ADF Perspectives

The *status quo* and *quo vadis* of mast cells

Abstract: Mast cells (MCs) have been intensely investigated over the past two decades, e.g. the numbers of PubMed-listed reports on MCs have steadily increased and doubled over the past twenty years. Surprisingly, many recent findings that have fundamentally changed our understanding of MC biology and functions have yet to be sufficiently recognized by scientists interested in cutaneous biology and clinical dermatologists. The aim of this study is to review recent hallmark contributions to the field of MC research, to outline the development of our current knowledge of MCs, and to predict the outcome of future MC research efforts. The development of straightforward rodent *in vivo* models has allowed for the identification and characterization of various novel MC functions. MC effects are not limited to the induction of pathology, but can serve important functions in maintaining health and preventing disease. Attempts to better define the role of MCs in the human system may lead to novel strategies for treating inflammatory disorders and could eventually allow us to utilize MCs for improving responses to environmental danger signals.

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Introduction

Hardly any other cell is more in need of an update than the mast cell: The general knowledge of this cell is still widely based upon findings of the 1960s and 1970s, although the results of numerous recent research efforts have fundamentally changed what we know about the biology of mast cells, especially their physiological functions. It is not the goal of the following feature to provide a traditional review of the work that contributed to the many recent changes of our understanding of mast-cell functions. Rather, we felt that it would be more worthwhile, for us and the reader, to sketch the development of today's status quo on mast cells from a historical perspective and to share our own personal view of where mast-cell research of the future should and will be headed. This overview summarizes the key messages of the *Quo Vadis* lecture 'Mast cells – What are they good for? presented at the 2004 annual meeting of the Arbeitsgemeinschaft Dermatologische Forschung.

Abbreviations: CLP, cecal ligation and puncture; MCs, mast cells.

Mast cells – at first sight

Common grounds

When we ask our medical students (as we often do) about the functions of mast cells, we are bound to get one answer and one answer only: Mast cells are allergy cells. And indeed, that is exactly what today's textbooks have to say about mast cells (MCs): They are activated upon rechallenge with environmental allergens (e.g. tree or grass pollen and many others) in pre-sensitized individuals who have produced specific IgE antibodies, which have then bound to high-affinity IgE receptors (Fc$_\varepsilon$RI) on the surface of MCs. And it is common knowledge that activated MCs release histamine, which is responsible, at least in part, for the elicitation of allergic symptoms that can be seriously annoying (e.g. in atopic eczema and allergic rhinitis) or dangerous (e.g. in allergic asthma and angioedema), or even deadly in the case of anaphylactic shock. How and why has this evolved – the view of MCs as rather simply structured characters with one single goal in life, to harm or even kill its host? First of all, because it is true. In fact, few

other cells express Fc$_\varepsilon$RI or receptors of equally high affinity, MCs are the main producers of histamine, and virtually no other cell can kill us quicker than MCs. And because MCs are such potent players in allergic diseases, MC research has focused on this role for the longest time. Can you think of another cell of the human body, whose pathological function, or better, one pathological function, is as thoroughly studied and understood as the MC's role in allergy? Histamine, the major MC mediator involved in the induction of allergic reactions was first described almost a hundred years ago and has been intensively investigated ever since. IgE was discovered in the 1960s, followed by extensive scrutiny including the identification and in depth characterization of its receptors including their downstream signaling pathways. As a result, the IgE/Fc$_\varepsilon$RI pathway of MC degranulation activation is now, by many, considered to be one of the best researched and understood mechanisms of receptor-mediated cell activation.

Challenging the Darwin dogma

'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.', Darwin said in *The descent of man* 6 years before MCs were discovered. Could it be that evolution has simply forgotten to get rid of 'the structure MC'? Could it be that this phylogenetically ancient cell used to serve functions that have ceased to be of importance or benefit? This question has troubled generations of MC biologists and is historically referred to as 'the riddle of the mast cell' (1).

MCs – the inside view

Steps to the era of enlightenment

It is not that MC biologists did not have good ideas on what MCs are good for. On the contrary, numerous hypotheses on physiological MC functions have been postulated during the first hundred years following the discovery of MCs, some very reasonable and backed by phenomenological evidence and *in vitro* findings (Table 1). The problem was that none of them were testable, as suitable *in vivo* models did not exist. In the mid 1980s, Kitamura, Galli, and co-workers developed a mouse model which made it possible to test MCs *in vivo* for their contribution to any inducible pathological or physiological process of interest (Fig. 1). This model made use of the

Table 1. Selected hypotheses for physiological functions of mast cells

Hypothetical mast-cell function	Author	Year
Protection from tumors	Ehrlich	1877
Phagocytosis of pathogens	Metchnikoff	1892
Endocrine functions	Cajal	1896
Lipid metabolism	Ciaccio	1913
Vitamin metabolism	Tuma	1928
Calcium metabolism	Pautrier	1931
Growth control	Sylven	1941
Blood clotting and coagulation	Baeckeland	1950
Hair growth	Montagna	1951
Haematopoesis	Messerschmitt	1955
Local detox	Higginbotham	1956
Regulation of blood pressure	Keller	1957
Regulation of pH	Caselli	1958
Regulation of temperature	LeBlanc	1959
Aging	Spicer	1960
Stress responses	West	1962
Containment of foreign bodies	Selye	1963
Regulation of sweat secretion	Szabo	1964
Peripheral 'memory bank'	Padawar	1978

Kit^W/Kit^{W-v} mouse, which is, due to mutations in both copies of *c-kit*, virtually MC-deficient but also anemic and devoid of germ cells, melanocytes, and interstitial cells of Cajal. However, by engrafting bone marrow-derived, cultured MCs locally and selectively into these genetically MC-deficient mice, so called MC knock-in mice could be generated. This allowed for testing groups of mice that differed solely in containing or lacking MCs at defined body sites. By comparing MC-deficient mice and MC knock-in mice (or MC-deficient and MC-reconstituted sites in MC knock-in mice) for differences, it was now possible to characterize the role of MCs in various pathological and physiological processes.
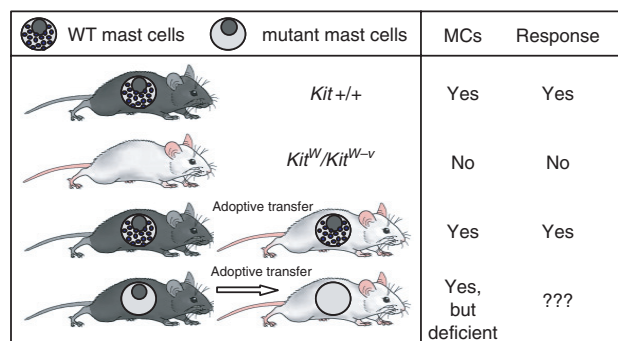

Figure 1. The Kit^W/Kit^{W-v} mouse model, a crucial tool to identify and characterize the role of mast cells (MCs) in physiology and pathology. Biologic response can be tested for differences in MC-deficient Kit^W/Kit^{W-v} and wildtype $Kit+/+$ mice, in which case Kit^W/Kit^{W-v} mice can be engrafted with wildtype MCs or, to characterize a putative role of MC mediators or receptors expressed by MCs, with MCs from mutant mice that are deficient for specific mediators or receptors. Symbols: black mice = WBB6F1-$Kit+/+$ mice ($Kit+/+$, wild type (WT)), white mice = WBB6F1-Kit^W/Kit^{W-v} mice (Kit^W/Kit^{W-v}), cell with granules = wildtype MCs, empty cell = mutant MCs.

Making use of the ever increasing number of knock-out mice, the MC knock-in mouse model was later developed further so that it became possible to test, which MC mediator or MC receptor contributes to a process identified as MC-dependent. Because this is achieved by engrafting Kit^W/Kit^{W-v} mice with MCs from genetically compatible knock-out mice that lack specific MC mediators or receptors, it was until recently impossible to use this approach in the case of lethal loss of function mutations. However, very elegant techniques developed by the Galli lab during the past years now allow for the *in vitro* generation of MCs that are deficient for mediators or receptors, even if the respective knock-out mouse can not be used to obtain bone marrow cells for the *in vitro* generation of MCs, e.g. because these mice die before or shortly after birth. This novel technology involves the generation of immature MCs from embryonic stem cells, which are then used for the engraftment of selected organs of MC-deficient mice where they differentiate, similar to adoptively transferred bone marrow-derived cultured MCs, to mature and functional MCs with tissue-specific characteristics (2,3).

Putting old concepts to new tests

For obvious reasons, the induction and promotion of allergic reactions were among the first MC functions that were put to the test using this new model. As was to be expected, type I allergic responses to allergens, i.e. IgE-mediated allergic reactions of the skin and other organs, were found to be almost entirely MC-dependent. Moreover, MCs were also shown to be critically involved in type III hypersensitivity responses (e.g. Arthus reaction) and in some type IV allergic reactions (e.g. contact hypersensitivity). Given the similarities of these processes and autoimmune responses [e.g. in rheumatoid arthritis, Sjogren syndrome, systemic sclerosis, multiple sclerosis, thyroid disease, chronic urticaria, pemphigus, bullous pemphigoid, and atherosclerosis (4)], it was not long before MC knock-in mice were also used to explore the role of MCs in models of autoimmune conditions. With great success: For example multiple sclerosis, an autoimmune disease characterized by the destruction of myelin and subsequent spasticity, pain, vision impairment, vertigo, and fatigue was shown to depend on MC support as demonstrated using the murine EAE model (5). Even more strikingly, MC-deficient mice are protected from the induction of autoimmune arthritis (AA), whereas prior engraftment of Kit^W/Kit^{W-v} mice with MCs restored their susceptibility for developing AA to wildtype levels (6). In fact, the abbreviation MCs for MCs was proposed to actually stand for 'Master Cells' in this context, as MCs were widely regarded to account for the elicitation and coordination of all subsequent pathogenic events following the induction of AA. In short, the reputation of MCs appeared to be ruined once and for all, and the constant flow of reports showing that MCs contribute to pathology in yet another disease model, from A as in alopecia areata to Z as in several zoonoses, certainly did not help.

MCs – what are they good for?

However, the force was strong with those that were unwilling to give MCs up to the dark side, and in the mid 1990s, two independent groups of researchers set out to use the MC knock-in model to test MCs for a health-promoting and disease-preventing function that had been postulated almost 100 years earlier: Antibacterial host defense. At that time, the results from two active areas of MC research had provided a number of additional good arguments for a role of MCs in the elicitation of innate immune responses to bacteria. MCs had been shown to be preferentially localized in organs that separate us from our environment, i.e. the skin, gut, and airways, and that are therefore primary target sites for bacterial infections. Indeed, MC populations of the skin exhibit two unique gradients of distribution in that cutaneous MC numbers increase (i) with proximity to the epidermis and (ii) with distance from the body center (7). In other words, the most superficial layers of hand, feet, and face skin, i.e. where the risk of bacterial infection is the highest, contain markedly more MCs than deep layers of truncal skin, i.e. where the risk of infection is lowest (Fig. 2). MCs had also been shown to respond to a large variety and number of signals other than IgE/allergen, including many that are up-regulated at sites of bacterial infection. In addition, numerous additional MC products had been discovered, many of which are nowadays regarded as first line of defense mediators in antibacterial innate immunity. Nonetheless, when Malaviya and Echtenacher and their respective co-workers proved in 1995 with their breakthrough back-to-back reports in *Nature* that MCs are critical effector cells in eliciting protective responses against bacteria, the MC world was taken by storm and, in retrospect, their reports of these findings must be called the beginning of a new era in MC research (8,9).
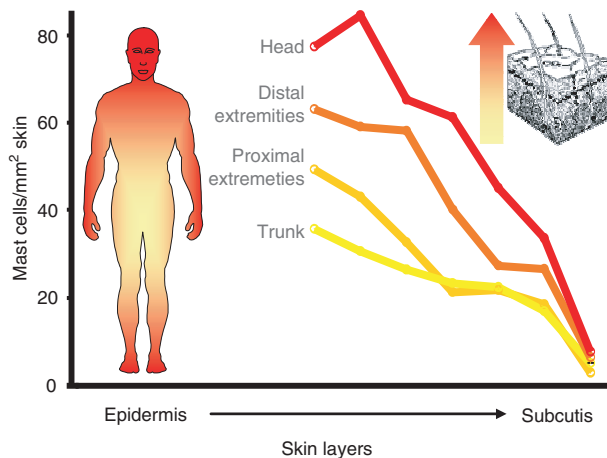
Figure 2. Mast cells (MCs) in the skin are preferentially located at sites with higher risks of bacterial infection. MC numbers in human skin are highest in the most superficial skin layers and lowest in the subcutis, and they increase with distance of anatomical site from body center.

Relieved by the fact that 'their cell' was finally found to be good for something and inspired by the 'life saving' effects of MCs, many MC laboratories around the globe focused their efforts on characterizing this 'new' important MC function. This renaissance of interest in MCs, which – let us not forget – was only made possible because of the models developed by the Galli and Kitamura laboratories 10 years earlier, was driven by two dominating questions: How do MCs know that they have to deal with invading pathogenic bacteria? How are bacteria eliminated by MCs?

The MC–complement connection in host defense

Hypothesizing that complement components could be involved in the activation of MCs at sites of bacterial infection, we assessed the outcome of septic peritonitis induced by cecal ligation and puncture (CLP) in complement 3 (C3)-deficient mice. Surprisingly, the phenotype that these mice developed in response to CLP was strikingly similar to the one in MC-deficient mice, and increased susceptibility to the pathological consequences of bacterial sepsis in C3-deficient mice was repaired in part by prior reconstitution with C3. Moreover, MC activation in C3-deficient mice subjected to CLP was found to be markedly reduced as compared with complement-reconstituted C3-deficient mice and wildtype mice (10). Given that MCs express complement receptors (CR) 1 and 2 and that mice deficient for these receptors also express reduced MC activation and increased mortality following CLP (11), complement must be regarded as a critical mediator of MC activation in the context of antibacterial host defense. Other important mechanisms of MCs to detect host-derived signals induced by bacterial challenge include their expression of endothelin-1 receptors and the direct detection of bacteria (e.g. via CD48) or bacterial products (e.g. via toll-like receptor 4). The fact that MC activation at sites of bacterial infection can be induced by several very different mechanisms further emphasizes the importance of early MC degranulation and mediator release for protective antibacterial host defense (Fig. 3).
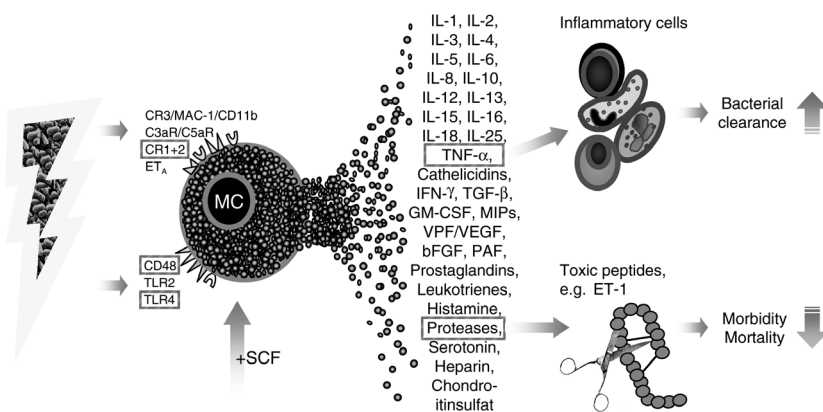

Figure 3. Mast cells (MCs) are key effector cells in innate immunity against bacteria. The activation of MCs (e.g. by bacteria and bacterial products) results in the release of a wide array of mediators, among them proteases that can degrade toxic peptides, and TNF-α which helps clear an infection by recruitment of inflammatory cells. MC receptors and mediators that have been proven to play a role in bacterial infections using the Kit^W/Kit^{W-v} mouse model are enclosed in a box. CR, complement receptor; ET_A, endothelin A receptor; TLR, toll-like receptor; MC, mast cell; SCF, stem cell factor; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; TGF, transforming growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; MIP, macrophage inflammatory protein; VPF/VEGF, vascular permeability factor/vascular endothelial growth factor; bFGF, basic fibroblast growth factor; PAF, platelet-activating factor; ET = Endothelin.

TNF-α – the MC's favourite weapon against bacteria

MCs can phagocytose and kill bacteria, but their relatively low numbers (e.g. less than 5% of skin cells) and their limited mobility and plasticity strongly argue against a major role for direct bactericidal MC action. By contrast, MC-mediated protection against bacteria has been consistently shown to be linked to MC degranulation early after infection. This suggests that MCs protect from bacteria (and the damage that results from bacterial invasion) by releasing mediators that are either pre-stored in cytoplasmic granules and/or rapidly synthesized upon activation. Even though MCs can produce and release dozens of mediators that meet these criteria, some appear to be more important than others, and TNF-α seems to be the most important of them all. TNF-α-deficient mice respond virtually identically to CLP as MC-deficient mice, and TNF-α can be adoptively transferred instead of MCs to normalize responses to bacteria. But what exactly is the function of MC-derived TNF-α? The kinetics of MC responses in bacterial infection imply that TNF-α must be released very early after MC activation, i.e. from pre-formed stores, and peritoneal TNF-α levels after CLP correlate with the early influx of neutrophils and the clearance of bacteria from the peritoneal cavity. Notably, neutralizing antibodies against TNF-α given early in acute septic peritonitis result in impaired neutrophil recruitment, reduced bacteria elimination rates, and increased morbidity and mortality (Fig. 3).

MCs – the more the better?

At this point, an interesting and intriguing question had to be dealt with (allergists are advised to read on at their own risk): Can antibacterial host defense be improved by increasing MC numbers? To answer this question, we applied the MC growth factor SCF (the ligand of Kit) to naïve mice for several weeks and then tested these mice for their responses to CLP. Interestingly, SCF treatment resulted in a dose-dependent increase in peritoneal MC populations, which correlated with survival of acute septic peritonitis, i.e. the more peritoneal MCs mice express, the lower their morbidity and mortality following CLP. SCF treatment did not increase protection in MC-deficient mice (unless they had been engrafted with MCs) indicating that MCs are required for SCF to improve antibacterial protection (12).
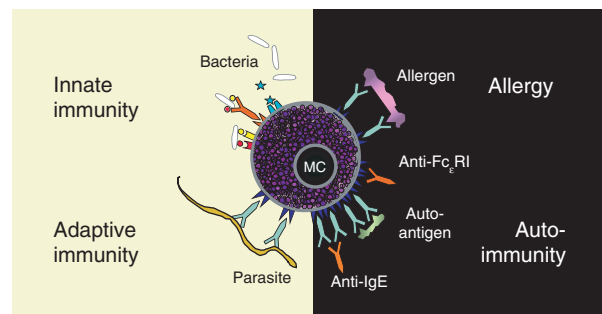

Figure 4. Mast cells – the good and the bad side. MCs can be detrimental in allergy (e.g. anaphylactic shock) and autoimmunity (e.g. autoimmune arthritis) but also have beneficial roles in innate (e.g. bacterial infections) and adaptive immunity (parasite infections). MC, mast cell; Fc$_\varepsilon$RI, high-affinity IgE receptor.

In summary, because of a large body of supporting work, MCs are now regarded to be 'good and bad guys', just like every other cell (13). Their best talent, i.e. to raise inflammatory responses can be annoying, dangerous, and even deadly in the context of allergic and autoimmune disorders; but it can also prevent and control disease and even save the host's life during the course of innate or adaptive host defense responses against pathogens (Fig. 4).

MC – Quo vadis?

Where do we go from here? What are the most important questions that remain to be answered and that will or should be asked? In our view, the most important answers that MCs have in store for us in the near future are the ones to the questions (1) how do all these novel findings from murine models affect us as humans and (2) what else are MCs good for and how can we find out?

From mice to man

At this point, we simply do not know whether or not murine and human MCs serve the same or different physiologic functions. MCs in mice and in humans are very similar in many respects including their distribution, responses to stimulating signals, and mediators. There are, however, important differences (e.g. human MCs do not contain serotonin, some proteases differ in structure and function, and cytokines such as IL-3 and IL-4 can differ in their effects on murine or human MCs) and uncritical extrapolation of findings from mouse studies to other species is not a good idea. We need research approaches

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aimed at clarifying the role of MCs in human innate and adaptive immune responses, and such investigations are well underway in many laboratories. We must also question our strategies for the treatment of MC-mediated diseases, allergies in particular, as shutting down MCs and antagonizing their effector functions may in theory result in impaired MC-mediated protection against environmental and endogenous danger signals. It may be a good thing that we have not found ways of completely annihilating MC populations or activation. Finally, our approaches to specifically target pathology promoting MC effects (while keeping their beneficial functions intact) should benefit from the ever-growing knowledge of what mechanisms MCs employ to respond to different signals and which mediators MCs use to do the job in question (14). In other words, a better understanding of the importance of defined MC receptors and their downstream signaling pathways as well as the characterization of the contribution of individual MC mediators in specific settings could greatly improve the efficacy and safety of treatment strategies for different inflammatory disorders.

The MC – jack of all trades?

As for predictions of what additional, as of yet unknown, MC functions will be discovered next, the MC may reveal itself as a 'jack of all trades' when it comes to detecting danger and providing damage control (Fig. 5). We now know that MCs provide protection against various pathogenic bacteria, a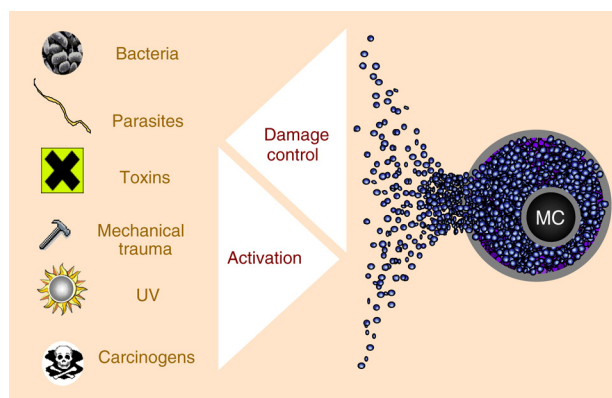nd MCs reportedly contribute to the control of a number of gastrointestinal and cutaneous parasites including *Leishmania*, *Schistosoma*, various nematodes, and larval ticks. Toxins, e.g. poison or bacterial as well as endogenous toxins, may be another group of danger signals that MCs protect us from. For example, we have recently found that the levels and pathogenic effects of endothelin-1 (ET-1), an endogenous toxin produced and released in a number of pathologic conditions (e.g. sepsis, where it also contributes to morbidity and mortality), are almost completely controlled by MCs (Fig. 3). Using the MC knock-in mouse model, we were able to demonstrate that MCs detect minute increases of ET-1 via their ET_A receptors and that ET-1 is degraded and 'detoxified' by proteases including chymase released as a consequence of MC degranulation (3). According to recent findings from our and other laboratories, other danger signals that MCs could detect and counteract include UV light, mechanical trauma, and even carcinogens (Fig. 5). While these are potential MC functions that we can be sure to hear more about in the near future, they are by far not the only ones. Hypotheses on what MCs are good for are as old as the first description of MCs by Ehrlich and von Recklinghausen and many promising suggestions from the pre-MC knock-in model era remain to be tested (Table 1). In any case, we can be sure (and we do look forward to this) that MCs will continue to surprise us with novel and/or unexpected functions and that this exciting challenge will drive the efforts of the rapidly increasing next generation of MC biologists.

Figure 5. Are mast cells (MCs) simply acting in large measure as sensors to environmental alteration? MCs are ideally located at sites where potential dangers threaten the host. Mediators from MCs that are activated by these danger signals can help control the damage. Thus, the main physiological function of mast cells located at body surfaces may be to help maintain homeostasis by detecting and controlling danger. MC, mast cell; UV, ultraviolet light.

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