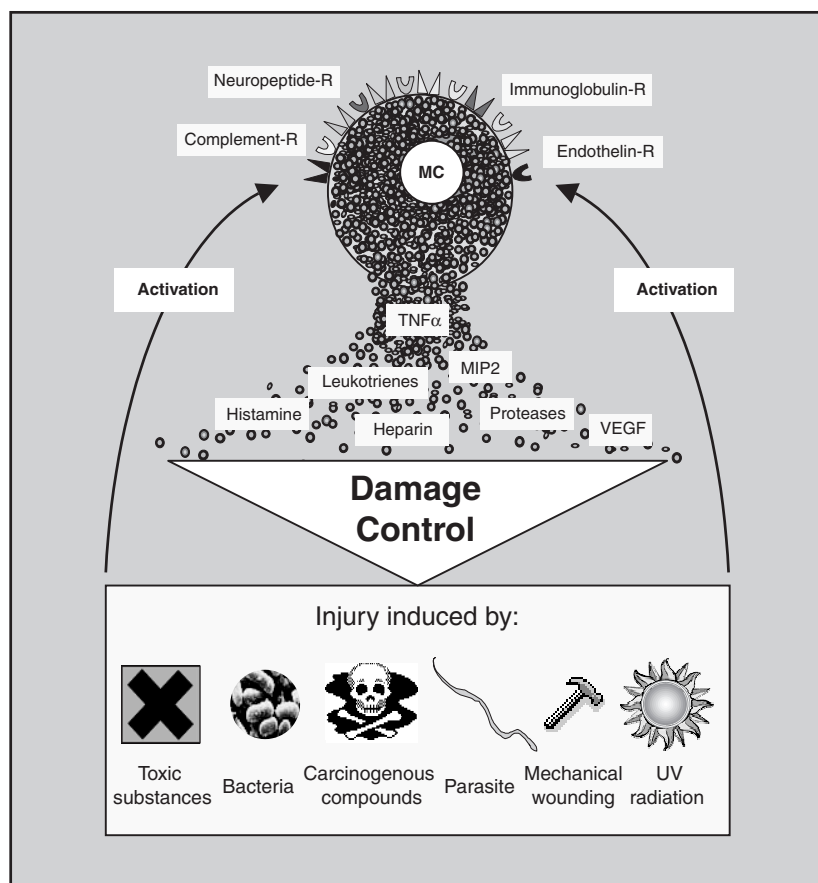


Figure 1. Physiological function of mast cells.

with preformed mediators (=degranulation), 3) many of which are produced by both cells (NGF, neuropeptides, and endothelin-1). 4) MCs and SNs can be long lived (with a life span that corresponds to that of their host) and 5) undergo repetitive cycles of de- and regranulation. 6) In the skin, MCs are preferentially colocalized with SNs (37) and in some instances are found to be innervated directly (38–40). Similarly, MCs have been described to reside within SNs (41,42). 7) Furthermore, MCs can be potently activated by SN products such as substance P (43) and endothelin-1 (44). Many MC products including serotonin (45) and tryptase (46) have been shown to activate SN activation, while histamine can up- and down-regulate SN activity, depending on what histamine receptors are targeted (47–49). In addition, MC proteases reportedly both activate and degrade nerve products by enzymatic cleavage (50,51). 8) MC/SN crosstalk has also been implicated in the regulation of MC proliferation, migration, and differentiation, histamine and cytokine production, and priming to other stimulatory signals (52,53), and MCs products are thought to modulate SN development, degeneration, and regeneration after trauma (54–56). 9) Finally, MCs and SNs have been suggested to cooperate in a number of pathological and physiological processes such as the regulation of hair follicle cycling and development (57), wound healing (35), responses to stress (58), and the pathogenesis of inflammatory diseases (59).

In our view, a better understanding of how MCs and SNs interact will be crucial to the elucidation of where such bi-directional crosstalk is of relevance under physiological and pathological conditions (60). Recently described models of selective cutaneous denervation (61) and the generation of mice deficient in neuropeptides or neuropeptide receptors and MCs should help to better characterize 1) how MC and SN activation is modulated, 2) how SNs control MC functions and vice versa, and 3) if and how MCs make use of SNs in inducing inflammation (Fig. 2).

Undoubtedly, MC-deficient mice have been and will be a most useful and unique tool in elucidating the physiological (and pathological) functions of MCs, indispensable for working out where and when and how MCs are beneficial or harmful to the host. Yet, as lucky as we are to be able to exploit such a (literally) black and white model (Fig. 3), there are two important caveats: 1) MC-deficient mice will not reveal everything MCs are good for. While these mice allow for the identification of settings where MCs are required and indispensable, they will not help us to characterize the contribution of MCs to reactions where MCs are involved, yet the lack of MCs will not result in detectable deficits, maybe because other cell populations take over MCs and compensate for their functions. 2) One must be careful in extrapolating from the murine system to the human situation. Fortunately, MCs in mice and humans are quite similar regarding localization and distribution, mediator production, storage, and release, as well as mechanisms of activation and functions. Yet, careful studies in humans are required for confirmation of findings made in the mouse model.

What MCs are good for has finally become a question that can be answered. Undoubtedly, there is more than one *raison d'être* for MCs and the testing of existing and new hypotheses will continue to rapidly increase our understanding of their functions in health and disease. The riddle of the MC is not yet solved, but now we do have the tools to do so.

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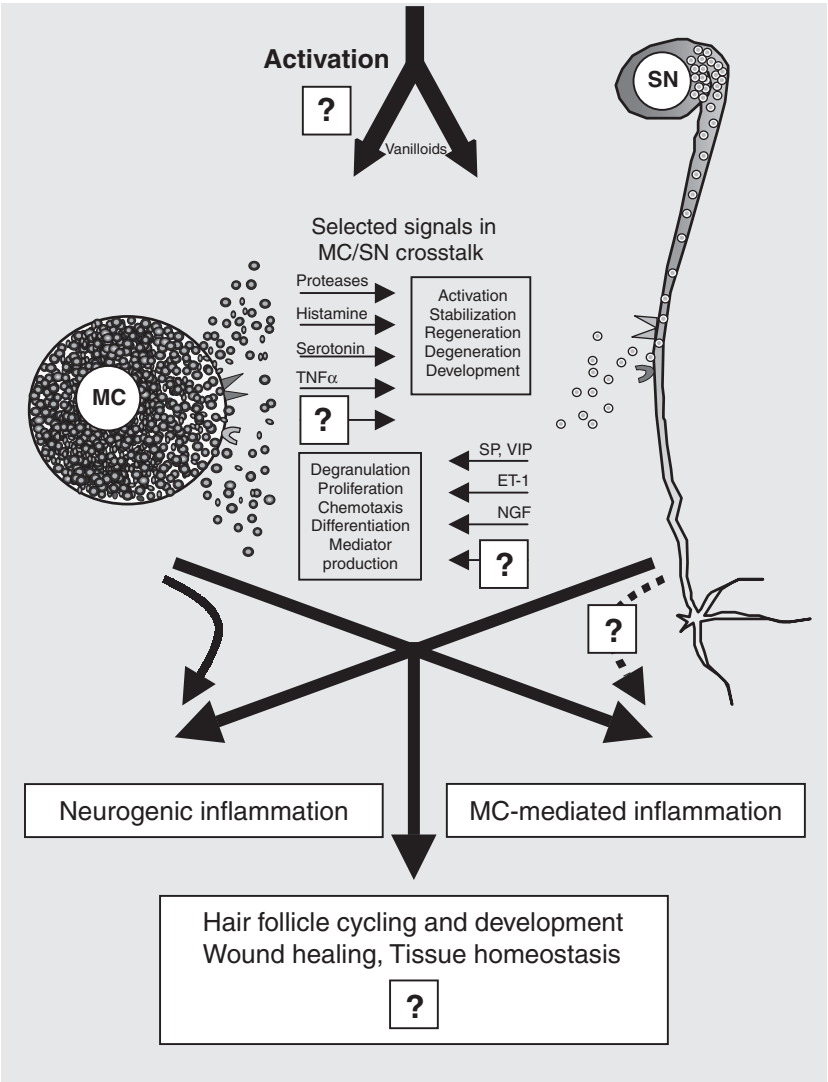


Figure 2. The mast cell nerve connection. Hints to its role in homeostasis and disease.

Mouse	Generation of mice used in this model	Are mast cells present?	Can mast cell-dependent reaction be elicited?
Kit ^{+/+} mouse		Yes	Yes
Kit ^W /Kit ^{W-v} mouse		No	No
Kit ^W /Kit ^{W-v} mouse & Kit ^{+/+} MCs		Yes	Yes
Kit ^W /Kit ^{W-v} mouse & MC-product ^{-/-} MCs		Yes, but deficient	???

Figure 3. Mouse model to test hypothetical mast cell functions.

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Viewpoint 2

Skin is the largest area of the body in constant contact with the outside world. Environmental and physical stressors are therefore first picked up by the skin; similarly, emotional stressors may find quick expression on the skin as a way of subconsciously discontinuing or modifying behavior. The skin is richly endowed with sensory nerve endings, stimulation of which activates local mast cells (1). One of the molecules released from nerve endings is substance P (SP) that has been shown to lead to mast cell-dependent granulocyte infiltration (2–4), possibly through tumor necrosis factor- α (TNF- α) that induces the expression of endothelial leukocyte adhesion molecule-1 (5).

Another molecule that activates skin mast cells is corticotropin-releasing hormone (CRH), the main factor released from the hypothalamus but also released from dorsal root ganglia (DRGs) under stress (6). For instance, we showed that intradermal administration of CRH activated skin mast cells and increased vascular permeability (7); the same was true for the structurally related peptide urocortin (Ucn) (8), and this action was more potent than all other peptides tested (Fig. 1). This process was receptor mediated and did not occur in mast cell-deficient mice (7,8).

Stimulated skin mast cells could trigger more release of CRH and/or Ucn from DRG (6), skin elements (9), or local immune cells (10), further stimulating mast cells directly or through the release of neuropeptides (11) (Fig. 1). Mast cell-derived vasoactive, pro-inflammatory, and neurosensitizing molecules (12) could then act on keratinocytes, endothelial cells, or neurons to release more such molecules, leading to chronic inflammation. Thus, the skin appears to contain the basic elements of a functional equivalent of the hypothalamic-pituitary-adrenal (HPA) axis, which has been termed the 'skin-stress response system' (9). CRH regulates the HPA axis by acting on two types of receptors, CRH-R1 and CRH-R2 α , R2 β ; both of these are primarily found in the brain, but CRH-R2 β has also been identified outside the brain (13). CRH itself is also found outside the brain (6) and has been postulated to have pro-inflammatory actions through the activation of mast cells (14,15). CRH and CRH-R gene expression has been documented in rodent (16) and human skin (17,18). CRH and/or Ucn could also act on capillaries directly, as CRH has been shown to be produced by (19) and act on endothelial cells (20,21).

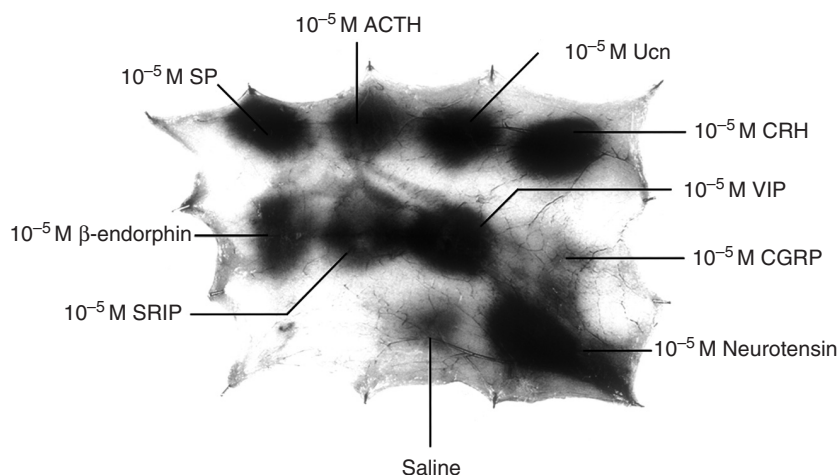


Figure 1. Photograph of rat skin showing vascular permeability in response to intravenously administered equimolar concentrations (0.01 mM) of various neuropeptides (ACTH, adrenocorticotropic hormone; CGRP, calcitonin-gene related peptide; CRH, corticotropin-releasing hormone; saline, 0.9% NaCl; SP, substance P; SRIF, somatotropin release inhibitory factor; Ucn, urocortin; VIP, vasoactive intestinal peptide).

Increasing evidence indicates that stress influences pathophysiological processes (22), especially inflammation, through neuropeptides (23,24), cytokines, or other chemical mediators (25). For instance, psychological factors increase the morbidity of allergic reactions (26) and that of many dermatoses (27–29), such as atopic dermatitis (29–31) and alopecia areata (32,33). Acute stress is well known to precipitate flushing and itching in systemic mastocytosis patients (34–36). We showed that acute stress (37) can trigger mast cell degranulation and increase vascular permeability in rodent skin; in fact, activated mast cells were found in close proximity to CRH-positive elements. This effect was blocked by depleting sensory nerves of their SP content with neonatal capsaicin administration or by pretreatment with a neurotensin (NT) receptor antagonist, implying the involvement of NT and SP. Plasma extravasation in the skin was also elicited by antidromic stimulation of the lumbosacral dorsal roots in the rat (38). While chronic stress is typically known to attenuate immune processes, acute stress was recently shown to stimulate them (39,40). For instance, it led to re-distribution of leukocytes from the blood to the skin (41) and enhanced delayed hypersensitivity reactions (42). In fact, even the involvement of the sympathetic system in stress is recast in view of recent findings indicating that it, too, may have bidirectional effects (43).

Murine skin mast cells are juxtaposed to nerve endings during hair follicle formation (44) and in alopecia, where nerve endings were found to be SP positive (45). Similarly, the expression of CRH and CRH-R in murine skin was associated with the hair cycle (46). A case in point is alopecia areata (33,47,48), which is strongly associated with atopy (47). We showed that skin biopsies from affected scalp areas from patients with stress-induced alopecia areata expressed intense signal only for CRH-2 β ; receptor message was seen specifically around hair follicles, while unaffected scalp areas from patients and normal controls showed only sparse distribution of faint signal (49). Activation of CRH receptors could lead to local inflammation. In fact, there is increased inflammatory infiltrate around the affected hair follicles in alopecia (50,51); this is notable for increased numbers of mast cells (50,52–56), many of which are activated (45,50). Mast cell infiltration and/or proliferation may be triggered by RANTES released from other immune cells (57) and by nerve growth factor (NGF) (58) released from nerve endings or leukocytes (59,60).

Aside from their well-known role in allergy, mast cells have emerged as versatile effector cells in inflammation (61), neuroimmunoenocrine processes (11), and homeostasis (62). The proposed role of mast cells as a universal sensor in the skin is supported by increasing evidence suggesting a similar function for brain mast cell regulation of the blood–brain barrier (63,64) and of the HPA axis (65,66). The median eminence of

the hypothalamus is particularly rich in mast cells (67–69) that contain most of the histamine (70), leukotrienes (71), and interleukin-6 (IL-6) (72) in this critical region. Hypothalamic mast cells (73) are located close to nerve endings containing CRH (74) and can be activated by acute stress (75). Stimulation of hypothalamic mast cells appears to occur by ‘intragranular activation’ without the massive degranulation typical of anaphylaxis (76) and could lead to selective or differential release of mediators (77,78), especially cytokines such as IL-6 (79). In fact, we recently showed that IL-1 can stimulate selective synthesis and release of IL-6 without degranulation through a unique vesicular mechanism (80). Histamine (81) and cytokines derived from skin mast cells (82) can then trigger CRH release (83), leading to HPA activation, or can act as CRH-independent activators of the HPA axis (84) (Fig. 2). Moreover, acute stress was shown to increase local release of CRH in rat skin (85). The CRH could derive either from local nerve endings or from mast cells themselves; as they were recently shown to synthesize and release both CRH and urocortin (86). CRH could act on mast cells directly, or through NK-1 receptors or endothelial cells (88), possibly with different effects. It was recently shown that CRH-1 receptor was involved in the modulation of chronic contact dermatitis (89).

Hypothalamic mast cells, and their counterparts in the skin, appear to act as sensors of stressful events with bidirectional regulation of the HPA axis and its equivalent regulatory system in the skin. A pathological process may reflect dysregulation of these systems due to either underlying processes or excessive stimulation or both. Hans Selye, who wrote definitive works on stress (90) and mast cells (91), would be pleased with the emerging intimate relationship between the two.

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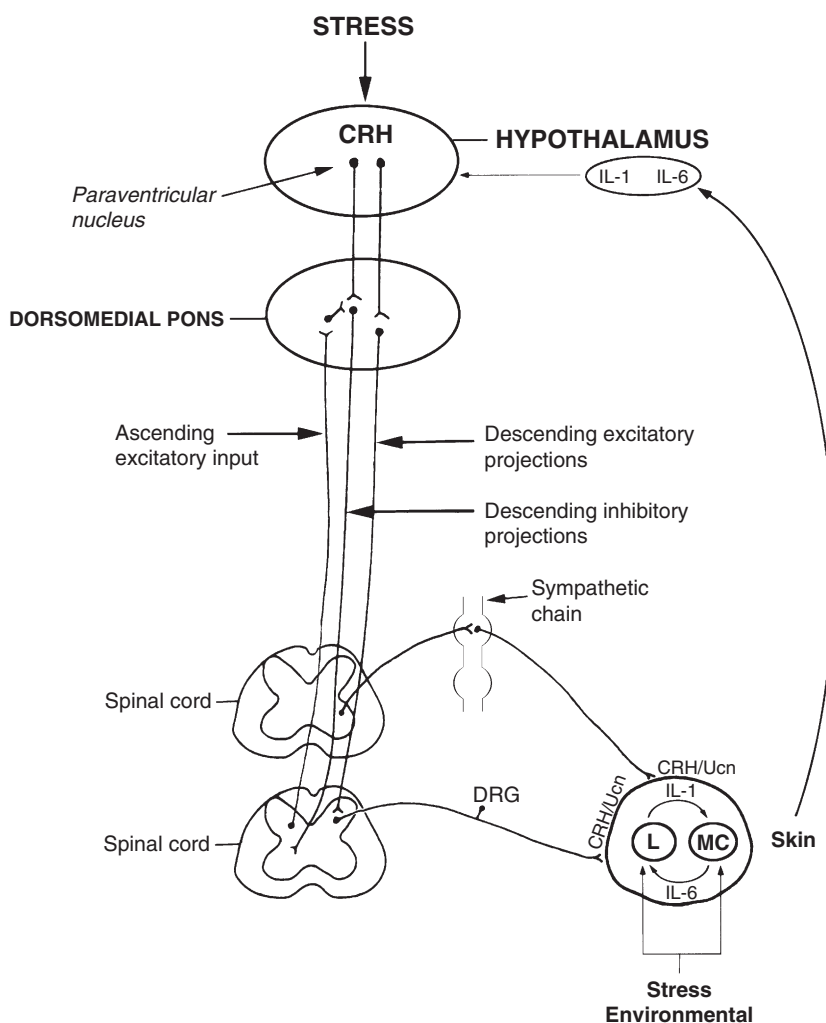


Figure 2. Schematic representation of the hypothesized effect of central or local stress on skin mast cell activation, corticotropin-releasing hormone (CRH)/urocortin (Ucn) release, and local inflammatory mediator release. DRG, dorsal root ganglion; IL-1, interleukin-1; IL-6, interleukin-6; L, lymphocyte; LT, leukotriene; MC, mast cell.

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Viewpoint 3

Mast cells (MCs) are connective tissue cells found most abundantly in the skin, lung, and gastrointestinal tract. Two types of MCs can be distinguished. The MC_T phenotype contains tryptase alone, while the MC_{TC} phenotype contains chymase and tryptase (1). Both subtypes can be found in a given tissue.

Within the skin, MCs occur most prominently just below the dermal–epidermal junction (2) and are concentrated around appendages, nerves, and blood vessels (3). Cutaneous MCs were the subject of a recent review in this journal (4). This article summarized the evidence that cutaneous MCs are involved in the initiation of immunity and host defenses (4). Although MCs have classically been thought of as playing a role in host defense through recruitment of cells and proteins from the vascular compartment, a role for cutaneous MCs in specific immunity is suggested by their ability to phagocytose and take up antigen (5), their expression of major histocompatibility complex (MHC) class I molecules, and their ability to express MHC class II molecules under some circumstances (6–9). Furthermore, anatomic contacts between MCs and lymphocytes have been demonstrated by electron microscopy, and MCs have been shown to traffic to lymph nodes in contact hypersensitivity (CHS) reactions (10,11). MCs have also been shown to express a number of costimulatory molecules and integrins (1,4,12–14). Additionally, MCs are capable of releasing several cytokines (4,15–17), as previously discussed in detail [4]. The spectrum of cytokines expressed appears to vary depending on the maturity

state of the MCs and the tissue of residence (1). The ability to express these various cytokines, of course, suggests a role in influencing immune reactions. Of particular significance, presentation of antigens (including bacterial antigens) by MCs to T cells has been demonstrated (18,19). It has also been reported that MC-deficient mice have reduced survival and are less efficient in clearing enterobacterial infections compared to wild-type control mice (1,17,19) and that MC-deficient mice were found to have increased morbidity and mortality after ligation and puncture of the cecum (20). Additionally, injection of normal mice (but not MC-deficient mice) with stem cell factor (a potent MC growth factor) increased survival after cecal ligation and puncture (21).

The observations summarized above [and reviewed in detail in Ref. (4)] suggest an important role for MCs in specific and innate host immune functions. However, recent studies have suggested that MCs play a crucial role in the down-regulation of immune responses and induction of tolerance after exposure of skin to ultraviolet radiation (UVR). This essay discusses the possibility that MCs have an important physiologic function in mediating cutaneous immune suppression following exposure to UVR.

Much work has been performed over the past two decades examining the mechanisms by which UVR induces suppression of immunity (22–24). Of particular importance is the putative role of UVR-induced immune suppression in the development of cutaneous malignancies.

It is now clear that exposure to UVR and, particularly, mid-range UVR (UVB radiation, 280–320 nm) produces immunosuppression in experimental animals and humans (22–24). Furthermore, substantial evidence from animal models and circumstantial evidence in humans suggest that this immune suppression plays a role in skin cancer development. Thus, it must be asked why evolution would select for such mechanisms to exist? The answer to this question is, of course, highly speculative. However, it is well known that exposure to UVR exacerbates autoimmune conditions such as lupus erythematosus and exposure to doses of UVR sufficient to damage or kill cutaneous cells will cause release of various autoantigens where they may, hypothetically, be available for immunologic recognition and induction of autoimmunity. Thus, one may speculate that the evolution of mechanisms by which UVR down-regulates immune mechanisms may serve to prevent the development of autoimmune states subsequent to exposure to sunlight.

The immunosuppressive effects of UVR have been well reviewed in recent years (22–24). Exposure of certain strains of mice to low doses of UVB radiation (insufficient to cause gross changes in the skin) followed by immunization with an epicutaneously applied hapten at the site of irradiation leads to a greatly suppressed CHS response. Furthermore, adoptive transfer of T cells from these mice to naïve recipients suppresses the induction of CHS in these secondary hosts in a hapten-specific manner. At higher doses of exposure (sufficient to cause gross changes in the skin), sensitization to an epicutaneously applied hapten even at a non-irradiated site leads to suppressed CHS along with the presence of transferable, hapten-specific suppressor T cells. In the high-dose regimen, similar suppression is observed upon subcutaneous immunization with protein antigens. The sequence of events that leads to these immunosuppressive effects is complex and appears to involve chromophores that include *trans*-urocanic acid and DNA as well as mediators including tumor necrosis factor- α (TNF α), interleukin-10 (IL-10), prostaglandins, and *cis*-urocanic acid. With regard to MCs, TNF α and IL-10 content may be of particular interest. Experiments utilizing neutralizing antisera to TNF α and/or IL-10 indicate that release of TNF α (presumably from cutaneous elements) subsequent to exposure to UVR plays a crucial role in inhibiting the induction of CHS in both the local and systemic models, while release of IL-10 (also presumably from cutaneous sources) is responsible for suppression of the induction of delayed-type hypersensitivity (DTH) to an injected protein (25–27). Additionally, there is evidence that IL-10 plays a role in the establishment of tolerance to epicutaneously applied haptens after UVR exposure (28,29).

Recently, a series of experiments have suggested an important role for MCs in these events. Most interestingly, interactions between MCs and elements of the nervous system appear to be involved in UVR-mediated immune suppression. Langerhans' cells are anatomically closely associated with unmyelinated nerves within the epidermis, and MCs are also frequently found in association with nerves (30–33). It has been recently demonstrated by immunohistochemical and immuno-ultrastructural analysis that a plexus of axons surrounds superficial dermal MCs and extends into the overlying epidermal layer with intimate associations with epidermal Langerhans' cells (34). Interestingly, capsaicin (which releases neuropeptides from nerves) applied to human skin induced release of chymase within 6 h and induction of E-selectin in adjacent microvascular epithelium, consistent with release of substance P from axons with subsequent stimulation of cytokine-mediated mast cell-endothelial interaction (34). However, application of capsaicin to human skin grafted onto immunodeficient mice, and thus lacking innervation, failed to show similar findings (34). These results were interpreted as demonstrating that

unmyelinated axons unite epidermal Langerhans' cells and dermal MCs.

Evidence of a role for neuron-derived products modulating cutaneous immunity comes from a number of observations. First, treatment of mice and rats with capsaicin to deplete skin of sensory neuropeptides was shown to lead to exaggerated contact and DTH responses, suggesting that sensory neuropeptides on balance are inhibitory (35–37). Second, treatment *in vitro* of epidermal cell populations containing Langerhans' cells with the neuropeptide calcitonin gene-related peptide (CGRP) resulted in decreased antigen-presenting capability in several essays (30,38). Third, injection of CGRP intradermally followed by epicutaneous application of a hapten to the injected site resulted in a suppressed level of CHS (38). As a whole, these data strongly suggest a regulatory function for the nervous system with regard to cutaneous immune function.

The first report suggesting that a product of nerves may play a role in UVR-induced immunosuppression was from Gillardon and colleagues (39), who reported that topical administration of the CGRP inhibitor CGRP_{8–37} prior to exposure to UVR prevented UVR-induced immune suppression in mice. In addition, it was found that topical application of CGRP reduced the density of I-A⁺ epidermal cells in a manner similar to exposure to UVB radiation. Separately, another group demonstrated that administration of an inhibitor of CGRP systemically prevented the systemic suppression of CHS induced by high-dose UVB radiation exposure (40).

How do MCs fit into UVB radiation-induced immunosuppression? This question may have been answered by a series of experiments performed by Streilein and his colleagues (41,42). These investigators demonstrated that intradermal administration of CGRP_{8–37} prior to UVR exposure and subsequent epicutaneous application of hapten at the injected and irradiated site leads to a normal CHS response, while injection of diluent alone prior to irradiation and sensitization yields a suppressed CHS response (43). They then demonstrated that the suppression of the induction of CHS observed after immunization at sites injected intradermally with CGRP does not occur in MC-deficient mice (43).

As a whole, these data suggest that MCs play a very significant role in the neurocutaneous immune axis. While these cells have classically been thought of as anaphylactic effector cells causing unwanted effects due to hypersensitivity responses, a role for involvement in immune reactions seems evident. Particularly exciting is the evidence that these cells may play a crucial role in UVR-induced immunosuppression. In this hypothesis (Fig. 1), it would appear that exposure of the skin to UVB radiation induces the release of CGRP from cutaneous nerves. CGRP then acts on MCs to cause degranulation and release of IL-10 and TNF α , which then initiate a further sequence of events leading to abnormal antigen presentation with altered recognition of encountered antigens, decreased immune expression, and a state of specific tolerance. Hypothetically, this down-regulation may involve tumor antigens on incipient neoplasms and thus may play a role in the development of cutaneous malignancies. The possibility that MCs play a role in the down-regulation of immunity to antigens encountered through other sites where these cells are prevalent, such as the lung and gastrointestinal tract, deserves study.

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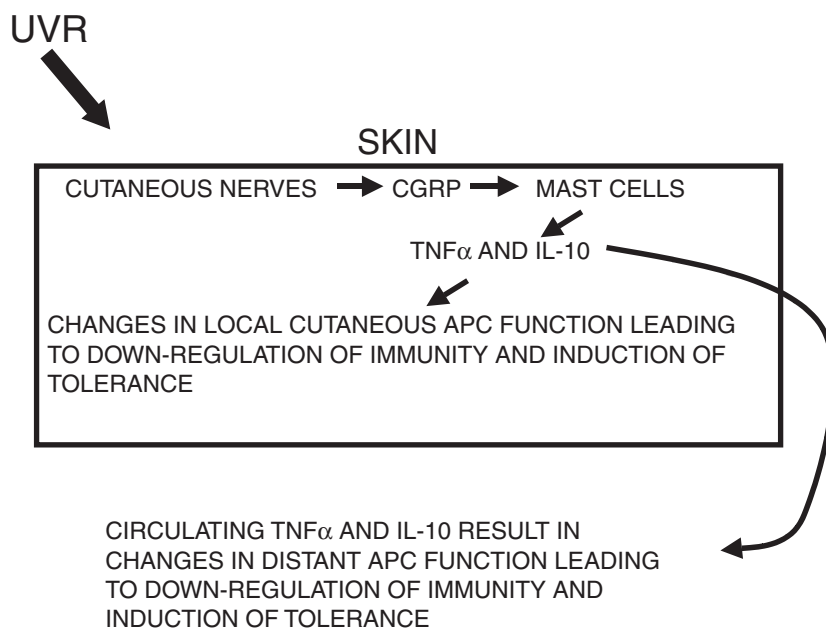


Figure 1. Hypothetical pathway showing involvement of mast cells in ultraviolet radiation (UVR)-induced immune suppression. APC, antigen-presenting cell; CGRP, calcitonin gene-related peptide; IL-10, interleukin-10; TNF α , tumor necrosis factor- α .

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Viewpoint 4

Mast cells are generally associated with sneezing, wheezing, itching, allergic reaction in general, and other inflammatory events. In the past, we learnt a lot about the mechanisms of allergic diseases and the role of mast cells in the pathophysiology of hypersensitivity reactions (1–5). It became clear that mast cells not only release histamine and eicosanoids upon crosslinking of surface-bound immunoglobulin E (IgE) by allergen but also many other mediators not readily associated with inflammation. Examples are Th2 cytokines such as interleukin-3 (IL-3), IL-5, and IL-13, tissue-modulating factors such as basic fibroblast growth factor (bFGF) and transforming growth factor- β (TGF- β), and proteoglycans. Other mast cell-derived mediators such as tumor necrosis factor- α (TNF- α) are found elevated during inflammatory processes but may also induce neutrophil recruitment, known to be of importance, e.g., for host defense against bacteria (6–9).

Mast cell biology becomes even more complex when realizing that not only the number of mast cell products but also the number of mast cell agonists is huge and by far not restricted to agents inducing IgE crosslinking, such as allergen. We now know that particular cytokines such as stem cell factor (SCF) and IL-4 are important regulators of the development of mast cell from myeloid progenitors and for the function of mature mast cells (9). SCF induces mast cell proliferation, prevents mast cell apoptosis, and enhances IgE-dependent mediator and cytokine release. To some extent, SCF at higher concentrations also induces mediator release; however, the *in vivo* significance of this *in vitro* finding is unclear yet. Most of these SCF effects on mature mast cells are enhanced by IL-4 (10). IL-3 has been described as another important mast cell cytokine in rodents, but these data could not be confirmed for human mast cells so far (11). However, own

unpublished data indicate that IL-3 in synergism with SCF may also regulate human mast cell functions (submitted for publication). Apart from cytokines, other mast cell regulators have been identified including parasites, bacteria, adhesion factors and extracellular matrix proteins, nitric oxide, as well as neurotransmitters and neurotrophins (12–19). Apart from such chemical mediators, physical changes such as irradiation and pH changes may affect mast cell function (20,21). These observations strongly suggest that the function of mast cells cannot be restricted to their pathophysiological role in allergy and inflammation. Oddly enough, our knowledge on physiological functions of mast cells is very limited compared to the enormous literature on pathophysiological functions. The question why we have mast cells has to be answered.

There is increasing body of evidence that mast cells play a role in host defense against different kinds of injuries including mechanical injuries such as ischemia and infection either by bacteria or by helminthes (12,13,21). In some cases, mast cell activation may be accompanied by an aggravated tissue dysfunction as described in circulation disorders causing ischemia or in postinflammatory tissue remodeling leading to fibrosis (21–23). However, the same mechanisms may be of importance in wound healing, regulation of tissue perfusion, and induction of host defense mechanisms (9,24,25). An intriguing example is the role of mast cells in host defense against bacteria. Mast cells, besides macrophages, are an important source of TNF- α , known to belong to the so-called pro-inflammatory cytokines. TNF- α is released by mast cells upon stimulation with IgE-crosslinking allergen or gram-negative bacteria typically located in the gut (6–8). One may argue that mast cells contribute to the destructive inflammatory process during

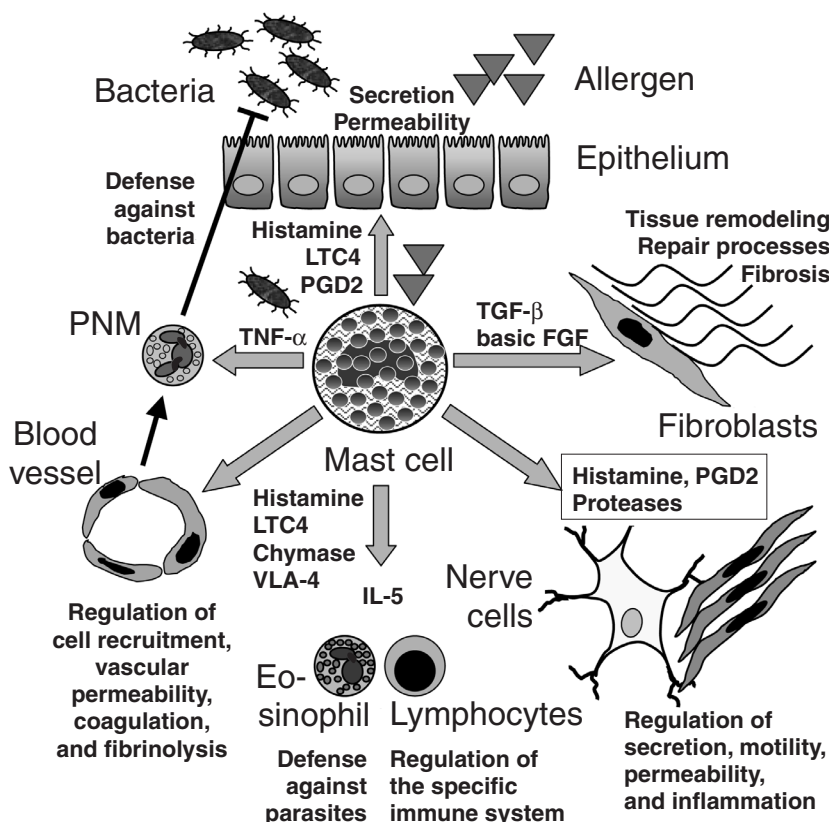


Figure 1. Physiological mast cell functions – current understanding. For details see text.

bacterial infection, but animal studies have clearly shown that the opposite is true. Mortality following bacterial peritonitis is increased in mast cell-depleted animals (W^v/W^v mice), and most likely, this is due to the lack of TNF- α produced by intraperitoneal mast cells. This mast cell-derived TNF- α seems to be crucial for the neutrophil recruitment to the site of inflammation, which is required for overcoming the infection (26,27). Although these animal data are very stimulating, they have to be confirmed for the human situation. We could show that gram-negative bacteria induce TNF- α production in human intestinal mast cells *in vitro*. The TNF production is accompanied by histamine secretion, but in contrast to IgE crosslinking by allergen, the bacteria do not induce leukotriene C4 production, suggesting an IgE-independent mechanism (9). Other studies revealed that bacterium-mast cell interactions are not restricted to an induction of TNF- α , because bacteria may be opsonized by mast cells that are capable of presenting bacterial antigen effectively to CD4 and CD8-positive T cells (28). Studies in our laboratory are in progress to address the question whether these rodent data hold true for human mast cells.

Mast cells are typically located nearby external and internal body surfaces such as skin, mucosa, and blood vessels (1,4,9,14). It is tempting to speculate that mast cells exert some physiological functions at these sites. Again, mast cells may have defense functions at these strategically important sites, but there is evidence that mast cells may act there in more general terms also without infection. In particular in the gut, it has been shown that mast cells regulate multiple organ functions such as fluid and electrolyte secretion by epithelial cells, motor functions of smooth muscle cells, and integrative functions mediated by the enteric nervous system (29,30). In addition, some recent studies have indicated that mast cells are a unique source of heparin and other coagulation-regulating factors (31–33). These properties, together with the mentioned anatomical vicinity and functional interaction between mast cells and endothelial cells, suggest that mast cells also play a role in regulating blood vessel function, which is of importance for the development of cardiovascular disease (14,34). Because mast cells located at barrier sites respond to multiple physical changes and chemical/biological mediators, this cell type may be regarded as a cellular 'watchdog', enabling quick reactions to any harmful event or even to any event.

If interpreted in this manner, mast cells belong to the innate immune system that recognizes multiple molecules and events, and mast cells interact with many other systems such as the specific immune system, the epithelial and endothelial system, the wound healing machinery, and the nervous system (16,34–36). Our current knowledge on the physiological functions of mast cells is summarized in Fig. 1. Within this context, it becomes clear that mast cells are primarily 'physiological cells' exerting multiple functions that have to be unraveled in future studies. While the mast cell fulfills all these functions, mistakes may

occur, leading to more or less harmful consequences such as allergy and many other diseases.

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Viewpoint 5

Mast cells are distributed throughout all the tissues of the body. In many of these, they are associated with nerves and in turn have a functional distribution and proximity to blood vessels. They seem to be distributed freely in some cavities such as the perito-

neal cavity, but it is generally agreed that they do not circulate in a recognizable, morphological form in the blood. Nevertheless, they are largely derived from stem cells in the bone marrow and reach their final form in the tissues under the influence of local

factors such as stem cell factor interleukin-3 (IL-3), IL-4 and IL-9. Different functional subset of mast cells are encountered in different tissues. The subsets are associated with different pharmacological responses to both stabilizing and degranulating agents, and the cells are characterized by different sets of responses which include synthesis and secretion of proteolytic enzymes such as tryptase and chymase and products of arachidonic acid metabolism such as prostaglandins and leukotrienes.

Consequently because of this vast heterogeneity of cell type, distribution, anatomic association and function, the physiological role of mast cells may be different in different tissues even under the same challenge. Therefore, the answer to the question posed at the beginning would be determined by the nature of the stimulation, the site in which it occurred, the extent and duration of the stimulus and the determination of whether the stimulus was acute or prolonged and was accompanied by other events such as those induced by different forms of inflammation.

Nevertheless, it is possible to identify a series of physiological functions that appear to be important.

Control of mast cell and immunoglobulin E levels

Activation and degranulation of mast cells appears to be associated with the notion that this physiological event, no matter how it occurs, promotes a general increase in the total body mass of mast cells (1). There is also a relationship between mast cell mass and total amount of immunoglobulin E (IgE), the major allergic antibody that binds to mast cells through a high-affinity receptor (2). Conceptually, this leads to the notion that control of mast cell activation leads to control of IgE levels and reciprocally, control of IgE levels as may now occur by treatment *in vivo* with anti-IgE will control mast cell mass.

Blood vessel tone and coagulation

Mast cells are not just present for the promotion of wheezing, sneezing and itch. Indeed, their association with blood vessels suggests that they are intimately involved in maintaining blood vessel tone, vascular permeability and angiogenesis (3). In addition, mast cells contain a variety of glycosaminoglycans such as heparin which have major physiological effects on coagulation and various cellular activities (4). Undoubtedly, mast cells are involved in perfusion of tissues by blood and in the regulation of blood pressure through these means. Mast cell activation has also been suggested to be a key factor in the initiation of migraine (5).

Resistance to infection

Mast cells are significantly involved in host resistance to infection (6). The presence of functional mast cells is associated with recovery from and expulsion of nematodes in the intestine, but the absence of mast cells simply prolongs the infection leading to eventual expulsion, presumably by other means. Evidence for involvement in host resistance comes from experiments involving cecal ligation and puncture, where prevention of morbidity and mortality is associated with adequate mast cell function, primarily through the secretion of tumour necrosis factor α (TNF α), which in turn promotes IL-8 synthesis and polymorphonuclear leukocyte influx.

Allergy and inflammation

Mast cells are involved in allergy through a variety of complex events which involve synthesis and release of both preformed mediators such as histamine, serotonin, prostaglandins, leukotrienes, TNF α , etc. as well as a whole series of cytokines, enzymes and so on which may or may not be preformed (7). The events

which occur are caused both directly by mediators such as histamine or indirectly through complex central and peripheral events. These end up causing increased vasodilatation, local secretion by mucosal tissues, increase in smooth muscle reactivity and lowering the threshold for subsequent stimulation (8).

Communication with the nervous system

Mast cells are involved in bidirectional communication with nerves (9). Mast cells are universally found in close proximity to nerves of a variety of sorts. Factors released from mast cells can communicate with nerves and hence via local ganglia to the spinal cord and then to the brain. Mast cells can also locally promote axon reflexes. Thus, they can act as sensory receptors for noxious agents such as allergens, toxins, etc. and convey accurate information, as to the encounter with these agents, its nature, extent and location, to the central nervous system (CNS) (10). In turn, the CNS can use the same mast cells associated with nerves to be activated as targets of efferent nervous regulatory pathways. These processes are not only found to occur in allergy, but also in delayed hypersensitivity reactions and in response to bacterial toxins such as that of *Clostridium difficile* (11).

Involvement in behaviour

There is a large literature describing the involvement of mast cells and various brain processes. Suffice to say, mast cells are found associated with blood vessels throughout the brain and especially in the meninges (12). They seem also to be involved in behavioural activity such as the courting behaviour of doves (13). Surprisingly, mast cells are found in abundance around the pituitary gland and are thought to act as an immune 'gate' for hypothalamic-pituitary-adrenal axis activity (14). The effects of stress which are largely mediated through corticotropin-releasing factor, either locally or centrally synthesized seem to be mediated through nerves and mast cells (15). Pavlovian conditioning has also been shown to be able to promote mast cell degranulation through as yet unknown mechanisms (16).

Tissue repair

Increased numbers of mast cells are always found at sites of tissue repair and fibrosis (3). Their exact activity in this promotion and regulation of this process is not clear.

Summary

Mast cells have multiple functions; however, most of these functions are not essential for life, as various mast cell-deficient strains of mice and rats seem to have normal life spans (17). However, mast cells can be regarded as the conductors of the symphony of information, which is being constantly gathered both in the periphery and in the centre. They are masters and mistresses of coordination and integration, but they have the added dimension of remarkable varied effector functions. When one adds to their capacity to be motile or sessile depending on local circumstances, they emerge as the kings and queens of varied repertoire amongst all the cells in the body. Thus, mast cells may be thought to be important ancillary aids against infection, promoters of tissue repair, regulators of blood vessel tone, permeability and angiogenesis, important mediators of stress and allergy, regulators of neuroendocrine function, purveyors of sensory information to the central nervous system, and as effector targets for efferent nervous pathways. Lastly, but by no means least, they appear to act as a switchboard for the regulation of inflammation.

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Viewpoint 6

Due to the ubiquitous distribution of mast cells in diverse tissues and their presence throughout the animal kingdom, they are particularly well equipped to contribute to the maintenance of tissue integrity and function. Awareness of this has been only slowly emerging until recently, when mast cells have been shown to function as mediators of pathological reactions such as anaphylaxis, type-I allergen presentation, delayed-type hypersensitivity, natural immunity against bacteria and parasites, and tissue repair and fibrosis (1,2).

In this forum of controversies, I would like to present evidence that mast cells play a key function in tissue physiology, focussing on their direct or indirect contribution to epithelial and connective tissue growth.

Mast cell mediators and epithelial growth

Clear data to this effect emerged several years ago in the field of hair growth where several lines of evidence pointed to a role of mast cells during the initiation of the murine hair follicle cycle (3). In additional studies, mast cell-derived mediators like histamine, serotonin and nerve growth factor (NGF) were implicated in the regulation of murine hair growth (4–6). Some doubt as to the normality of these observations lurk, however, in the back of my mind, as in the model employed, anagen is induced by plucking of hair and thus by mechanical trauma with an associated massive mast-cell degranulation. Possibly, tissue traumatization and associated mast-cell-dependent processes in the context of wound healing may therefore be operative in the model employed (7).

Nevertheless, numerous other compelling lines of evidence have emerged in recent years that mast cells are able to induce hair growth as well as epithelial proliferation and differentiation in general. This holds in particular with regard to a number of mast cell mediators. Histamine is the most prominent among them and an obvious candidate for a physiological role, since this preformed and readily released vasoactive amine induces increased blood flow and leakiness of small vessels, allowing for the constant renewal of tissue fluid and thus the nutrition of diverse organs. Via the up-regulation of adhesion molecules on endothelial cells, histamine can also induce the immigration of formed elements from the blood which would then engage in tissue surveillance and renewal. Beyond that, we could recently show that at low concentrations which are likely to prevail under physiological conditions, histamine functions also as an epithelial

growth factor (8), as suggested already earlier by demonstration of epidermal keratinocyte proliferation in murine skin (6).

Furthermore, histamine up-regulates human keratinocyte expression of several major integrins ($\alpha 2$, $\alpha 3$, $\alpha 6$ and $\beta 1$ chains) at mRNA and partly also at protein level (8), a process that is viewed as an indication of keratinocyte differentiation (9). Expression of the differentiation-related dioxin receptor AhR and of the proliferation marker PCNA was also markedly increased under these conditions (8,10). Finally, partial inhibition by an H1 receptor-specific antihistamine suggests that histamine is also one of the mast-cell mediators contributing to fibroblast proliferation (11).

Mast cells and connective tissue homeostasis

Besides histamine, human mast cells also store several proteases in their secretory granules, specifically tryptase, chymase, carboxypeptidase A and cathepsin G (12), enzymes that are implicated in defence against helminthic, allergic, cardiovascular and chronic inflammatory diseases. Beyond this, these molecules are able to inactivate mediators of inflammation such as neuropeptides and numerous cytokines (13). Specifically, chymase can activate collagenase and stromelysin, destruct vitronectin and fibronectin, and induce fibroblast proliferation (13,14), suggesting an important role of this mast-cell-specific protease in tissue matrix turnover and renewal. Normally, several protease inhibitors within the connective tissue ensure tissue homeostasis by inhibiting excessive activities of this enzyme in the immediate mast cell environment. Such natural inhibitors are not known for mast cell-specific tryptase which is less active than chymase in connective tissue remodelling and fibroblast proliferation (13). It can, however, also stimulate type-I collagen synthesis (15) and, at low concentrations, it can't induce keratinocyte integrin expression just like histamine and thus epithelial differentiation (8).

Mast cell-derived growth factors affect numerous other cell types

In addition to the molecules discussed so far, mast cells are able to secrete numerous other mediators that can affect the growth of cells residing within epithelial and connective tissues (Table 1). Among these, interleukin-8 (IL-8) is of special interest, as it is also

Table 1. Mast-cell-derived factors likely to be involved in cutaneous physiology

Keratinocyte growth and differentiation factors	Fibrogenic factors	Angiogenic factors	Melanocyte growth factors
Histamine*	Platelet-derived growth factor (PDGF)	VEGF ₁₂₁ *, 165*, 189*, 206	SCF*
Tryptase*	Tryptase*	FGF-2	IL-8*
IL-3, IL-6, IL-8*	Chymase*	PDGF	Melanocyte growth stimulating activity/growth related oncogene
TGF-β	TGF-β	TGF-β	FGF-2
FGF-2, -5, -7, -10	FGF-2	GM-CSF	
HB-EGF	IL-4	TNF-α*	
EGF	Histamine*	IL-3, IL-8*	
Leukotrienes (B ₄ , C ₄ , D ₄)	Carboxypeptidase A	Histamine*	
		Heparin*	
		Tryptase*	

Mediators are either present in a preformed state*, generated rapidly, as holds for the lipid mediators like leukotrienes, or synthesized several hours after appropriate stimulation of the cells (1).

GM-CSF, granulocyte/macrophage-colony stimulating factor; IL, interleukin; TGF, transforming growth factor, TNF, tumour necrosis factor.

stored within mast-cell granules and thus readily available even on minor stimulation of the cells (16,17). Lipid mediators with keratinocyte growth-promoting activity like leukotrienes are not preformed, but they are generated within seconds via enzymatic action on membrane-associated arachidonic acid. The remaining mast-cell-derived cytokines and growth factors affecting keratinocyte growth (see Table 1), including the recently described HB-EGF and several members of the FGF family (8), are synthesized de novo on appropriate stimulation, a process that takes at least several hours (18). As none of these factors are detectable in freshly isolated skin mast cells, their direct contribution to skin physiology via mast cells is unlikely. Interestingly, they may however, be generated secondarily in neighbouring cells via rapidly released mast-cell mediators, as, e.g. recently shown for tryptase-induced FGF-2 and histamine-induced FGF-7 (KGF) synthesis in human fibroblasts (8).

Besides their well-established role in the maintenance of epithelial and connective tissue integrity, mast cells can also contribute via several mediators to endothelial growth, ensuring thus the needed tissue vascularization and proper nutrition. Furthermore, they produce several factors that stimulate melanocyte growth and melanin production (Table 1) and thus the necessary protection against UV-induced damage. Their important role in these normal physiological functions is underlined by the fact that many of the mediators listed are preformed within mast-cell granules (Table 1), including the three major isotypes of VEGF (19).

The picture of an all-round activity of mast cells in tissue physiology is completed in that mast-cell products can also affect nerve cell growth and differentiation via NGF, Langerhans cell migration and maturation via granulocyte/macrophage-colony stimulating factor and IL-4 and lymphocyte homing into the epidermis and proliferation via several distinct chemokines and cytokines (2).

Mast cell interact with numerous cells directly

On viewing the data discussed so far in perspective, one is struck by the emerging picture of mast cells acting within a network or along several axes involving all major tissue cell types. This is particularly evident for stem cell factor (SCF) which, in the skin, can be generated by keratinocytes, endothelial cells, fibroblasts, mast cells and melanocytes and which stimulates melanocytes and mast cells via its c-Kit receptor on these cells (1,20). Like IL-8, which can also be produced by several of these same cell types and for which mast cells also express specific chemokine receptors (21), SCF can thus stimulate its own secretion by mast cells in an autocrine fashion. This raises a number of questions as to the importance of mast cells vs. other cell types in the maintenance of

tissue growth and homeostasis. The same holds for the many different mediators potentially participating in these activities. Redundance of nature ensuring normal tissue function may well account for what seems puzzling at first sight.

Physiological stimuli of mast cell mediators

An important remaining issue is the clarification of the types of stimuli that might regulate mast-cell function in normal physiology, as in most of the literature cited so far, artificial laboratory conditions prevailed, with a veritable bombardment of the cells with agents inducing mast cell mediator release. For the skin, several possible, more physiological stimuli are readily apparent. The various stimuli inducing mast cell mediator release under physiological conditions are C3a/C5a, UV light, substance P, bacterial products, αMSH, IL-8, NGF and SCF. Thus, considering the fact that the skin and other tissues are continuously bombarded by pressure or shearing forces, neurosensory effector mechanisms may have an important impact on low-level mast-cell secretion that should be operative in a more normal physiological setting. Substance P is a possible player in this game, as are proopiomelanocortin-derived peptides like αmelanocyte stimulating hormone (MSH) both of which induce histamine secretion even at very low concentrations (16,22). As a result of constantly impacting UV light on the exposed skin surface, release of mediators like αMSH and SCF from keratinocytes must be considered as another likely scenario (23). Furthermore, the potent mast cell secretagogues C3a and C5a are known to also be constantly and rapidly generated in response to minor trauma, and finally products of the bacterial flora located in the upper layers of the epidermis or defensins produced by epithelial cells encompass mast-cell secretagogues as well (24,25) and may thus contribute to mast-cell function in normal physiology.

Conclusion: Mast cells are important players in tissue physiology

To sum up, mast cells are veritable powerhouses of growth factor production, as they can potentially generate and release their numerous potent mediators also under physiological conditions. The cells are thus superbly equipped to maintain tissue growth, differentiation and survival, either directly or through interaction with major cell types in their immediate surrounding. The unravelling of further details as to the conditions under which these diverse mast cell functions become operative in the context of tissue physiology remains an enticing future challenge.

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Commentary 7

This commentary focuses on the question ‘what – if any – is the physiological function of coronary mast cells?’ Our laboratory has investigated the role of mast cells in the pathogenesis of coronary atherosclerosis and its clinical complications (1). The pathogenesis of this chronic and long-lasting inflammatory disease involves the inner layer of the arterial wall, the intima (2). In its early stage, the disease is characterized by the accumulation of intracellular lipid (fatty streaks), in its later stages by extracellular accumulation of lipid (atheromas or atherosclerotic plaques) (3), and in its final clinically relevant stages by erosion or rupture of the plaques, with ensuing thrombotic complications (4). For the definition of a physiologic role for the mast cell in coronary atherogenesis, we have attempted to identify the crossroads at which the physiology of the mast-cell system meets with the pathology of the atherosclerotic process.

Regarding a physiologic role of mast cells in the normal human arterial intima, we have been puzzled by what is meant by ‘physiologic’ or ‘normal’. This same uncertainty may be felt by other scientists who are searching for a possible role of mast cells in other normal tissues. Although we still lack detailed studies on the age- and disease progression-related temporal sequence in which mast cells appear in the human arterial intima, it seems that, at the stage of lesion initiation, no mast cells or only a few mast cells are present in the intima (5). Thus, in the normal arterial intima, the average density of mast cells is only about 1 cell/mm², a number which contrasts sharply with that in the normal skin, which has been reported to be, on average, 50 cells/mm² (6). From the paucity of mast cells in the normal coronary intima, we conclude that these cells have little, if any, physiological role in this tissue site.

When the arterial intima is converted from a normal into a pathologic tissue, macrophages, T lymphocytes, and also mast cells arrive on the scene. In such lesions, the number of mast cells may be locally up to 10–50 or even to 100 cells/mm², and most of them show some degree of degranulation, i.e. they are activated (7). Unfortunately, we still have only a very rough idea of the phenotypic characteristics of mast cells in the plaque: with immunohistochemistry, we have shown that they contain heparin and tryptase, and sometimes also chymase and tumor necrosis

factor- α (8). Also, more studies are required for identifying the factors that regulate the different aspects of the life and death (immigration, differentiation, activation, and apoptosis) of a mast cell in the human coronary plaque. Thus, a very limited knowledge on the life cycle and actions of mast cells in the coronary plaques is available. Actually, a driving force for hypothesis generation have been the results obtained with rat serosal mast cells in cell culture and the results of biochemical experiments with purified mast-cell proteases. They, together with immunohistochemical data, have to guide us further in our search for a role of mast cells in the pathogenesis of atherosclerosis.

However, we have realized that, depending on the state of the disease, these potent effector cells may possess either disease-promoting or disease-inhibiting properties. Among the hypothetical disease-promoting properties is the potential ability of the heparin and neutral proteases released from activated mast cells to induce and maintain accumulation of intracellular cholesterol in early atherosclerotic lesions (9). Not only would the mast cells increase the uptake of cholesterol by intimal cells, but they would also inhibit the release of cholesterol from cholesterol-loaded cells. Similarly, degradation of the extracellular matrix by the neutral proteases released from activated mast cells and ensuing exposure of the subendothelial thrombogenic surface would definitely be harmful (10). In contrast, once the thrombogenic subendothelial layers have been exposed, the heparin proteoglycans released from activated mast cells may effectively prevent collagen-induced platelet aggregation (11), and hence prevent the formation of an occluding thrombus with ensuing myocardial damage. Thus, the mast cells in human coronary plaques appear to be at first harmful and then beneficial, but they only make good the harm that they have caused.

If we assume that the mast cells in coronary plaques are, after all, normal (in contrast to, at least, malignant mast cells), we must conclude that, in the human atherosclerotic plaque, physiologic cells reside in a pathologic environment. We need to know whether such mast cells have participated in the worsening of the pathologic environment or whether the pathologic environment was responsible for their local accumulation. Most

likely, the disease has progressed by a series of mutually perpetuating events. Then both of the above suggestions would be correct.

In summary, in human coronary plaques, mast cells may accelerate certain stages and prevent other stages in the disease progression. Probably, we should regard their disease-preventing functions as physiologic and their disease-aggravating functions as pathologic. Maybe, this functional dichotomy could then also be applied to the vast number of sentinel mast cells present in the body surfaces where they react to foreign invaders. Whereas the initial activation of mast cells in the normal surface tissue is physiologic, their chronic activation in a setting of prolonged inflammation may be either physiologic or pathologic.

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Commentary 8

Mast cells: from disease to physiology

Mast cells are bone marrow-derived cells that are well-recognized as triggers of the allergic process. The allergic process is initiated when the antigen causes the aggregation of occupied high affinity immunoglobulin E (IgE) receptors (FcεRI) expressed on the mast cell surface. This event is followed by the immediate release of pro-inflammatory mediators, such as histamine and tryptase, and the *de novo* synthesis and consequent release of prostaglandins and leukotrienes, a stage known as the early phase of allergy. Recent works have also clearly indicated the contribution of mast cells to the development of the late and chronic phases of allergy through the release of cytokines, chemokines, and other mediators that can influence the recruitment, activation, and survival of inflammatory cells (1), such as eosinophils (2), CD4⁺ T cells (3), basophils (4), neutrophils (5), and macrophages (6). Furthermore, mast cells have been shown to be related to the pathogenesis of a number of fibrotic conditions developing in the lung, such as idiopathic lung fibrosis and bleomycin or radiation-induced fibrosis in the skin such as scleroderma, hypertrophic scars, keloids, and chronic graft vs. host disease (cGvHD) in the intestine such as Crohn's disease, and postoperative peritoneal adhesions (7–9).

Mast cells have also been linked to the growth of new blood vessels through the production of angiogenic factors, such as vascular endothelial growth factor, heparin, basic fibroblast growth factor, transforming growth factor, tumor necrosis factor (TNF)-α, and interleukin (IL)-8 (10). Mast cells might, therefore, also contribute to the development of pathological conditions, in which blood vessel formation is present, such as tumor growth and others (10).

New evidences are indicating that mast cells are also involved in human immunodeficiency (HIV)-1 infection. The HIV-1 glycoprotein gp120 stimulates IL-4 and IL-13 release from mast cells via gp120 interaction with the V_H3 region of IgE. In addition, the Tat protein, secreted by HIV-1-infected cells, is a potent chemoattractant for FcεRI⁺ cells and up-regulates the expression of CCR3 on their surface. Mast cells also express the CXCR4 and

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CCR5 receptors and can be infected *in vitro* by M-trophic HIV-1 strains (11).

Taking into consideration all these factors, the original question posed in this commentary: 'What is the physiological role of mast cells?' is not simple to answer.

This commentary will describe in detail mast-cell contribution to physiological conditions in which consistent evidences demonstrate that mast cells take part in innate and adaptive immunity as well as in tissue repair.

Mast cells and innate immunity

Innate defense mechanisms against foreign invaders include mechanical barriers, secreted products, and inflammatory cells. Innate immunity present at all times in normal individuals does not distinguish among microorganisms of different species and does not change in intensity upon re-exposure (12). Mast cells are mainly found in the host–environment interface from which microorganisms can penetrate mucosa and submucosa of the gastrointestinal tract, lamina propria of the respiratory tract, skin, and peritoneum (13). Therefore, mast cells can constitute one of the first inflammatory cells establishing a direct contact with the invading pathogen.

Nevertheless, the role of mast cells in innate immunity has been clearly demonstrated with the assistance of the 'unlucky' mast-cell-deficient *Kit^W/Kit^{W-v}* mice. These mice do not express a functional receptor for stem cell factor (SCF) (14), a critical regulator of mast-cell development and survival (15). In addition, *Kit^W/Kit^{W-v}* mice are anemic and lacking tissue mast cells, germ cells, melanocytes, and interstitial cells of Cajal (16). Mast-cell deficiency of *Kit^W/Kit^{W-v}* mice can be repaired by directly injecting mast cells of wild-type origin into the so-called 'knock-in' mice. Using this model, it has been shown that during a bacterial infection, mast cells secrete TNF-α to recruit neutrophils to the infection site (17). In addition to these studies, Maurer et al. found that repetitive administration of SCF can improve the survival of normal mice subjected to cecal ligation and puncture (CLP), a model of acute bacterial peritonitis (18).

Using the CLP model, a mechanism has also been suggested, by which the mast-cell population is increased during a bacterial infection. Rosenkranz et al. have demonstrated that peritoneal mast cells express Mac-1 (CD11b/CD18 and CR3), a β_2 integrin with a role in the migration of mast-cell progenitors by their interaction with endothelium. However, it cannot be ruled out that Mac-1 also has a significant influence on the proliferation and survival of differentiated mast cells (19).

How mast cells are activated to release TNF- α and to initiate the resolution of the bacterial infection still remains unclear. Mast cells can be triggered during bacterial infections by direct and indirect mechanisms. For example, mast cells are directly activated by interaction of CD48, a glycosylphosphatidylinositol-anchored molecule expressed in mast cells, and FimH, a bacterial adhesin expressed in many members of the *Enterobacteriaceae*, such as *E. coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Salmonella Typhimurium* (20). In the indirect mechanism, mast-cell response seems to be mediated through bacterial activation of the host's complement system, because *in vivo* inflammatory response to CLP was significantly reduced in C4 or C3-deficient mice compared with wild-type mice (21). The resolution of CLP has also been shown to require high levels of IgM (22). In fact, IgM is a potent activator of complement that results in opsonization of the bacterial surface with C3d fragments that in turn activate complement receptor-bearing cells.

Both direct and indirect mechanisms could be amplified by the activation of coreceptors, such as CD21/CD35/CD19, suggesting new mechanisms for the regulation of mast-cell activity in the context of bacterial infections (23).

Mast cells in acquired immunity

The adaptative immune system is directed specifically to the infection agent. This system is inactive until stimulated by a specific infection. Afterwards, the adaptative immune system is altered in its intensity and response time upon re-exposure.

The role of mast cells in acquired immunity has been shown to be regulated by IL-3 that is probably derived from T cells that recognize the infecting agent. IL-3 contributes to an increase in the number of tissue mast cells, enhances basophil production, and increases immunity in mice infected with the intestinal nematode *Strongyloides venezuelensis* (24). However, recent studies have indicated that IL-3 is probably not the only factor responsible for the induction of mast-cell response against parasites. Indeed, it has been demonstrated that IL-4 and IL-13 are required for the mast-cell response that induces the expulsion of *Trichinella spiralis* and the cytokine responses that induce intestinal mastocytosis (25).

These findings suggest that the pattern of cytokines that induce mastocytosis during a worm infection varies according to the type of parasite. In fact, it has been observed that gastrointestinal nematodes such as *T. spiralis* and *Trichuris muris*, which live within cells, induce considerably more interferon (IFN)- γ production than gastrointestinal nematodes that have an entirely extracellular existence, such as *Nippostrongylus brasiliensis* and *S. venezuelensis* (26).

Another controversial issue in the context of acquired immunity is the role of mast cells as antigen-presenting cells (APC). Human mast cells have been shown to constitutively express MHC class I molecules (27) and can be induced to express MHC class II molecules after stimulation with IFN- γ and TNF- α (28). The functionality of MHC class II molecules was investigated by Dimitriadou V et al. They found that MHC class II molecules are expressed in the human mast-cell line HMC-I and bind the staphylococcal enterotoxin A (SEA) significantly. The ligation of MHC class II molecules with specific monoclonal antibodies or with SEA leads to ultrastructural changes that suggest mast-cell degranulation (29). Nevertheless, additional

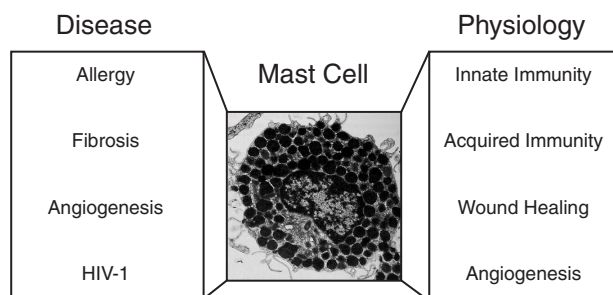


Figure 1. Some examples of different mast cell roles in health and disease.

studies must be done in order to better understand the physiological relevance of this finding.

Mast cells in the repair process: physiological role of mast cells in wound healing

The repair process is a normal physiological response to injury and generally leads to the restoration of the normal structure and function of a tissue. In certain disorders, the repair process leads finally to an altered restitution of tissue structure and function that is associated with the development of remodeling and fibrosis. As mentioned above, mast cells are related to the pathogenesis of many fibrotic disorders. However, mast cells have also been shown to participate in the physiological wound repair. The physiological role of mast cells in this process could be demonstrated in *in vitro* and *in vivo* models of wound healing. Wound healing is a very complex event that involves interactions of various cell types, such as lymphocytes, monocytes, epithelial cells, and fibroblasts. Three main overlapping phases have been identified in tissue response to injury: inflammation, granulation tissue and matrix formation, and remodeling. During granulation tissue formation, fibroblasts proliferate and migrate into wound space (30). We have demonstrated *in vitro* that mast cells influence the wound healing process by increasing this fibroblast migration and proliferation (31). This effect is partially mediated by histamine that acts on H₂-receptors on fibroblasts (32). Besides histamine, mast-cell-derived IL-4 was also found to stimulate fibroblasts to proliferate and migrate (33). We have recently found that nerve growth factor that is found preformed in mast cells (34), selectively increases fibroblast migration and α -smooth muscle actin expression (35).

New evidences are indicating that skin mast cells and mast-cell chymase are important for the formation of granulation tissue and synthesis of collagen fibers that occur at the edges of the wound in the burn-healing model in mice (36). This finding is supported by the fact that burn wound repair is delayed or poorly regulated in mast-cell-deficient mice (36). In addition, it has also been suggested that tryptase and chymase-positive mast cells (MC_{TC}) do indeed participate in tissue remodeling during the early stages of this process of wound closure. Afterwards, this type of mast cells disappear and an increase of tryptase-positive mast cells (MC_T), possibly infiltrating cells, is detected. In fact, the monocyte chemoattractant protein-1 seems to be responsible for mast-cell migration (33). Therefore, the oncoming MC_T are probably related to the later stages of wound healing and even fibrosis (37).

In conclusion, we have provided consistent evidences for a physiological role of mast cells in the processes of innate and acquired immunity as well as in wound healing.

The fact that mast cells are present in the tissues of all normal individuals leads us to think that their role as important cells in the regulation of physiological processes might be wider than what we discussed in this paper.

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Commentary 9

Mast cells work as a 'Koban', a residential police office distributed widely in the society for the maintenance of community order.

Mast cells are widely distributed in the body, especially at serosal and mucosal surfaces of gastrointestinal tract, conjunctiva membrane of eyes and surface of airways, which are the frontier defensive lines against the invasion of various foreign enemies into our body. Moreover, mast cells are resident cells of submucosal and dermal connective tissue keeping a constant distance with each other as if preparing for invaders breaking through the first defence lines. From their distribution, we can easily speculate that mast cells play important roles in host defence against bacteria and parasite infections by releasing various kinds of mediators (1,2). For example, histamine released immediately after their stimulation induces vasodilatation, increased vasopermeability and smooth muscle contraction resulting in edema at local affected sites. The localization of mast cells near vessels seems ideal for the enhancement of local permeability by released histamine together with certain newly generated lipid mediators and cytokines with vasoactive properties such as vascular endothelial growth factor (3). As a result, trespassers from outside were made floating in the cell interstitial fluid, washed out into lymphodactal tracts and finally trapped by lymph nodes. For the protection systems against outer world, we have not only innate immunity but also much more specialized, acquired immunity. Using the latter system efficiently, invaders must be recognized by antigen-

presenting cells. Mast cell-induced local edema seems very practical to make invaders encounter the antigen-presenting cells at trapped lymph nodes.

By using genetically mast-cell-deficient *W/W^v* mice, Malaviya et al. (4) found that mast cells may take a part in absolving intranasal or intraperitoneal infection with pathogenic *Klebsiella pneumoniae*. This effect was correlated with an ability of mast cells to produce tumour necrosis factor- α (TNF- α) and recruit neutrophils to the site of infection. Reconstitution of the *W/W^v* mice with purified mast cells improved the bacterial clearance rates back to normal levels by restoring TNF- α production and neutrophil recruitment. Echtenacher et al. (5) also used *W/W^v* mice in a model of acute septic peritonitis induced by surgical caecum ligation and puncture. When this treatment was performed in *W/W^v* mice, a striking 100% mortality occurred compared with the 25% mortality in control mice. Reconstitution with bone marrow-derived mast cells before surgery appeared to protect *W/W^v* mice from the lethal infection. This effect was mediated by TNF- α because the injection of its neutralizing antibodies suppressed the mast-cell-mediated protection, while injection of TNF- α protected unreconstituted *W/W^v* mice from peritonitis. These two papers proposed the hypothesis that pathogenic bacteria invading tissues activated the mast cells to release stored TNF- α , which then induced the rapid recruitment of neutrophils resulting in the early clearance of bacteria. However, there is a big enigma remaining in these studies with *W/W^v* mice. Even if these mast-cell-deficient mice were maintained in

conventional condition, they are quite healthy and never die from infections, suggesting that mast-cell contribution to innate immunity is, if any, restricted and may not be essential for threatening of our survival.

Once the fight was over, the tissue must be repaired to prepare for the next chance of invasion. Tryptase, a neutral protease specifically stored in human mast-cell granules, induces fibroblast proliferation and type-I collagen synthesis (6,7). Mast cells also produce some fibrogenic cytokines such as transforming growth factor- β and basic fibroblast growth factor, indicating that mast cells contribute to fibrosis (8,9). On the other hand, mast cells can produce matrix metalloproteinases and may also contribute to fibrolysis (10,11). Although the most relevant biologic substrates of tryptase remain uncertain, tryptase can cleave fibronectin as its substrate especially at acidic pH optimum (12), which supports the hypothesis that mast cells can take a part in the absorption of degraded matrix after inflammation. Thus, mast cells contribute to maintain the tissue healthy, as 'Koban' policemen keep cleaning around their community. However, we know that mast cells are not only cells that have such functions related to the tissue remodelling and that other types of cells can manage fibrosis and fibrolysis more efficiently than mast cells.

What is, then, the indispensable function of mast cells? Today in developed countries, mast cells are considered to work only for evoking of allergic disorders, such as allergic rhinitis, urticaria and probably asthma and atopic dermatitis. Therefore, some ironists may infer that the importance of mast cells is just for feeding allergologists, who get more patients visiting their office year by year because people are suffering from allergic disorders.

Gastrointestinal stromal tumour (GIST) expresses a mutated type of Kit tyrosine kinase (13), leading to autophosphorylation of the receptor for stem cell factor (SCF), which is the only growth factor that by itself supports the growth and differentiation of human mast cells *in vitro*. The tyrosine kinase inhibitor STI-S71 (Imatinib Mesilate), which is employed for the treatment of PR⁺ leukemia, is reported to be effective for the treatment of GIST by inhibiting the signal transduction through Kit. *W/W^v* mice, mentioned above as genetically mast-cell-deficient mice, are in fact the mice having little activity of Kit. Human mast cells are highly dependent on the signal through Kit and they will die within a few days after the withdrawal of

SCF (14,15). By using such kinds of drugs, we can even terminate all the mast cells in our body as the possible treatment of allergic disorders. However, mast cells are not simple troublemakers in allergic reactions but are smart guards for maintenance of the order in our body by protecting from invaders, scavenging debris after fights and keeping our tissue clean and comfortable through remodelling. Who can declare that we do not need 'Koban' system anymore because we have developed more powerful and effective defence systems? 'Koban' can still provide close and quick services in our society because they reside in the community, as do mast cells in our body.

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Commentary 10

Mast cells (MCs) are widely known as major effectors of inflammation. However, dramatic responses elicited by explosive MC degranulation have overshadowed evidence that, through subtle activities, MCs orchestrate a plethora of physiological processes. MCs can affect cell maturation, differentiation, survival, proliferation, and migratory, secretory and contractile responses. They exhibit several phenotypes, with differences primarily in mediator content and responsiveness to stimuli. This heterogeneity is induced by microenvironmental factors that tailor gene expression in MCs to enable functions specific to a particular organ or tissue site. Plasticity of phenotype and function allows MCs to be sensitive, tissue-specific regulators of physiological processes.

Although these concepts are not new, data are limited and often based on associations between MC numbers and mediator levels, rather than on mechanistic investigations. Future advances must consider the selected release of individual mediators at lower levels than in anaphylactic degranulation and tissue specific responsiveness, perhaps mediated by site-specific heterogeneity

of receptor expression. MC activities in reproductive physiology provide excellent examples of such complexities in the modulation of cycling, abortion, parturition and menstruation.

Estrogen levels correlate with MC numbers in tissues (1). Furthermore, estrogen (2,3) and luteinizing hormone (LH) (4) administrations both increase MC numbers. MCs in the genital tract are activated through tissue-specific signals, such as estrogens and LH, and also through corticotropin-releasing hormone, endothelin-1, neuropeptides such as substance P and embryo-derived factors (5).

Several MC mediators modulate reproductive physiology. Histamine increases cystic ovarian follicle formation, and LH-mediated histamine release from MCs might induce polycystic ovarian disease in an estrogen receptor knockout mouse (6). Endometrial MCs release proteases that can activate matrix metalloproteinases (MMP) (7); MMP levels increase during the late proliferative phase and cause focal tissue breakdown during menstruation. MCs are the main source of iNOS in the uterus (8); expression is regulated by estrogens and fluctuating during

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gestation. Early in gestation, nitric oxide promotes smooth muscle relaxation and increases uterine blood flow, whereas later it induces cervical ripening (9).

MCs also appear to translate signals for premature and stress-induced abortions. MCs adjacent to smooth muscle increase uterine contractility through histamine and serotonin, MC proteases and protease-activated receptors. Uterine MC numbers and histamine content increase late in pregnancy (2) when myometrium becomes hyper-responsive to histamine and serotonin (10). MC mediators increase gap junctional conductance in smooth muscle and could therefore coordinate uterine contractions at parturition (11).

Research has been hampered by the lack of good animal models. *W/W^v* and *Sl/Sl^d* mice are infertile because c-kit/SCF are important for germ cell development. Microphthalmic mice (*mi/mi*) that also lack MCs have abnormal ovulation and show uterine inversion during delivery; ovulation can be corrected by bone marrow transplantation but the cells responsible are not known (12).

Thus, MCs appear to play diverse roles in reproductive physiology. Their primary function is to orchestrate a variety of physiological processes in the genital tract. Moreover, we could have easily developed similar conceptual overviews of MC functions for other systems, including bone growth, development and remodelling; hypothalamic-pituitary neuroendocrine pathways; intestinal secretion and motility, etc. Given this broader vision of physiologic MC activation and functions, future experiments must incorporate more mechanistic rather than merely descriptive approaches.

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Commentary 11

Mast cells and basophils in immunoglobulin E-associated disorders

There is now little doubt that tissue mast cells (and basophils, a circulating granulocyte that can produce a spectrum of mediators that is similar but not identical to that of mast cells) can promote pathology, which, in certain circumstances (e.g. anaphylaxis), can even result in death (1–5). Moreover, evidence is increasing that mast cells can contribute importantly not only to the early events but also to the ‘late phase’ and chronic features of allergic asthma, an increasingly prevalent disorder associated with enormous morbidity and significant mortality (1–7).

The ability of mast cells and basophils to contribute to both acute and chronic aspects of asthma and other immunoglobulin E (IgE) antibody-associated disorders can be understood, at least in part, in terms of the products that these cells release upon stimulation with IgE and specific antigen. The reaction of bi- or multi-valent antigen with IgE bound to the surface of mast cells or basophils activates mediator release by inducing aggregation of the cells’ high-affinity IgE receptors (FcεRI) (8–10). Mast cells and basophils can secrete mediators that are either preformed and granule associated (e.g. histamine, proteoglycans and neutral proteases) or are synthesized de novo (e.g. leukotriene C₄ [LTC₄], platelet-activating factor [PAF] and prostaglandin D₂ [PGD₂] (in mast cells only)) (1–5). Furthermore, mouse or human mast cells represent potential sources of many cytokines, chemokines and growth factors with effects on inflammation, immunity, hematopoiesis, tissue remodeling and other diverse biological processes [e.g. interleukin-1 (IL-1), IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-11, IL-13, IL-16, tumor necrosis factor-α (TNF-α), bFGF, VPF/

VEGF, transforming growth factor-β (TGF-β) and many C-C chemokines, including macrophage inflammatory protein-1α (MIP-1α) and monocyte chemoattractant protein-1 (MCP-1)] (1–5,11); by contrast, the spectrum of basophil-derived cytokines appears to be more limited but includes IL-4 and IL-13 (12–14).

While such information helps us to explain how mast cells and basophils can produce pathology, it does not tell us why these cells continue to exist and, in the case of mast cells, populate almost all vascularized tissues. To address this fascinating question, we must first consider what qualifies as compelling evidence for an important role for mast cells or basophils in health or disease.

Genetic approaches for studies of mast-cell or basophil function

Because the strongest evidence for the roles of individual cells in biological responses *in vivo* usually is derived from *in vivo* studies, efforts to ascertain whether, and to what extent, mast cells or basophils actually contribute to specific physiological or pathological processes would be greatly facilitated by being able to analyze those processes in animals that differ only in containing or not containing the cell type of interest. At present, there is no animal model that exhibits a selective and complete deficiency in basophils. However, there is a very useful animal model for the analysis of mast-cell function that employs genetically mast-cell-deficient WBB6F1-*Kit^W/Kit^{W-v}* mice (*W/W^v* mice) and the congenic normal mice.

W/W^v mice exhibit markedly reduced c-kit receptor function due to spontaneous mutations in both copies of *c-Kit* (15,16). Adult *W/W^v* mice have less than 1% the wild-type levels of cutaneous mast cells, and ordinarily have no detectable mast cells in the peritoneal cavity, respiratory system, gastrointestinal tract or other sites (17). These mice also have other abnormalities due to the lack of adequate c-kit function, including a virtually complete lack of germ cells, interstitial cells of Cajal and cutaneous melanocytes, a moderate anemia, and additional, more subtle, abnormalities (4,16,17).

However, the mast-cell activity in *W/W^v* mice can be selectively reconstituted by the adoptive transfer of genetically compatible, *in vitro*-derived mast cells; such mast cells can be generated in at least two ways: first, cultured mast cells (CMCs) can be produced from the bone marrow or other hematopoietic cells of either the congenic wild-type mice or the various mutant, transgenic or knockout mice (18); or, second, embryonic stem cell-derived mast cells (ESMCs) can be generated directly from embryonic stem cells (19,20). Such *in vitro*-derived mast cells can be administered by intravenous, intraperitoneal, or intradermal injection, or by direct injection into the anterior wall of the stomach, thus producing so-called 'mast cell knock-in' mice (1,5,21–23). Experiments with such mice should include attempts to ascertain the numbers, anatomical distribution, and, in some cases, the phenotype of the adoptively transferred mast cells, as these features may not necessarily be identical to those of the mast cell populations in the same anatomical compartments in wild-type mice. Nevertheless, this approach permits one to assess directly how the expression of biological responses *in vivo* differs in wild-type mice (WBB6F1^{+/+} mice) or in WBB6F1-*W/W^v* mice in the presence or absence of adoptively transferred wild-type mast cells or mast cells with certain genetic alterations (including 'embryonic lethal' mutations) that affect mast-cell phenotype or function (1,18–20).

Unfortunately, there is no analogous 'basophil knock-in' system available at this time. Although IL-3^{-/-} mice fail to develop the striking basophilia that occurs in response to infections with certain intestinal nematodes, IL-3^{-/-} mice typically express normal (albeit very low) baseline levels of bone marrow basophils and circulating basophils (21). As a result, the normal expression in these mice of a biological response that is thought to involve basophils could reflect the residual function of the small numbers of basophils present in these animals.

Functions of mast cells and basophils in acquired and innate immunity

It appears very likely that mast cells and basophils contribute importantly to acquired immunity against certain pathogens, especially those that can induce a T-helper 2 (Th2)-type, IgE-associated immune response. While the strongest evidence supports a role for mast cells and basophils in acquired immunity to ectoparasites (ticks) (22–24), these cells also probably represent important effectors in immune responses to helminths (21). Recent work shows that mast cells can also play a critical role in innate immunity to bacterial infection (25–29) and that mast cells and basophils can be activated by viral proteins (30,31). In both acquired and innate immunity, one (but probably not the only) mechanism by which mast cells contribute to host defense is by functioning as 'sentinels' that detect invading pathogens and then help orchestrate protective local inflammatory/immune responses (32). Indeed, this ability to respond to a very localized signal and to amplify greatly the inflammatory response to that signal probably represents a fundamentally important aspect of mast-cell function. Moreover, evidence is accumulating that mast cells and basophils may also mediate immunoregulatory functions, both through their ability to produce certain cytokines and by other mechanisms (1–5,10,11,33).

Diverse mechanisms can elicit mast-cell function

In addition to IgE and antigen, a large variety of other agents can induce activation and mediator and/or cytokine release from mast cells and/or basophils. These agents include products of pathogens (bacteria, viruses, and parasites), products of complement activation, some neuropeptides and neurotrophins, certain hormones, endothelins, products of leukocytes, components of venoms and other toxic substances, several cytokines and chemokines, various types of physical and chemical injury, and many others (1–5,16,30,32), perhaps including "monomeric" IgE itself (10,34,35). In mice, mast cells also can be activated by IgG1 antibody-dependent mechanisms, that can recruit mast cells to participate in immune responses that occur independently of IgE (1,5,9,10), including models of 'autoimmune' disorders (36,37).

Notably, many of these IgE-independent mechanisms of mast-cell activation can induce a pattern of mediator and/or cytokine release that differs in composition, magnitude, or kinetics from that induced by IgE and antigen in the same cell populations [e.g. some bacterial products induce mast cells to release certain cytokines, but not stored mediators, whereas certain neuropeptides induce the release of stored products preferentially over products that must be synthesized *de novo* (1–5,16,30,32)].

The large number of agents that can induce mast-cell and/or basophil-mediator/cytokine release, when taken together with the large number of settings in which such mast-cell or basophil-activating agents might be produced and the diverse functions of the various mediators, cytokines and growth factors that can be elaborated by mast cells and basophils, has generated an enormous number of hypotheses about how mast cells and/or basophils might contribute to health and disease [(1–6,16,30,32,36,37) and other essays in this volume]. Moreover, mast cells have been reported to express functions that appear to be independent of their ability to release biologically active products [whether in association with classical degranulation or via other mechanisms, such as vesicular transport (38)]. These additional functions include phagocytosis (39), the endocytic uptake of exogenous substances (some of which may be subsequently released) (40) and antigen presentation (33); these represent some of the additional potential mechanisms by which mast cells can participate in pathological or physiological processes.

What do mast cells and basophils contribute to biological responses that are not involved in host defense?

This question has evoked speculation and controversy ever since Paul Ehrlich's description of some of the histochemical staining characteristics of these cells in the late nineteenth century (41,42). Space does not permit a detailed discussion of all of the current hypotheses in this area [of which there are many (1–6,10,16,30,32,36,37) and the other essays in this volume about the possible physiological functions of mast cells], hence I will instead outline a few general principles. The functions of mast cells and basophils *in vivo* will fall into three general categories: (1) responses in which the cell is essential (i.e. without that cell, no response will occur); (2) responses in which the cell can make a significant contribution (e.g. by altering the threshold for initiation, or the kinetics, intensity, anatomical distribution and/or duration of the entire response or of particular aspects of the response), but the cell is not essential for the occurrence of the response; and (3) responses in which the cell has functions that are redundant (i.e. largely overlapping) with those of other elements.

In the case of mast cells, their essential or contributory functions in various biological responses can be revealed by appropriate experiments in 'mast cell knock-in mice'. However, in the

third type of response (in which the cell's relevant function(s) can also be supplied by other participants), one would have to be able to manipulate selectively the involvement of the other important elements, as well as the mast cell, to identify the redundantly expressed role(s). Moreover, mast cells are long-lived cells that can participate in multiple cycles of activation over time and are likely to undergo complex alterations in phenotypic and functional characteristics in response to a variety of circumstances (1–5). These include: (1) prior exposure to a series of stimuli of functional activation (e.g. multiple rounds of IgE-dependent or -independent activation); (2) 'priming' (i.e. by agents that do not themselves activate mediator release, but alter the cell susceptibility to other agents that can activate mediator release); (3) exposure to many microenvironmental changes (e.g. alterations in local levels of growth factors, and other molecules, including IgE itself, that can affect mast-cell biology during inflammatory, reparative, and immunological responses); and/or (4) exposure to diverse systemic factors that can influence mast-cell biology, including certain growth factors or hormones (1–5,10,34,35). Moreover, as many complex physiological, adaptive, or pathological responses may be associated with the expression of different factors that can influence mast cells at different times during the course of the response, mast cells may have distinct roles in these processes at various times during their natural history.

It is likely that the number of biological responses in which mast cells and/or basophils have contributory or redundant functions exceeds those in which either cell is essential. Accordingly, some of these functions may be revealed only by carefully (or fortuitously) designed and executed experiments. A recent example is the recognition that mast cells can contribute significantly to multiple important characteristics of asthma in certain mouse models of the disorder, but that some of these mast-cell-dependent effects are not observed in other asthma models that employ different (generally stronger) procedures of antigen sensitization and/or challenge (6,7).

Given the economy of nature, it is to be expected that mast cells (and basophils, which are likely to express some physiological functions that overlap with those of mast cells) can express essential, contributory or redundant functions in many of the diverse physiological and pathological responses in which these cells have been implicated. Moreover, all of the cell-surface receptors and secreted products of mast cells and basophils, as well as those functions of the cells that are independent of their ability to release biologically active products (of which there may be many), probably have specific roles in at least some of these settings.

Remarkably, there have been few studies employing mast cell knock-in mice to address the roles of mast cells in physiological or adaptive responses. However, other lines of evidence suggest that mast cells may express beneficial functions by limiting the pathologic effects of certain endogenous or exogenous agents (other than pathogens). The potential mechanisms by which mast cells might influence such processes include: (1) helping to initiate, orchestrate, and/or resolve inflammatory responses that eliminate or contain the pathologic agent; (2) directly or indirectly altering the structure, biodistribution, or metabolism of biologically active endogenous or endogenous molecules [e.g. it should be emphasized that we are just beginning to understand the possible functions of the many serine proteases that can be produced by mast cells (43)]; (3) directly or indirectly influencing the phenotype/function of the many potential target cells in the tissues in which the pathology occurs (including fibroblasts and myofibroblasts, epithelial cells, endothelial cells, smooth, striated or cardiac muscle, and lymphoid and hematopoietic cells, as well as nerves); or (4) other functions, such as the internalization and intracellular sequestration and/or degradation of the potentially toxic agent.

In the search for physiological or adaptive mast-cell functions, it is attractive to hypothesize that many roles that have been identified for mast cells in pathological reactions reflect the dis-

ordered, nonadaptive, expression of complex cell-cell interactions that evolved to promote homeostasis or host defense. For example, studies in *W/W^v* mice whose mast-cell deficiency was repaired (albeit non-selectively) by adoptive transfer of wild-type bone marrow cells indicate that mast cells can promote epithelial chloride secretion in a model of intestinal anaphylaxis, probably in part via bidirectional interactions with enteric nerves (44), can promote colonic mucin release in a mouse model of immobilization stress (45) and can contribute to both neutrophil recruitment and intestinal fluid secretion in a model of enteritis that is induced by *Clostridium difficile* toxin A (46). Studies in mast-cell knock-in mice show that mast cells can significantly contribute to the enhanced vascular permeability and tissue swelling, leukocyte recruitment, and local fibrin deposition in response to injections of substance P (47,48). These, and many of the other studies conducted with mast cell knock-in mice, may well contain clues about the roles of mast cells in health, as well as in disease.

Clearly, there are still more questions than answers about the 'physiological' (as well as the pathological) functions of mast cells and basophils. Happily, in the case of the mast cell at least, we now have approaches that not only will help us to define whether and to what extent mast cells actually contribute to diverse biological responses *in vivo*, but will permit us to explore exactly how they express such functions. While these certainly are encouraging developments, past experience suggests that the mast cell, which has fascinated so many scientists for so long, will continue to surprise us.

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