Published in final edited form as:

Trends Immunol. 2012 December ; 33(12): 613–625. doi:10.1016/j.it.2012.09.008.

New models for analyzing mast cell functions *in vivo*

Laurent L. Reber^{1,3}, Thomas Marichal^{1,3}, and Stephen J. Galli^{1,2}

¹Department of Pathology, Stanford University School of Medicine, Stanford, California 94305-5324, USA

²Department of Microbiology & Immunology, Stanford University School of Medicine, Stanford, California 94305-5324, USA

Abstract

In addition to their well-accepted role as critical effector cells in anaphylaxis and other acute IgE-mediated allergic reactions, mast cells have been implicated in a wide variety of process that contribute to disease or help to maintain health. While some of these roles were first suggested by analyses of mast cell products or functions *in vitro*, it is critical to determine whether, and under which circumstances, such potential roles actually can be performed by mast cells *in vivo*. This review discusses recent advances in the development and analysis of mouse models to investigate the roles of mast cells and mast cell-associated products during biological responses *in vivo*, and comments on some of the similarities and differences in the results obtained with these newer versus older models of mast cell deficiency.

Keywords

Basophils; *c-kit*; mouse model; Cre recombinase

The spectrum of ‘potential’ mast cell functions: an embarrassment of riches

Trying to figure out what mast cells (MCs) do *in vivo* has been challenging. This is not for want of hypotheses. Indeed, given what has been reported about MCs based on *in vitro* or *in vivo* evidence, the possibilities appear to be almost endless (Box 1). Taking such information into account, one can come up with a nearly limitless list of “potential” or “possible” MC functions – spanning many if not all aspects of health, host defense, and disease. But what, in fact, are the important functions of MCs *in vivo*, and how can these be identified? To answer these questions, one must first agree on terminology, including the definition of “important functions”. In Box 2, we propose working definitions of a range of possible MC contributions to biological responses or to the individual features of such processes.

© 2012 Elsevier Ltd. All rights reserved.

Address correspondence to: Stephen J. Galli, Department of Pathology, L-235, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305-5324, Tel: 650-723-7975, Fax: 650-725-6902, sgalli@stanford.edu.

³These authors contributed equally to this work.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

What kinds of experimental approaches can permit one to identify the actual contributions of MCs when investigating their potential roles in particular biological settings? The simplest would be to be able to ablate MCs selectively *in vivo*, e.g., with a drug or an antibody, or genetically. Moreover, one ideally would be able to ablate selectively either all MCs (producing a fully MC-deficient host, in which potential local and/or systemic effects of MCs could be tested) or only the MC populations of interest (e.g., those in the skin or lungs). Once it is established that MCs have a detectable role in a biological response, it is useful then to define how that MC role is expressed in that setting. To address this question, one ideally would be able to delete selectively elements of MC activation pathways, or MC products, or to block specifically those MC-derived products by which MCs might express that function.

To date, no agent that can solely and specifically suppress MC activation has been discovered. Notably, recent findings indicate that even the so-called “MC stabilizer” cromolyn is neither an effective nor selective inhibitor of mouse MC activation *in vitro* and *in vivo* [1]. For this reason, genetic approaches probably represent a more definitive way to identify and characterize MC functions in mice *in vivo*. While progress has been made in devising genetic approaches that address that goal, particularly over the last few years, each of the new approaches (as well as older models that have been widely used for many years) have known or potential limitations that must be kept in mind when interpreting the results of such work.

In this review, we will compare and contrast the advantages and potential disadvantages of both older and newer approaches to investigate the roles of MCs *in vivo*. We will discuss some examples of MC functions that now have been supported by evidence derived both from studies in older models (consisting of mice that have mutations affecting *c-kit* structure or expression and that consequently exhibit a profound MC deficiency together with a variety of other phenotypic abnormalities) and from work employing newer models in which the MC deficiency is not dependent on mutations affecting *c-kit* structure or expression. We also will discuss some MC functions that were proposed based on evidence obtained in “*kit* mutant MC-deficient mice” which have not been confirmed in initial studies employing the new “KIT-independent” models of MC deficiency. Finally, we will comment on some early results of work attempting to probe the roles of MCs in particular biological responses using more than one model system.

“Kit mutant” MC-deficient mice and “MC knock-in mice”

To date, mice whose sole abnormality is a specific lack of all populations of MCs have not been reported. However, we and others have used mice deficient for KIT, the receptor for the main MC growth and survival factor, *stem cell factor* (SCF) [2, 3] (which sometimes are collectively called “*kit* mutant mice”) to analyze the functions of MCs *in vivo* [4–8]. The two types of MC-deficient mice used most commonly for such studies are WBB6F₁-*Kit*^{W/W^{-v} and C57BL/6-*Kit*^{W-sh/W-sh} mice [5–12]. *Kit*^W is a point mutation that produces a truncated KIT that is not expressed on the cell surface [13]; *Kit*^{W^{-v} is a mutation in the *c-kit* tyrosine kinase domain that substantially reduces the kinase activity of the receptor [14] and *Kit*^{W-sh} is an inversion mutation that affects the transcriptional regulatory elements upstream of the *c-kit* transcription start site on mouse chromosome 5 [15, 16]. Both WBB6F₁-*Kit*^{W/W^{-v} and C57BL/6-*Kit*^{W-sh/W-sh} mice are profoundly deficient in MCs and melanocytes and have several other phenotypic abnormalities that reflect the biological distribution and functions of KIT in cells within and outside of the immune system of these mice, including some abnormalities affecting hematopoietic cells other than MCs that contribute to innate or adaptive immune responses (Box 3) [6, 8, 12, 16–18]. However, some of these “non-MC” phenotypic abnormalities differ between the two most commonly used types of *kit* mutant}}}

MC-deficient mice. For example, WBB6F1-*Kit*^{W/W^{-v}} mice are anaemic, have reduced numbers of neutrophils [8, 12, 16, 18] and basophils [8, 19, 20], and are sterile [5, 6]. By contrast, C57BL/6-*Kit*^{W-sh/W-sh} mice are neither anaemic nor sterile, but have increased numbers of neutrophils [6, 8, 12, 16] and basophils [8].

Differences in the biological responses in *kit* mutant mice compared with wild-type (WT) mice of course may reflect any one (or more) of the abnormalities that result from the alterations of KIT structure or expression in these animals, in any of the directly or indirectly affected cell lineages, and may not be due solely or even partly to their deficiency in MCs. However, at many anatomical sites, the deficiency in MCs in *kit* mutant mice can be selectively “repaired” by the adoptive transfer of genetically-compatible, *in vitro*-derived WT or mutant MCs [4–6, 10, 21]. Such *in vitro*-derived MCs, for example bone-marrow-derived cultured MCs (BMCMCs), can be administered intravenously (i.v.), intraperitoneally (i.p.) or intradermally (i.d.) to create so-called ‘MC knock-in mice’. Since their description in 1985 [21], such MC knock-in mice have been widely employed to assess the importance of MCs in regulating the expression of biological responses *in vivo*.

However, it has long been known that, depending on the route of injection of MCs and/or the numbers of MCs injected, the numbers and/or anatomical distribution of adoptively transferred MCs after transfer to *kit* mutant mice can differ from those of the corresponding native MC populations in the corresponding WT mice [6, 17, 22, 23]. With direct injection of BMCMCs into the skin or peritoneal cavity of WBB6F1-*Kit*^{W/W^{-v}} or C57BL/6-*Kit*^{W-sh/W-sh} mice, the numbers and anatomic distribution of adoptively transferred MCs in the dermis or in the peritoneal cavity and mesentery, when assessed 4–8 weeks after MC transfer, can be similar to those of native MCs in WT mice [6, 17]. By contrast, at 4–28 weeks after injection of BMCMCs i.v. into WBB6F1-*Kit*^{W/W^{-v}} or C57BL/6-*Kit*^{W-sh/W-sh} mice, few or no MCs are detectable in the trachea of the mice (and numbers are much less than those in the corresponding WT mice) whereas the numbers of MCs in the periphery of the lung are substantially greater than, and the numbers of MCs around the bronchi can be similar to, those in the corresponding WT mice [6, 17, 22, 24]. Such differences in MC numbers and anatomical distribution of adoptively transferred versus corresponding WT MC populations should be taken into account when considering the results obtained in MC knock-in versus corresponding WT mice. One must also consider the possibility that the native and adoptively transferred MC populations differ in certain aspects of phenotype. Although direct comparisons of such populations have in general shown that, over time, the phenotype of the adoptively-transferred MCs comes to closely resemble that of the native population [21, 25], there have been relatively few studies of that type. Moreover, it is not currently possible to define every aspect of the phenotype of either native or adoptively transferred MC populations *in situ*. Therefore, one can’t formally rule out the possibility that the two MC populations might express phenotypic differences that in turn might influence the results obtained in a particular biological response.

Mutant mice with constitutive MC deficiency unrelated to c-kit abnormalities

KIT has pleiotropic functions unrelated to MCs (Box 3). Therefore, even when MC engraftment results in MC numbers and anatomical distributions in the recipient *kit* mutant mice that are very similar to those of the corresponding WT mice, it is possible that such adoptively transferred MCs can “normalize” some of the biological responses that are abnormal in *kit* mutant mice because the transferred MCs compensate in the mutant mice for abnormalities in lineages other than the MC -- abnormalities that do not exist in the corresponding WT mice. To return to the example of the round table with 10 legs (Box 2), MCs may play more critical roles in some biological responses in *kit* mutant mice than in

WT mice because that biological response has less redundancy in the *kit* mutant mice than is present in the WT animals.

Because of the potential complexities and caveats inherent in interpreting findings based on work employing *kit* mutant MC-deficient mice, several groups have sought to develop mice that are MC-deficient but which lack abnormalities related to KIT structure or expression. A common approach has been to generate mice in which Cre-recombinase (Cre) is expressed under the control of promoters thought to be “MC-specific” or at least “MC-associated” [26–29]. To date, three new strains of mutant mice with marked *constitutive* deficiencies in MCs have been reported (Table 1) [19, 28, 30].

Mcpt5-Cre; R-DTA mice

Dudeck *et al.* (2011) crossed *MC protease (Mcpt)5-Cre* transgenic mice with *R-DTA^{fl/fl}* mice [31] to generate a mouse strain in which the diphtheria toxin alpha chain (DTA) is produced only in Cre-expressing cells, thereby driving Cre-specific ablation of such cells [30]. Naive *Mcpt5-Cre;R-DTA* mice displayed a constitutive lack of peritoneal and ear skin MCs as well as >90% reductions in the numbers of abdominal and back skin MCs in comparison to the *Cre⁻* counterparts [30]. The effect of Cre-mediated DTA expression on mucosal MCs (MMCs, which are thought not to express MCPT5) or other hematopoietic cell types in steady-state or inflammatory conditions, and the efficiency of DTA-induced deletion of connective tissue type MCs (CTMCs) during inflammatory responses associated with increased numbers of MCs, remain to be described.

Cpa3^{Cre/+} - “Cre-Master” mice

“Cre-Master” stands for “Cre-mediated mast cell eradication”. Feyerabend *et al.* (2011) used an elegant knock-in strategy to induce *Cre* expression under the control of the *Cpa3* promoter while deleting 28 nucleotides of the first exon of *Cpa3*, which encodes for the MC-associated protease carboxypeptidase A3 (CPA3) [19]. Unexpectedly, heterozygous *Cpa3^{Cre/+}* mice exhibited a virtually complete lack of MCs, multiple MC-associated proteases, and a MC gene expression signature, as assessed in the peritoneal cavity and skin. Skin MCs were still undetectable under inflammatory conditions that can be associated with the development of skin MCs in *WBB6F1-Kit^{W/W-v}* mice [32] and MMCs remained absent in the intestine after helminth infection [19]. In addition, *Cpa3^{Cre/+}* mice did not detectably respond in an IgE-dependent model of passive cutaneous anaphylaxis (PCA) and exhibited neither reduced body temperature nor mortality when subjected to an IgE-dependent model of passive systemic anaphylaxis (PSA) [33], unless they were engrafted with BMCMCs.

This profound depletion of MCs appears to be mediated by Cre-induced genotoxicity [34]. However, although CPA3 is highly expressed in MCs [35], it is also expressed in basophils [36] and some populations of T-cell progenitors and thymic T cells [27, 37], and in certain hematopoietic progenitor cells [38]. Consistent with this, the authors also detected some Cre activity in T cells [27], as well as a 60% reduction in numbers of spleen basophils [19]. While Cre expression in basophils was not sufficient to ablate the entire population, it must be kept in mind that the residual basophils may not be fully functional.

Cpa3-Cre; Mcl-1^{fl/fl} – “Hello Kitty” mice

Our group generated transgenic mice expressing Cre recombinase under the control of a *Cpa3* promoter fragment [28] and crossed them with mice in which the gene coding the anti-apoptotic factor *myeloid cell leukemia sequence 1* (Mcl-1) [39, 40] was floxed [28]. The resulting *Cpa3-Cre; Mcl-1^{fl/fl}* mice exhibited a marked *kit*-independent constitutive reduction in numbers of MCs (92–100% reduction in all anatomical sites tested except the spleen, that, like the spleen of the corresponding control mice, contained small numbers of

MCs); *Cpa3-Cre; Mcl-1^{fl/fl}* mice also exhibited a substantial reduction in bone marrow, blood and spleen basophils (reductions of 78, 74, and 58%, respectively, in comparison to the *Cpa3-Cre* controls). Because these phenotypes are seen in the absence of mutations affecting *c-kit* structure or expression, these mice are informally called “Hello *Kitty*” MC- (and basophil)-deficient mice.

Assessing the responses of these markedly MC-deficient mice in three models of IgE-dependent inflammation revealed, as expected, that they were markedly deficient in two responses that previously had been characterized (in *kit* mutant MC-deficient mice) as IgE- and MC-dependent [41–43], specifically, IgE-dependent PCA (except at sites engrafted with WT MCs) [41] and IgE-dependent PSA [43]. However, these studies also revealed that the reduction in numbers of basophils in Hello *Kitty* mice, although modest compared to the deficiency in tissue MCs, was associated with a profound impairment in the animals’ ability to orchestrate a response that is IgE- and basophil-dependent, but MC-independent [28, 44]. The latter finding illustrates that mutant mice with less than full ablation of a certain type of effector cell (in this case, the basophil) may nevertheless exhibit a marked abnormality in a biological response that is particularly dependent on that cell type.

These three new types of MC-deficient mice represent welcome new tools for investigating the role of MCs in biological responses *in vivo*. Nevertheless, in designing experiments employing such mice (or the older models), and in interpreting the results of such work, it will be prudent to keep in mind the already identified and additional potential limitations of these models, issues that may turn out to be more important in some types of biological responses than in others (Table 1, Box 3). One potential problem common to each of the three new strains, and to the *kit* mutant MC-deficient mice, is that the effects on certain biological responses of a constitutive deficiency of MCs may be different than those observed when the MCs are ablated just before or during the response. The latter situation generally has more clinical relevance than the former, as in most cases one would not attempt to reduce MC numbers or functions in human subjects unless there was compelling clinical evidence that MCs are important in the pathology associated with a particular disorder.

Inducible depletion of MCs

Employing mouse models to test the hypothesis that MCs represent an important therapeutic target in a particular setting should ideally be performed using mice in which inducible and selective MCs ablation can be achieved. Depletion of MCs from mice by conventional techniques, such as the injection of depleting antibodies, is limited by the lack of surface markers that have been shown to be unique to the MC population. For example, repeated treatment with antibodies that neutralize SCF [45] or block KIT [46, 47] can result in the depletion of MCs *in vivo*, but can also have potential effects on other cell types such as hematopoietic stem cells [48].

One promising approach for achieving a more selective and efficient depletion of a particular cell population is the injection of diphtheria toxin (DT) into transgenic mice bearing the DT receptor (DTR) only in that particular cell type [49]. This approach was recently used by two different groups to deplete MCs in adult mice (Table 1) [30, 50, 51].

Mcpt5-Cre; iDTR mice

Dudeck *et al.* mated *Mcpt5-Cre* mice with *iDTR^{fl/fl}* mice expressing a floxed simian DTR transgene inserted into the *Gt(ROSA)26Sor* (ROSA26) locus, to achieve Cre-dependent expression of DTR in MCs [30]. The authors reported that a single i.p. injection of DT leads to nearly complete ablation of peritoneal MCs in *Mcpt5-Cre; iDTR* mice after 24 h, however

they didn't comment on MC numbers in other organs or whether there were any effects on other cell types. Repeated i.p. injections of DT (once a week for four weeks) led to complete ablation of MCs in the peritoneal cavity and abdominal skin of *Mcpt5-Cre; iDTR* mice as compared to *Mcpt5-Cre⁻* mice, when assessed one week after the last DT injection. However, achieving complete deletion of ear skin MCs required combining repeated i.p. and subcutaneous treatment with DT [30]. Moreover, analysis of the small intestine and stomach of DT-treated *Mcpt5-Cre; iDTR* mice showed depletion of subepithelial CTMCs but not intraepithelial MMCs, most likely reflecting a lack of *Mcpt5-Cre* transgene expression in MCs of the mucosal type [30]. Nevertheless, these animals should represent a valuable tool for studying the effects of a local depletion of MCs in various acute biological processes. These mice may even be used to study the role of MCs in more chronic settings since only about 10% of peritoneal and skin MCs reappeared 3 weeks after cessation of treatment with DT under steady-state conditions. The authors reported that numbers of other major hematopoietic cells, including bone marrow basophils, were not affected by DT-treatment. However, this analysis was performed one week after the last DT injection, and it would be of interest to know whether DT injections resulted in any transient depletion of other cell types.

“Mas-TRECK” mice

Otsuka *et al.* and Sawaguchi *et al.* described a new transgenic strain, named “Mas-TRECK” (for Mast cell-specific enhancer-mediated Toxin Receptor-mediated Conditional cell Knock out), in which expression of the human *DTR* gene is under the control of an intronic enhancer (IE) element of the *IL-4* gene [50, 51]. They previously reported that this IE element was essential for IL-4 expression in MCs but not basophils, natural killer (NK) T cells or Th2 cells [52]. Repeated i.p. treatment of Mas-TRECK mice with DT for 5 consecutive days completely depleted MCs in the skin, peritoneal cavity, stomach and mesenteric windows, as assessed 3 days after the last injection, and abrogated IgE-dependent PCA and PSA reactions [50]. They also showed that skin MCs remain depleted for at least 12 days after cessation of DT treatment [51]. However, DT treatment in these mice also leads to a transient depletion of blood basophils and virtually completely inhibited the development of a model of basophil-dependent, IgE-mediated chronic allergic inflammation of the skin [44, 50]. Other major types of leukocytes (dendritic cells, B, T, NKT cells, eosinophils and neutrophils) did not express *DTR* mRNA and were not affected by DT treatment, although numbers of these cells were only reported for analyses done 12 days after the end of DT treatment [50, 51].

Mutant mice with deletion of MC-associated products

If a mediator is selectively expressed by MCs (to prove this, expression needs to be analyzed in other cell types under both baseline and pathological conditions), its role can be investigated *in vivo* by testing animals in which that mediator has been knocked out. However, many of these highly MC-associated (if not truly MC-selective) mediators (such as MC-associated proteases) show strong interdependence in terms of proper storage in the cytoplasmic granules and this clearly must be kept in mind when interpreting results obtained with mice genetically deficient in such mediators (Table 2). Analyzing to what extent MCs represent important sources of products that can also be derived from other cell types, such as leukotrienes, prostaglandins, cytokines, chemokines, and growth factors, would require deletion of that product specifically in MCs. In this regard, the newly developed “MC-specific Cre” mice [26–29, 53] may allow for specific deletion of “floxed” genes in MCs.

Dudeck *et al.* (2011) were, to our knowledge, the first to take advantage of this system in order to reduce secretion of MC-derived interleukin (IL)-10 *in vivo* by crossing *Mcpt5-Cre*

transgenic mice [26] with *Il-10^{fl/fl}* mice [30]. Other researchers used this approach with *Mcpt5-Cre* mice in order to drive expression of a gain-of-function mutation of KIT (*Kit^{D814V}*) [54] or to specifically delete *SH2 domain-containing phosphatase-2 (SHP2)* gene [55] in CTMCs. Recently, a new mouse strain expressing Cre under the control of the *high affinity receptor for IgE, β chain* promoter (*Fc ϵ RI- β Cre*) has been generated and used to delete the *phosphatase and tensin homolog (Pten)* gene in the MC compartment [53].

Two critical issues have to be taken into consideration when interpreting the results obtained using a Cre/lox approach. First, Cre activity must be efficient in, and ideally selectively restricted to, MCs, both in naive animals and under inflammatory conditions, since MC promoter-driven Cre expression may vary depending on the conditions and models tested. In this regard, using a reporter mouse is a valuable tool for the assessment of Cre-mediated recombination under different conditions *in vivo*.

In the study by Dudeck *et al.* (2011), *Mcpt5-Cre⁺* mice were crossed to the Cre excision reporter mice *ROSA26 Stop^{fllox} EYFP (R26Y)* and EYFP expression was assessed by flow cytometry in naive animals, confirming a highly efficient recombination in peritoneal and skin MCs but also revealing an unexpected recombination in a small population of blood NK cells. Lilla *et al.* (2011) crossed transgenic *Cpa3-Cre* mice [28] with a *mT/mG* reporter line [56], thus revealing the steady-state detection of Cre expression in a small population of basophils, eosinophils and neutrophils in addition to MCs [28]. The breeding of *Cpa3-Cre* mice with particular ‘floxed’ mice might therefore result in gene inactivation in certain populations of granulocytes (as well as in MCs) in double transgenic mice and this might limit the ability of this approach to reveal specific roles of MC-derived products in settings in which such other cells also may have important roles. Transgenic mice expressing Cre recombinase under the control of the conserved *baboon alpha-chymase* promoter (*Chm:Cre*) [29] displayed Cre expression specifically in lung and colon tissues by using *Chm:Cre/ROSA26R* reporter mice. However, in the lung of *Chm:Cre/ROSA26R* naive mice, 26% of Cre positive cells were KIT negative, strongly suggesting that Cre activity might not be fully MC-specific.

Second, the Cre-mediated gene inactivation should be demonstrated in MCs and only in MCs. Dudeck *et al.* used an elegant, sensitive method of single-cell PCR in order to assess the specificity and efficiency of Cre-mediated *Il-10* gene inactivation in several cell types using nested primers [30, 57]. The authors showed thereby an efficient inactivation of the functional *Il-10* locus in peritoneal and skin MCs, but not in peritoneal B cells, macrophages or skin T cells from *Mcpt5-Cre⁺; Il-10^{fl/fl}* mice, or any cell type tested in *Mcpt5-Cre⁻; Il-10^{fl/fl}* mice. However, blood NK cells, which exhibited some Cre-mediated recombination, apparently have not been tested for *Il-10* gene inactivation [30].

“Kit mutant” vs. “KIT-independent” MC-deficient mice in models of host defense and disease: Concordance, controversies, and opportunities

As reviewed above, individual mouse models of MC deficiency, or models to alter the expression of MC-associated products, differ in their features and may vary in their advantages and limitations for studies of MC function. The newer models of MC deficiency are particularly attractive because they lack the KIT-related phenotypic alterations associated with *kit* mutant MC-deficient mice. However, because the newer models only recently have been described, it is likely that there is still more to be learned about their phenotype, including features that may influence the interpretation of experiments designed to investigate MC functions. In addition, one often can select from among a wide variety of experimental protocols and conditions to investigate particular hypotheses about MC functions in examples of host defense or disease.

Both factors, i.e., (1) the choice of MC-deficient mouse model(s) and (2) the selection of particular experimental conditions for investigating the roles of MCs in various types of biological responses, can influence the results of such work. Moreover, the choice of experimental protocol may be particularly important in biological responses in which the MC is more likely to have a redundant rather than unique (non-redundant) role (Box 2). This rather obvious point was evident even before the introduction of the new models of MC deficiency, as is illustrated by the results of efforts to employ *kit* mutant MC-deficient mice to investigate the roles of MCs in cutaneous contact hypersensitivity (CHS) responses or in allergic inflammation of the airways (Table 3).

There are at least two ways to view the fact that work by different groups (or even different experiments performed by the same group [58, 59]) support “different conclusions” about the importance of MCs in various biological responses. The first is that this constitutes a “controversy” regarding the roles of MCs in that type of biological response [60, 61]. The second (which is not necessarily incompatible with the first) is that such discrepancies identify interesting opportunities for understanding the basis for the differences, and thereby to gain additional insights into the regulation of these biological responses.

For example, in the case of both CHS and allergic airway inflammation, current findings are compatible with the conclusion that the ability of the MC to enhance particular features of the models is most readily detected when one attempts to elicit “relatively weak” responses, particularly in mice of suitable genetic background (Table 3). In the case of allergic inflammation of the airways, inducing reactions with relatively low doses of antigen for sensitization and challenge may more closely mimic clinical settings than do protocols which use strong adjuvants for sensitization and large amounts of antigen for airway challenge. With respect to models of antibody-dependent arthritis, it seems plausible, although not yet formally proven, that the relative neutrophil deficiency of WBB6F1-*Kit^{W/W-v}* mice (as well as perhaps other abnormalities affecting hematopoietic cells in addition to MCs and neutrophils) contributes to the inability of these mice fully to develop the features of the pathology (Table 3).

In more general terms, it seems reasonable both to think that evolution has engineered redundancy into the mechanisms needed to sustain many critical biological processes in order to ensure that they remain robust, as well as to propose that such mechanistic redundancy also applies to many pathological processes. Indeed, we speculate that there may be only a small number of biological responses in which MCs are uniquely critical (e.g., as an important source of proteases that may be highly expressed in MCs), to the extent that little or no response would be detectable in the absence of MCs under any conditions of testing. Accordingly, in many types of complex biological responses in which the roles of MCs may be partially overlapping with those of other effector elements, we expect that the choice of experimental model, including the intensity of the stimulus used to elicit the response, may be critical in determining whether the contributions of MCs will be sufficiently important that their absence will be reflected in a significant impairment of the response. Similarly, it seems reasonable to propose that the more critical the MC's contributions to a particular biological response, the more likely one will find abnormalities in that response when it is tested in each of the different types of MC-deficient mice.

For these reasons, we recommend attempting to test hypotheses about MC function using more than one model of MC deficiency. In our lab, we used to perform pilot experiments for any new project in both WBB6F1-*Kit^{W/W-v}* and WCB6F1-*Mgf^{Sl/Sl-d}* mice. We then switched to testing WBB6F1-*Kit^{W/W-v}* and C57BL6-*Kit^{W-sh/W-sh}* mice and we are now transitioning to doing pilot experiments in C57BL6-*Kit^{W-sh/W-sh}* and *Cpa3-Cre; Mcl-1^{fl/fl}* mice. If we obtain concordant results in both types of MC-deficient mice, we then proceed

to further studies, which can include using mice genetically deficient in particular MC products of interest. An example is our recent study of the roles of MCs and the MC-associated chymase, MCPT4, in enhancing resistance to the venoms of the Gila monster and two scorpions, and to the endogenous peptide, VIP [62].

By contrast, if tests in two different types of MC-deficient mice yield discordant findings, we generally do not continue. While pursuing such a project (or such an “opportunity”) might reveal interesting information about why the MC’s role may be “revealed” in one type of MC-deficient mouse and not in the other, there is also the risk that we might not succeed in explaining the discrepancy and thereby consume in an ultimately futile effort resources which could be used for more promising lines of inquiry. While in many settings it may not be practical also to test multiple models or protocols of the type of host defense or disease of interest, one certainly should keep in mind, as discussed above, that the roles of MCs in influencing different features of particular biological responses clearly can vary based on the selection of experimental conditions to examine.

Although we are in early days with respect to some of the newer models that can be employed for MC research, there already are examples where work in older and newer models have provided either very similar or rather discordant evidence for particular MC functions. Some of the possible reasons for why discrepant results have been obtained regarding the roles of MCs in particular biological responses are noted in Table 3. We have summarized in Box 4 some conclusions about the nature and importance of the roles of MCs in different types of biological responses that we think are compatible with current evidence derived from work in multiple older and newer models of MC-deficient mice and/or in mice deficient in certain MC-associated products.

Concluding remarks

This is an exciting time in MC research. The continued availability of “old” models (including “MC knock-in *kit* mutant mice” and various MC protease-deficient mice), combined with the introduction of several promising “new” models of MC deficiency or for MC-targeted deletion of MC products, offers a wealth of opportunities to enhance progress in solving the long-standing “riddle of the mast cell”, at least in mice. Some of this work may even suggest new approaches for the treatment of diseases in which MCs have been implicated. However, based on the results obtained so far with both the older and newer models for MC research, we think that the most robust conclusions about what MCs can do (or don’t do) in various biological responses *in vivo*, and regarding the importance of such MC contributions, are likely to be derived from investigations that employ multiple informative model systems. This approach increases the cost of such work, but permits one to exploit the attractive features of the various models while keeping in mind the known and potential limitations in each of them.

Acknowledgments

We thank the members of the Galli lab and our collaborators and colleagues for their contributions to some of the work reviewed herein and we apologize to the many contributors to this field whose work was not cited because of space limitations. We also thank Dr. Mindy Tsai for critical reading of the manuscript. L.L.R. is the recipient of fellowships from the French “Fondation pour la Recherche Médicale FRM” and the Stanford Pediatric Research Fund and is funded by the grant #SPO106496 from the Arthritis National Research Foundation; T.M. is supported by a fellowship from the Belgium American Educational Foundation and a Marie Curie International outgoing Fellowship for Career Development; S.J.G. acknowledges support from National Institutes of Health grants AI023990, CA072074 and AI070813.

References

1. Oka T, et al. Evidence questioning cromolyn's effectiveness and selectivity as a 'mast cell stabilizer' in mice. *Lab. Invest.* 2012 (*In Press*).
2. Oliveira SH, Lukacs NW. Stem cell factor: a hemopoietic cytokine with important targets in asthma. *Curr. Drug Targets Inflamm. Allergy.* 2003; 2:313–318. [PubMed: 14561150]
3. Reber L, et al. Stem cell factor and its receptor c-Kit as targets for inflammatory diseases. *Eur. J. Pharmacol.* 2006; 533:327–340. [PubMed: 16483568]
4. Kitamura Y. Heterogeneity of mast cells and phenotypic change between subpopulations. *Annu. Rev. Immunol.* 1989; 7:59–76. [PubMed: 2653378]
5. Galli SJ, et al. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu. Rev. Immunol.* 2005; 23:749–786. [PubMed: 15771585]
6. Grimaldeston MA, et al. Mast cell-deficient *W-sash c-kit* mutant *Kit^{W-sh/W-sh}* mice as a model for investigating mast cell biology *in vivo*. *Am. J. Pathol.* 2005; 167:835–848. [PubMed: 16127161]
7. Dawicki W, Marshall JS. New and emerging roles for mast cells in host defence. *Curr. Opin. Immunol.* 2007; 19:31–38. [PubMed: 17126541]
8. Piliponsky AM, et al. Mast cell-derived TNF can exacerbate mortality during severe bacterial infections in C57BL/6-*Kit^{W-sh/W-sh}* mice. *Am. J. Pathol.* 2010; 176:926–938. [PubMed: 20035049]
9. Galli SJ, et al. Mast cells in the development of adaptive immune responses. *Nat. Immunol.* 2005; 6:135–142. [PubMed: 15662442]
10. Grimaldeston MA, et al. Effector and potential immunoregulatory roles of mast cells in IgE-associated acquired immune responses. *Curr. Opin. Immunol.* 2006; 18:751–760. [PubMed: 17011762]
11. Grimaldeston MA, et al. Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. *Nat. Immunol.* 2007; 8:1095–1104. [PubMed: 17767162]
12. Zhou JS, et al. Mast cell deficiency in *Kit^{W-sh}* mice does not impair antibody-mediated arthritis. *J. Exp. Med.* 2007; 204:2797–2802. [PubMed: 17998392]
13. Hayashi S, et al. Exon skipping by mutation of an authentic splice site of *c-kit* gene in *W/W* mouse. *Nucleic Acids Res.* 1991; 19:1267–1271. [PubMed: 1709486]
14. Nocka K, et al. Molecular bases of dominant negative and loss of function mutations at the murine *c-kit*/white spotting locus: *W37*, *W^v*, *W41* and *W*. *EMBO J.* 1990; 9:1805–1813. [PubMed: 1693331]
15. Nagle DL, et al. Physical mapping of the *Tec* and *Gabrb1* loci reveals that the *W^{sh}* mutation on mouse chromosome 5 is associated with an inversion. *Hum. Mol. Genet.* 1995; 4:2073–2079. [PubMed: 8589683]
16. Nigrovic PA, et al. Genetic inversion in mast cell-deficient (*W^{sh}*) mice interrupts *corin* and manifests as hematopoietic and cardiac aberrancy. *Am. J. Pathol.* 2008; 173:1693–1701. [PubMed: 18988802]
17. Tsai M, et al. Using mast cell knock-in mice to analyze the roles of mast cells in allergic responses *in vivo*. *Chem. Immunol. Allergy.* 2005; 87:179–197. [PubMed: 16107772]
18. Chervenick PA, Boggs DR. Decreased neutrophils and megakaryocytes in anemic mice of genotype *W/W^v*. *J. Cell. Physiol.* 1969; 73:25–30. [PubMed: 5765776]
19. Feyerabend TB, et al. Cre-mediated cell ablation contests mast cell contribution in models of antibody- and T cell-mediated autoimmunity. *Immunity.* 2011; 35:832–844. [PubMed: 22101159]
20. Lantz CS, et al. Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites. *Nature.* 1998; 392:90–93. [PubMed: 9510253]
21. Nakano T, et al. Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal, and intravenous transfer into genetically mast cell-deficient *W/W^v* mice. Evidence that cultured mast cells can give rise to both connective tissue type and mucosal mast cells. *J. Exp. Med.* 1985; 162:1025–1043. [PubMed: 3897446]
22. Martin TR, et al. Mast cell activation enhances airway responsiveness to methacholine in the mouse. *J. Clin. Invest.* 1993; 91:1176–1182. [PubMed: 8450046]

23. Tanzola MB, et al. Mast cells exert effects outside the central nervous system to influence experimental allergic encephalomyelitis disease course. *J. Immunol.* 2003; 171:4385–4391. [PubMed: 14530364]
24. Wolters PJ, et al. Tissue-selective mast cell reconstitution and differential lung gene expression in mast cell-deficient *Kit^{W-sh/Kit^{W-sh}}* sash mice. *Clin. Exp. Allergy.* 2005; 35:82–88. [PubMed: 15649271]
25. Otsu K, et al. Phenotypic changes of bone marrow-derived mast cells after intraperitoneal transfer into *W/W^v* mice that are genetically deficient in mast cells. *J. Exp. Med.* 1987; 165:615–627. [PubMed: 3102674]
26. Scholten J, et al. Mast cell-specific Cre/loxP-mediated recombination in vivo. *Transgenic Res.* 2008; 17:307–315. [PubMed: 17972156]
27. Feyerabend TB, et al. Deletion of Notch1 converts pro-T cells to dendritic cells and promotes thymic B cells by cell-extrinsic and cell-intrinsic mechanisms. *Immunity.* 2009; 30:67–79. [PubMed: 19110448]
28. Lilla JN, et al. Reduced mast cell and basophil numbers and function in *Cpa3-Cre; Mcl-1^{fl/fl}* mice. *Blood.* 2011; 118:6930–6938. [PubMed: 22001390]
29. Musch W, et al. Generation and characterization of alpha-chymase-Cre transgenic mice. *Genesis.* 2008; 46:163–166. [PubMed: 18327770]
30. Dudeck A, et al. Mast cells are key promoters of contact allergy that mediate the adjuvant effects of haptens. *Immunity.* 2011; 34:973–984. [PubMed: 21703544]
31. Voehringer D, et al. Homeostasis and effector function of lymphopenia-induced "memory-like" T cells in constitutively T cell-depleted mice. *J. Immunol.* 2008; 180:4742–4753. [PubMed: 18354198]
32. Gordon JR, Galli SJ. Phorbol 12-myristate 13-acetate-induced development of functionally active mast cells in *W/W^v* but not *Sl/Sl^d* genetically mast cell-deficient mice. *Blood.* 1990; 75:1637–1645. [PubMed: 2328315]
33. Ando A, et al. Effects of chronic treatment with the c-kit ligand, stem cell factor, on immunoglobulin E-dependent anaphylaxis in mice. Genetically mast cell-deficient *Sl/Sl^d* mice acquire anaphylactic responsiveness, but the congenic normal mice do not exhibit augmented responses. *J. Clin. Invest.* 1993; 92:1639–1649. [PubMed: 7691882]
34. Schmidt-Supprian M, Rajewsky K. Vagaries of conditional gene targeting. *Nat. Immunol.* 2007; 8:665–668. [PubMed: 17579640]
35. Serafin WE, et al. Carboxypeptidase A in mouse mast cells. Identification, characterization, and use as a differentiation marker. *J. Immunol.* 1987; 139:3771–3776. [PubMed: 3680950]
36. Voehringer D, et al. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity.* 2004; 20:267–277. [PubMed: 15030771]
37. Taghon T, et al. Mast cell lineage diversion of T lineage precursors by the essential T cell transcription factor GATA-3. *Nat. Immunol.* 2007; 8:845–855. [PubMed: 17603486]
38. Franco CB, et al. Distinguishing mast cell and granulocyte differentiation at the single-cell level. *Cell Stem Cell.* 2010; 6:361–368. [PubMed: 20362540]
39. Steimer DA, et al. Selective roles for antiapoptotic MCL-1 during granulocyte development and macrophage effector function. *Blood.* 2009; 113:2805–2815. [PubMed: 19064728]
40. Zhou P, et al. Mcl-1 in transgenic mice promotes survival in a spectrum of hematopoietic cell types and immortalization in the myeloid lineage. *Blood.* 1998; 92:3226–3239. [PubMed: 9787159]
41. Wershil BK, et al. ¹²⁵I-fibrin deposition in IgE-dependent immediate hypersensitivity reactions in mouse skin. Demonstration of the role of mast cells using genetically mast cell-deficient mice locally reconstituted with cultured mast cells. *J. Immunol.* 1987; 139:2605–2614. [PubMed: 3655368]
42. Takeishi T, et al. Differences in the expression of the cardiopulmonary alterations associated with anti-immunoglobulin E-induced or active anaphylaxis in mast cell-deficient and normal mice. Mast cells are not required for the cardiopulmonary changes associated with certain fatal anaphylactic responses. *J. Clin. Invest.* 1991; 88:598–608. [PubMed: 1864969]
43. Miyajima I, et al. Systemic anaphylaxis in the mouse can be mediated largely through IgG₁ and FcγRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death

- associated with active or IgE- or IgG₁-dependent passive anaphylaxis. *J. Clin. Invest.* 1997; 99:901–914. [PubMed: 9062348]
44. Mukai K, et al. Basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of T cells and mast cells. *Immunity.* 2005; 23:191–202. [PubMed: 16111637]
 45. Newlands GF, et al. Stem cell factor contributes to intestinal mucosal mast cell hyperplasia in rats infected with *Nippostrongylus brasiliensis* or *Trichinella spiralis* but anti-stem cell factor treatment decreases parasite egg production during *N brasiliensis* infection. *Blood.* 1995; 86:1968–1976. [PubMed: 7544650]
 46. Brandt EB, et al. Mast cells are required for experimental oral allergen-induced diarrhea. *J. Clin. Invest.* 2003; 112:1666–1677. [PubMed: 14660743]
 47. Gekara NO, Weiss S. Mast cells initiate early anti-Listeria host defences. *Cell. Microbiol.* 2008; 10:225–236. [PubMed: 17714516]
 48. Czechowicz A, et al. Efficient transplantation via antibody-based clearance of hematopoietic stem cell niches. *Science.* 2007; 318:1296–1299. [PubMed: 18033883]
 49. Buch T, et al. A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. *Nat. Methods.* 2005; 2:419–426. [PubMed: 15908920]
 50. Sawaguchi M, et al. Role of mast cells and basophils in IgE responses and in allergic airway hyperresponsiveness. *J. Immunol.* 2012; 188:1809–1818. [PubMed: 22250079]
 51. Otsuka A, et al. Requirement of interaction between mast cells and skin dendritic cells to establish contact hypersensitivity. *PLoS One.* 2011; 6:e25538. [PubMed: 21980488]
 52. Yagi R, et al. Regulation of the *Il4* gene is independently controlled by proximal and distal 3' enhancers in mast cells and basophils. *Mol. Cell. Biol.* 2007; 27:8087–8097. [PubMed: 17908791]
 53. Furumoto Y, et al. PTEN deficiency in mast cells causes a mastocytosis-like proliferative disease that heightens allergic responses and vascular permeability. *Blood.* 2011; 118:5466–5475. [PubMed: 21926349]
 54. Gerbaulet A, et al. Mast cell hyperplasia, B-cell malignancy, and intestinal inflammation in mice with conditional expression of a constitutively active kit. *Blood.* 2011; 117:2012–2021. [PubMed: 21148330]
 55. Sharma N, et al. SH2 Domain-Containing Phosphatase-2 is a Critical Regulator of Connective Tissue Mast Cell Survival and Homeostasis in Mice. *Mol. Cell. Biol.* 2012; 32:2653–2663. [PubMed: 22566685]
 56. Muzumdar MD, et al. A global double-fluorescent Cre reporter mouse. *Genesis.* 2007; 45:593–605. [PubMed: 17868096]
 57. Haff LA. Improved quantitative PCR using nested primers. *PCR Methods Appl.* 1994; 3:332–337. [PubMed: 7920237]
 58. Galli SJ, Hammel I. Unequivocal delayed hypersensitivity in mast cell-deficient and beige mice. *Science.* 1984; 226:710–713. [PubMed: 6494907]
 59. Bryce PJ, et al. Immune sensitization in the skin is enhanced by antigen-independent effects of IgE. *Immunity.* 2004; 20:381–392. [PubMed: 15084268]
 60. Brown MA, Hatfield JK. Mast cells are important modifiers of autoimmune disease: With so much evidence, why is there still controversy? *Front. Immunol.* 2012; 3:147. [PubMed: 22701454]
 61. Rodewald HR, Feyerabend TB. Widespread immunological functions of mast cells: fact or fiction? *Immunity.* 2012; 37:13–24. [PubMed: 22840840]
 62. Akahoshi M, et al. Mast cell chymase reduces the toxicity of Gila monster venom, scorpion venom, and vasoactive intestinal polypeptide in mice. *J. Clin. Invest.* 2011; 121:4180–4191. [PubMed: 21926462]
 63. Wiener Z, et al. Highly reduced peritoneal mast cell number and decreased *c-kit* expression in histidine decarboxylase knock out mice. *Inflamm. Res.* 2001; 50(Suppl 2):S55–S56. [PubMed: 11411602]
 64. Ohtsu H, et al. Mice lacking histidine decarboxylase exhibit abnormal mast cells. *FEBS Lett.* 2001; 502:53–56. [PubMed: 11478947]

65. Wastling JM, et al. Histochemical and ultrastructural modification of mucosal mast cell granules in parasitized mice lacking the beta-chymase, mouse mast cell protease-1. *Am. J. Pathol.* 1998; 153:491–504. [PubMed: 9708809]
66. Younan G, et al. The inflammatory response after an epidermal burn depends on the activities of mouse mast cell proteases 4 and 5. *J. Immunol.* 2010; 185:7681–7690. [PubMed: 21076070]
67. Tchougounova E, et al. The chymase, mouse mast cell protease 4, constitutes the major chymotrypsin-like activity in peritoneum and ear tissue. A role for mouse mast cell protease 4 in thrombin regulation and fibronectin turnover. *J. Exp. Med.* 2003; 198:423–431. [PubMed: 12900518]
68. Stevens RL, et al. Transgenic mice that possess a disrupted mast cell protease 5 (mMCP-5) gene can not store carboxypeptidase A (mMC-CPA) protein in their granules. *FASEB J.* 1996; 10:17772.
69. Abonia JP, et al. Mast cell protease 5 mediates ischemia-reperfusion injury of mouse skeletal muscle. *J. Immunol.* 2005; 174:7285–7291. [PubMed: 15905575]
70. Thakurda SM, et al. The mast cell-restricted tryptase mMCP-6 has a critical immunoprotective role in bacterial infections. *J. Biol. Chem.* 2007; 282:20809–20815. [PubMed: 17456473]
71. Shin K, et al. Mouse mast cell tryptase mMCP-6 is a critical link between adaptive and innate immunity in the chronic phase of trichinella spiralis infection. *J. Immunol.* 2008; 180:4885–4891. [PubMed: 18354212]
72. Hunt JE, et al. Natural disruption of the mouse mast cell protease 7 gene in the C57BL/6 mouse. *J. Biol. Chem.* 1996; 271:2851–2855. [PubMed: 8576265]
73. Feyerabend TB, et al. Loss of histochemical identity in mast cells lacking carboxypeptidase A. *Mol. Cell Biol.* 2005; 25:6199–6210. [PubMed: 15988029]
74. Schneider LA, et al. Molecular mechanism of mast cell mediated innate defense against endothelin and snake venom sarafotoxin. *J. Exp. Med.* 2007; 204:2629–2639. [PubMed: 17923505]
75. Humphries DE, et al. Heparin is essential for the storage of specific granule proteases in mast cells. *Nature.* 1999; 400:769–772. [PubMed: 10466726]
76. Forsberg E, et al. Abnormal mast cells in mice deficient in a heparin-synthesizing enzyme. *Nature.* 1999; 400:773–776. [PubMed: 10466727]
77. Metz M, et al. Mast cells can enhance resistance to snake and honeybee venoms. *Science.* 2006; 313:526–530. [PubMed: 16873664]
78. Kung TT, et al. Mast cells modulate allergic pulmonary eosinophilia in mice. *Am. J. Respir. Cell Mol. Biol.* 1995; 12:404–409. [PubMed: 7695919]
79. Takeda K, et al. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. *J. Exp. Med.* 1997; 186:449–454. [PubMed: 9236197]
80. Kobayashi T, et al. An essential role of mast cells in the development of airway hyperresponsiveness in a murine asthma model. *J. Immunol.* 2000; 164:3855–3861. [PubMed: 10725747]
81. Williams CM, Galli SJ. Mast cells can amplify airway reactivity and features of chronic inflammation in an asthma model in mice. *J. Exp. Med.* 2000; 192:455–462. [PubMed: 10934234]
82. Yu M, et al. Mast cells can promote the development of multiple features of chronic asthma in mice. *J. Clin. Invest.* 2006; 116:1633–1641. [PubMed: 16710480]
83. Nakae S, et al. Mast cell-derived TNF contributes to airway hyperreactivity, inflammation, and TH2 cytokine production in an asthma model in mice. *J. Allergy Clin. Immunol.* 2007; 120:48–55. [PubMed: 17482668]
84. Becker M, et al. Genetic variation determines mast cell functions in experimental asthma. *J. Immunol.* 2011; 186:7225–7231. [PubMed: 21572035]
85. Lee DM, et al. Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science.* 2002; 297:1689–7692. [PubMed: 12215644]
86. Elliott ER, et al. Deletion of Syk in neutrophils prevents immune complex arthritis. *J. Immunol.* 2011; 187:4319–4330. [PubMed: 21918195]

87. Mancardi DA, et al. Cutting Edge: The murine high-affinity IgG receptor FcγRIV is sufficient for autoantibody-induced arthritis. *J. Immunol.* 2011; 186:1899–1903. [PubMed: 21248252]
88. Secor VH, et al. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. *J. Exp. Med.* 2000; 191:813–822. [PubMed: 10704463]
89. Bennett JL, et al. Bone marrow-derived mast cells accumulate in the central nervous system during inflammation but are dispensable for experimental autoimmune encephalomyelitis pathogenesis. *J. Immunol.* 2009; 182:5507–5514. [PubMed: 19380799]
90. Li H, et al. Kit (*W^{sh}*) mice develop earlier and more severe experimental autoimmune encephalomyelitis due to absence of immune suppression. *J. Immunol.* 2011; 187:274–282. [PubMed: 21646293]
91. Piconese S, et al. Exacerbated experimental autoimmune encephalomyelitis in mast-cell-deficient *Ki^{W-sh}/W^{sh}* mice. *Lab. Invest.* 2011; 91:627–641. [PubMed: 21321538]
92. Askenase PW, et al. Defective elicitation of delayed-type hypersensitivity in *W/W^v* and *Sl/Sl^d* mast cell-deficient mice. *J. Immunol.* 1983; 131:2687–2694. [PubMed: 6605986]
93. Biedermann T, et al. Mast cells control neutrophil recruitment during T cell-mediated delayed-type hypersensitivity reactions through tumor necrosis factor and macrophage inflammatory protein 2. *J. Exp. Med.* 2000; 192:1441–1452. [PubMed: 11085746]
94. Norman MU, et al. Mast cells regulate the magnitude and the cytokine microenvironment of the contact hypersensitivity response. *Am. J. Pathol.* 2008; 172:1638–1649. [PubMed: 18467702]
95. Abt MC, Artis D. The intestinal microbiota in health and disease: the influence of microbial products on immune cell homeostasis. *Curr. Opin. Gastroenterol.* 2009; 25:496–502. [PubMed: 19770652]
96. Abt MC, et al. Commensal bacteria calibrate the activation threshold of innate antiviral immunity. *Immunity.* 2012; 37:158–170. [PubMed: 22705104]
97. Hill DA, et al. Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat. Med.* 2012; 18:538–546. [PubMed: 22447074]
98. Gilfillan AM, Beaven MA. Regulation of mast cell responses in health and disease. *Crit. Rev. Immunol.* 2011; 31:475–529. [PubMed: 22321108]
99. Ribatti D, Crivellato E. Mast cells, angiogenesis and cancer. *Adv. Exp. Med. Biol.* 2011; 716:270–288. [PubMed: 21713661]
100. Kennelly R, et al. Mast cells in tissue healing: from skin to the gastrointestinal tract. *Curr. Pharm. Des.* 2011; 17:3772–3775. [PubMed: 22103847]
101. Galli SJ, et al. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nat. Rev. Immunol.* 2008; 8:478–486. [PubMed: 18483499]
102. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat. Med.* 2012; 18:693–704. [PubMed: 22561833]
103. Abraham SN, St John AL. Mast cell-orchestrated immunity to pathogens. *Nat. Rev. Immunol.* 2010; 10:440–452. [PubMed: 20498670]
104. Supajatura V, et al. Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J. Clin. Invest.* 2002; 109:1351–1359. [PubMed: 12021251]
105. Enoksson M, et al. Mast cells as sensors of cell injury through IL-33 recognition. *J. Immunol.* 2011; 186:2523–2528. [PubMed: 21239713]
106. Lunderius-Andersson C, et al. Mast cells respond to cell injury through the recognition of IL-33. *Front. Immunol.* 2012; 3:82. [PubMed: 22566963]
107. Schafer B, et al. Mast cell anaphylatoxin receptor expression can enhance IgE-dependent skin inflammation in mice. *J. Allergy Clin. Immunol.* 2012 Jun 22. [Epub ahead of print].
108. Fantozzi R, et al. Mast cell and neutrophil interactions: a role for superoxide anion and histamine. *Agents Actions.* 1985; 16:260–264. [PubMed: 2409774]
109. Gri G, et al. CD4+CD25+ regulatory T cells suppress mast cell degranulation and allergic responses through OX40-OX40L interaction. *Immunity.* 2008; 29:771–781. [PubMed: 18993084]
110. Gaudenzio N, et al. Cell-cell cooperation at the T helper cell/mast cell immunological synapse. *Blood.* 2009; 114:4979–4988. [PubMed: 19805617]

111. Hill PB, et al. Stem cell factor enhances immunoglobulin E-dependent mediator release from cultured rat bone marrow-derived mast cells: activation of previously unresponsive cells demonstrated by a novel ELISPOT assay. *Immunology*. 1996; 87:326–333. [PubMed: 8698398]
112. Ito T, et al. Stem cell factor programs the mast cell activation phenotype. *J. Immunol*. 2012; 188:5428–5437. [PubMed: 22529299]
113. Galli SJ, et al. The kit ligand, stem cell factor. *Adv. Immunol*. 1994; 55:1–96. [PubMed: 7508174]
114. Komai-Koma M, et al. Interleukin-33 amplifies IgE synthesis and triggers mast cell degranulation via interleukin-4 in naive mice. *Allergy*. 2012; 67:1118–1126. [PubMed: 22702477]
115. Okayama Y, et al. Expression of a functional high-affinity IgG receptor, FcγRI, on human mast cells: Up-regulation by IFN-γ. *J. Immunol*. 2000; 164:4332–4339. [PubMed: 10754333]
116. Baccari GC, et al. Mast cells in nonmammalian vertebrates: an overview. *Int. Rev. Cell. Mol. Biol*. 2011; 290:1–53. [PubMed: 21875561]
117. Galli SJ, et al. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. *Nat. Immunol*. 2011; 12:1035–1044. [PubMed: 22012443]
118. Bowie MB, et al. Steel factor responsiveness regulates the high self-renewal phenotype of fetal hematopoietic stem cells. *Blood*. 2007; 109:5043–5048. [PubMed: 17327414]
119. Ikuta K, Weissman IL. Evidence that hematopoietic stem cells express mouse *c-kit* but do not depend on steel factor for their generation. *Proc. Natl. Acad. Sci. USA*. 1992; 89:1502–1506. [PubMed: 1371359]
120. Ogawa M, et al. Expression and function of *c-kit* in hemopoietic progenitor cells. *J. Exp. Med*. 1991; 174:63–71. [PubMed: 1711568]
121. Galli SJ, et al. Regulation of mouse and human mast cell development, survival and function by stem cell factor, the ligand for the c-kit receptor. *Int. Arch. Allergy Immunol*. 1995; 107:51–53. [PubMed: 7542101]
122. Okayama Y, Kawakami T. Development, migration, and survival of mast cells. *Immunol. Res*. 2006; 34:97–115. [PubMed: 16760571]
123. Tsai M, et al. Induction of mast cell proliferation, maturation, and heparin synthesis by the rat c-kit ligand, stem cell factor. *Proc. Natl. Acad. Sci. USA*. 1991; 88:6382–6386. [PubMed: 1712491]
124. Tsai M, et al. The rat c-kit ligand, stem cell factor, induces the development of connective tissue-type and mucosal mast cells in vivo. Analysis by anatomical distribution, histochemistry, and protease phenotype. *J. Exp. Med*. 1991; 174:125–131. [PubMed: 1711559]
125. Oliveira SH, et al. Stem cell factor induces eosinophil activation and degranulation: mediator release and gene array analysis. *Blood*. 2002; 100:4291–4297. [PubMed: 12453875]
126. Krishnamoorthy N, et al. Activation of c-Kit in dendritic cells regulates T helper cell differentiation and allergic asthma. *Nat. Med*. 2008; 14:565–573. [PubMed: 18454155]
127. Heinemann A, et al. Stem cell factor stimulates the chemotaxis, integrin upregulation, and survival of human basophils. *J. Allergy Clin. Immunol*. 2005; 116:820–826. [PubMed: 16210056]
128. Manova K, et al. Gonadal expression of *c-kit* encoded at the *W* locus of the mouse. *Development*. 1990; 110:1057–1069. [PubMed: 1712701]
129. Manova K, Bachvarova RF. Expression of *c-kit* encoded at the *W* locus of mice in developing embryonic germ cells and presumptive melanoblasts. *Dev. Biol*. 1991; 146:312–324. [PubMed: 1713863]
130. Huizinga JD, et al. *W/kil* gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature*. 1995; 373:347–349. [PubMed: 7530333]
131. Milenkovic N, et al. Nociceptive tuning by stem cell factor/c-Kit signaling. *Neuron*. 2007; 56:893–906. [PubMed: 18054864]
132. Takagi K, et al. Involvement of stem cell factor and its receptor tyrosine kinase c-kit in pain regulation. *Neuroscience*. 2008; 153:1278–1288. [PubMed: 18423881]
133. Peters EM, et al. Kit is expressed by epithelial cells in vivo. *J. Invest. Derm*. 2003; 121:976–984. [PubMed: 14708595]

134. Stokman G, et al. Stem cell factor expression after renal ischemia promotes tubular epithelial survival. *PLoS One*. 2010; 5:e14386. [PubMed: 21200435]
135. Cozzi E, et al. The naive airway hyperresponsiveness of the A/J mouse is Kit-mediated. *Proc. Natl. Acad. Sci. USA*. 2011; 108:12787–12792. [PubMed: 21768379]
136. Finkelman FD. Anaphylaxis: lessons from mouse models. *J. Allergy Clin. Immunol.* 2007; 120:506–515. quiz 516–517. [PubMed: 17765751]
137. Nigrovic PA, Lee DM. Synovial mast cells: role in acute and chronic arthritis. *Immunol. Rev.* 2007; 217:19–37. [PubMed: 17498049]
138. Mekori YA, Galli SJ. Undiminished immunologic tolerance to contact sensitivity in mast cell-deficient *W/W^v* and *Sl/Sl^d* mice. *J. Immunol.* 1985; 135:879–885. [PubMed: 3159796]
139. Mekori YA, et al. Studies of the role of mast cells in contact sensitivity responses. Passive transfer of the reaction into mast cell-deficient mice locally reconstituted with cultured mast cells: effect of reserpine on transfer of the reaction with DNP-specific cloned T cells. *Cell Immunol.* 1987; 109:39–52. [PubMed: 3498543]
140. Yu M, et al. Identification of an IFN- γ /mast cell axis in a mouse model of chronic asthma. *J. Clin. Invest.* 2011; 121:3133–3143. [PubMed: 21737883]

Box 1: Some general observations about mast cell (MC) biology

In vivo and *in vitro* studies have shown that:

- MCs are distributed throughout nearly all tissues, and often in close proximity to potential targets of their mediators such as epithelia and glands, smooth muscle and cardiac muscle cells, fibroblasts, and blood and lymphatic vessels and nerves [5].
- MCs can store and release upon degranulation and/or secrete *de novo* a wide spectrum of biologically active mediators (many of which also can be produced by other cell types) that individually have been shown to have potential positive or negative effects on the function of various target leukocytes or structural cells, and that thereby have the potential to influence inflammation, hemostasis, tissue remodeling, cancer, metabolism, reproduction, behavior, sleep, and many other biological responses [98–101].
- MCs can be activated to secrete biologically active products not only by IgE and specific antigen (the main mechanism which accounts for their function in allergic disorders [102]) but by a long list of other stimuli including physical agents, products of diverse pathogens [103], many innate danger signals [104], certain endogenous peptides and structurally similar peptides found in invertebrate and vertebrate venoms [62, 74, 77], and products of innate and adaptive immune responses including immune complexes of IgG, certain chemokines and cytokines (including IL-33 [105, 106]), and products of complement activation [107].
- The ability of MCs to secrete biologically active mediators can be enhanced or suppressed by many factors, including interactions with other granulocytes [108], regulatory T cells [109] or other lymphocytes [110], and certain cytokines, including the main MC development and survival growth factor, the Kit ligand stem cell factor [5, 111–113], IL-33 [114], and interferon- γ [115].
- MCs in different anatomical locations and in different species can vary in multiple aspects of phenotype, including their responsiveness to signals regulating their proliferation and function, their content of stored mediators, and their potential to produce various newly synthesized mediators [116, 117].
- The numbers, anatomical distribution, phenotype, and function of MCs can be modulated, or “tuned”, by a wide variety of genetic or environmental factors, so that the properties of MCs may be different depending on the genetic background of the host and/or the local or systemic levels of factors with effects on MC biology (including those generated during ongoing innate or adaptive immune responses or diseases) [5].

Box 2. A hierarchy of mast cell (MC) roles in biological responses

We propose that the following working definitions of MC functions might be useful in conceptualizing how MCs can influence particular biological responses*:

1. **“unique” or “non-redundant”**: i.e., only MCs can perform that function;
2. **“important”**: that aspect of the response would be substantially different (e.g., by 50% or more) in the absence of MCs;
3. **“redundant” or “overlapping”**: the MC can contribute to the assessed feature of the response along with other effector or regulatory elements, but the potential contribution(s) of the MC may only be revealed if one or more of the other partially redundant or overlapping elements is impaired. One can think here of a round table supported by 10 legs that are equally spaced under its periphery. By selecting the correct ones to remove, as many as 7 of the legs could be eliminated without causing the table to fall; but remove one more leg and the table will collapse. Note that, in this simple example, by planning properly any 3 of the 10 legs could be selected to be the last ones keeping the table up – i.e., all 10 of them are equally redundant. However, in actual biological responses, some “legs” may be more important than others.
4. **“non-contributory”**: the MC has no role in that feature of the response.

* In particular biological responses, such as models of host defense or disease, MCs may influence some features of the response more importantly than others. Indeed, in some biological responses, MCs might have any combination of the 4 roles listed, depending on which features of the response one measures. The number of biological responses in which essentially all features of the response are fully and uniquely dependent on MCs may be small.

Box 3: KIT expression within and outside the immune system

Within the immune system

- KIT is highly expressed in hematopoietic stem cells (HSCs) in the bone marrow and activation of KIT by its ligand stem cell factor (SCF) contributes to regulation of the self-renewal, survival and differentiation of HSCs [48, 118–120].
- While most immune cells lose detectable KIT expression upon cell differentiation, MCs remain KIT⁺ throughout their life and SCF can promote MC maturation, survival and proliferation, as well as influence MC migration and functional responses [113, 121–124].
- KIT expression has also been described in purified eosinophils from mice infected with *Schistosoma mansoni* [125] and in mouse dendritic cells after stimulation with cholera toxin or house dust mite extract [126], and has been reported at low levels in some human basophils [127].

Outside the immune system

- KIT is expressed in melanocytes and germ cells [128, 129]. Both *Kit*^{W/W^{-v} and *Kit*^{W^{-sh}/W^{-sh} mice are deficient in melanocytes but only *Kit*^{W/W^{-v} mice are sterile [6, 17].}}}
- KIT is also expressed in interstitial cells of Cajal (ICC) in the gastrointestinal tract and *kit* mutant mice have marked reductions in ICC and have abnormal electrical pacemaker activity in the small intestine [130].
- KIT expression has been described in many other cell types in mice, such as subpopulations of sensory neurons [131], certain nerves in the CNS [132], keratinocytes [133], and tubular epithelial cells in the kidney [134]. *Kit*^{W/W^v mice backcrossed on the A/JxB6 F₁ background display reduced naïve airway hyperresponsiveness to metacholine as compared to *Kit*^{+/+} littermate controls in a MC-independent manner, suggesting the existence of a yet unidentified nonhematopoietic KIT⁺ cell type [135].}

Box 4. Concordant and discordant findings regarding mast cell (MC) functions *in vivo*

1. Findings in multiple model systems (including *kit* mutant MC-deficient mice and MC knock-in mice, and in the newer MC-deficient mice with normal KIT) support the conclusion that MCs have **important** and perhaps in some cases even **non-redundant** roles in many **acute, IgE-dependent responses** [12, 19, 28, 41–43, 50, 136] but are not required for development of a more chronic IgE-dependent cutaneous response that is dependent on basophils [28, 44].
2. Studies in both various *kit* mutant MC-deficient mice and in MC-associated protease-deficient mice are consistent in supporting an **important** role for mast cells [62, 77] and their proteases (namely, CPA3 [74] and MCPT4 [62]) in **reducing the pathology and mortality induced by the venoms of certain reptiles and arthropods**.
3. Different models of MC deficiency have yielded different results when the possible contributions of MCs have been examined in certain complex biological responses. In each of these responses, it is likely that multiple types of immune cells may have **partially redundant or overlapping** roles. Such responses include **antibody-dependent arthritis** [12, 85–87, 137], **EAE** [19, 60, 88–91], and **cutaneous contact hypersensitivity** [11, 30, 51, 58, 59, 92–94, 138, 139]. In models of **airway inflammation and airway hypersensitivity**, it has been reported that both the **details of the model system** [50, 78–83, 140], and **mouse strain background** [84], can influence the extent to which MCs are important for the development of various features of the responses; this is also likely to be true regarding the ability to discern the importance of the MC's contributions to many other biological responses.

Table 1

Characteristics of “KIT-independent” mast cell (MC)-deficient mice

Deficiency	Mice	Construct	MC numbers	IgE-dependent MC function	Basophil numbers	Basophil function	Known or potential limitations for MC studies	Refs
CONSTITUTIVE	<i>Mcp15-Cre; R-DTA</i> Tg(Cmal-cre) ARoer; B6.129P2- <i>Gt(ROSA)26</i> <i>Sortm1(DTA)JkyJ</i>	Cross between R-DTA floxed mice and <i>transgenic</i> mice expressing the Cre under the <i>Mcp15</i> promoter	Steady-state: marked reductions in peritoneal (98%) and skin (89–96.5%) MCs, mucosal MCs (MMCs) unlikely to be depleted	Not assessed	Not assessed (basophils thought not to express <i>Mcp15</i>)	Not assessed	<ul style="list-style-type: none"> Probable presence of MMCs Are there cell-intrinsic defects of NK cells or other cell types? 	[30]
	“Cre-Master” <i>Cpa3^{Cre/+}</i> Cpa3tm3(cre)Hrr	<i>Gene targeting:</i> Cre expression under the control of the <i>Cpa3</i> promoter while deleting 28 nucleotides of the first exon of <i>Cpa3</i> locus	Steady-state: absence of connective-tissue and mucosal MCs (in skin, peritoneum, intestine) Inflammatory conditions: remain deficient in skin MCs after PMA-induced dermatitis and in intestinal MMCs following helminth infection	Don't develop IgE-dependent models of PSA or PCA; PSA response restored by systemic engraftment of WT BMCMCs	60 % reduction in spleen basophil numbers (blood and bone-marrow basophil status not reported)	Not assessed	<ul style="list-style-type: none"> <i>Cpa3</i> expressed in other cell types Reduced basophil numbers Are there functional abnormalities of basophils or cell-intrinsic defects in other cell types? 	[19]
	“Hello Kitty” <i>Cpa3-Cre; Mcl-1^{fl/fl}</i> Tg(Cpa3-cre)3Glii; B6.129- <i>Mcl^{flm3skJ}</i>	Cross between <i>Mcl-1</i> floxed mice and <i>transgenic</i> mice expressing Cre under a <i>Cpa3</i> promoter fragment	Steady-state: marked reductions (92–100%) in connective-tissue and mucosal MCs in the skin, trachea, lung, peritoneum, digestive tract, etc., but no reduction in splenic MCs	Markedly reduced features of IgE-dependent models of PSA and PCA; PCA response restored by intradermal engraftment of WT BMCMCs	Reductions in basophil numbers in spleen (58%), blood (74%), and bone-marrow (75%)	Markedly reduced IgE-dependent chronic allergic inflammation of skin	<ul style="list-style-type: none"> <i>Cpa3</i> expressed in other cell types Reduced basophil numbers and function Increased spleen neutrophils & mild anemia Are there cell-intrinsic defects in other cell types? 	[28]
INDUCIBLE	<i>Mcp15-Cre; iDTR</i> Tg(Cmal-cre) ARoer; C57BL/6- <i>Gt(ROSA)26</i> <i>Sortm1(HBEGF)AwaiJ</i>	Cross between inducible DTR floxed mice and	Steady-state: 1 week after 4 weekly i.p. and 2 s.c. DT	Not assessed	Bone-marrow basophils	Not assessed (basophils thought not	<ul style="list-style-type: none"> Presence of MMCs 	[30]

Deficiency	Mice	Construct	MC numbers	IgE-dependent MC function	Basophil numbers	Basophil function	Known or potential limitations for MC studies	Refs
		<i>transgenic</i> mice expressing Cre under the <i>Mcpt5</i> promoter	<i>treatments</i> deficient in peritoneal and skin MCs (97.5%); stomach and intestinal MMCs present. Repopulation: 10% of pre-treatment skin and peritoneal MC numbers 3 weeks after the last DT treatment		not affected 1 week after 4 weekly i.p. treatments with DT	to express <i>Mcpt5</i>	<ul style="list-style-type: none"> Are there “off target” or other side effects of repeated treatment with DT? 	
	“Mas-TRECK”	<i>Transgenic</i> mice expressing human <i>DTR</i> under an intronic enhancer of the <i>Il4</i> gene	Steady-state: 3 <i>days after 5 daily i.p. DT treatments:</i> deficient in peritoneal, skin, stomach and mesenteric window MCs Repopulation: Skin MCs undetectable 12 days after the last DT treatment	Markedly reduced features of IgE-dependent models of PSA and PCA 2 days after 5 daily i.p. treatments with DT	Transient >95% reduction in blood basophil numbers 5 days after start of DT treatment and recovery 12 days after the last DT treatment	Markedly reduced features of IgE- and basophil-dependent chronic allergic inflammation of skin (induced 2 days after 5 daily i.p. treatments with DT)	<ul style="list-style-type: none"> Transient reduction in basophil numbers and function Are there “off target” or other side effects of repeated treatment with DT? 	[50, 51]

Table 2

Genetic deletion of mast cell (MC)-associated products

Mutant mice	Gene name	Phenotype and/or limitations	Refs.
<i>HDC</i> ^{-/-}	Histidine decarboxylase	<ul style="list-style-type: none"> No histamine produced (mice should be maintained under histamine free diet, since histamine can also be acquired through ingestion) Decreased MC numbers Altered storage of various proteases in MC granules Histamine can also be produced by other cell types, including some other hematopoietic cells such as basophils or neutrophils 	[63, 64]
<i>Mcpt1</i> ^{-/-}	MC protease 1	<ul style="list-style-type: none"> Markedly reduces esterase activity in intestinal mucosal MCs Histochemical and ultrastructural changes in granules of mucosal MCs 	[65]
<i>Mcpt4</i> ^{-/-}	MC protease 4 (chymase)	<ul style="list-style-type: none"> Does not affect the number or morphology of MCs in multiple anatomical sites tested Increased tryptase activity in peritoneal MCs 	[66, 67]
<i>Mcpt5</i> ^{-/-}	MC protease 5	<ul style="list-style-type: none"> Markedly reduced storage of CPA3 and CPA activity in peritoneal MCs Increased tryptase activity in peritoneal MCs Decreased chymase activity in peritoneal MCs 	[66, 68, 69]
<i>Mcpt6</i> ^{-/-}	MC protease 6	<ul style="list-style-type: none"> Does not affect the number or morphology of MCs in multiple anatomical sites tested Does not affect histamine and MCPT4 levels in peritoneal MCs 	[70, 71]
<i>Mcpt7</i> ^{-/-} (= C57BL/6)	MC protease 7	<ul style="list-style-type: none"> C57BL/6 mice are unable to express MCPT7 because of a point mutation in the exon/intron 2 splice of the <i>Mcpt7</i> gene Does not affect the number or morphology of MCs in multiple anatomical sites tested 	[72]
<i>Cpa3</i> ^{-/-}	Carboxypeptidase A3	<ul style="list-style-type: none"> Markedly reduced storage of MCPT5 in MC granules 	[73]
<i>Cpa3</i> ^{Y356L,E378A}	Carboxypeptidase A3	<ul style="list-style-type: none"> Inactive CPA3 due to two point mutations in the catalytic domain Doesn't affect storage of MCPT5 in MC granules 	[62, 74]
<i>NDST-2</i> ^{-/-}	N-deacetylase/N-sulfotransferase-2	<ul style="list-style-type: none"> MCs are unable to synthesize heparin Decreased numbers of connective tissue-type MCs Reduced storage of other proteases (MCPT4, MCPT5, CPA3) in MC granules 	[75, 76]

Table 3
Examples of consistent or discrepant conclusions about mast cell (MC) functions based on *in vivo* studies in MC-deficient mice

Model/disease	Mutant mice used	Test response	Main findings/conclusions	Refs.	Possible reason(s) for any discrepancies in results*
IgE-dependent passive anaphylaxis	MC knock-in <i>kit</i> mutant mice	PCA and/or PSA	WT BMCMCs (i.d.) restored ability of MC-deficient <i>Kit^{W/W^{-/-}}</i> mice to express robust PCA response	[41]	<ul style="list-style-type: none"> Does not apply (to date, all results are concordant)
	Cre-Master		PCA & PSA could not be elicited in <i>kit</i> mutant mice; WT BMCMCs (systemic engraftment) restored ability of MC-deficient mice to express PSA responses	[19]	
	Hello <i>Kit^{ty}</i>		WT BMCMCs (i.d.) restored ability of MC-deficient mice to express robust PCA response	[28]	
	Mas-TRECK		PCA & PSA could not be elicited in MC-deficient mice	[50]	
Innate resistance to venoms and/or their toxic components	MC knock-in <i>kit</i> mutant mice	Toxicity of venoms of 3 snakes & honey bee and sarafotoxin 6b (S6b)	MCs enhanced resistance to each venom and to S6b in <i>kit</i> mutant mice, and potato carboxypeptidase inhibitor reduced resistance to snake venoms and S6b in WT mice	[77]	<ul style="list-style-type: none"> Does not apply (to date, all results are concordant)
	<i>Kit^{W/W^{-/-}}</i> , <i>Cpa3^{Y356L.E378A}</i>	Toxicity of S6b	CPA3 can cleave S6b and neutralize this venom component	[74]	
	MC knock-in <i>kit</i> mutant mice, <i>Mcpt4^{-/-}</i> mice	Toxicity of Gila monster & 2 scorpion venoms and helodermin	MCs and MC chymase (MCPT4) can reduce toxicity of each venom and of helodermin and VIP	[62]	
“Asthma” (allergic inflammation and hyper-responsiveness of the airways)	<i>Kit^{W/W^{-/-}}</i>	17 day-long model, sensitization with adjuvant (alum)	Possible contributory role for MCs (but no studies in MC knock-in mice)	[78]	<ul style="list-style-type: none"> Importance of MC roles in such mice may vary based on differences in doses of antigen used for sensitization and/or challenge and/or amount of adjuvant, etc. (i.e., “strength” of the protocol in inducing involvement and activation of immune cells in addition to MCs).
	<i>Kit^{W/W^{-/-}}</i>	30 day-long model, sensitization with adjuvant (alum)	Redundant or no roles for MCs (no studies in MC knock-in mice)	[79]	
	MC knock-in <i>Kit^{W/W^{-/-}}</i>	20 day-long model, sensitization with adjuvant (alum)	Contributory roles for MCs with low dose (but not with high dose) antigen challenge	[80]	

Model/disease	Mutant mice used	Test response	Main findings/conclusions	Refs.	Possible reason(s) for any discrepancies in results*
	<i>Ki^lW^{+/+}</i>	31-day long model, sensitization with adjuvant (alum)	Redundant or no role for MCs with high dose antigen challenge (no studies in MC knock-in mice)	[81]	<ul style="list-style-type: none"> Differences in the models (duration of model; sensitization with or without use of adjuvant (alum)) that may influence the “strength” of the protocol in inducing involvement and activation of immune cells in addition to MCs
	MC knock-in <i>Ki^lW^{+/+}</i>	44 day-long model, sensitization w/o <i>I</i> adjuvant	Contributory role for MCs in AHR ² and airway inflammation		
	MC knock-in <i>Ki^l</i> mutant mice	9 week-long model, sensitization w/o <i>I</i> adjuvant	Important roles for MCs in multiple features of the pathology	[82]	
		47 day-long model, sensitization w/o <i>I</i> adjuvant		[83]	
	<i>Ki^lW^{-sh}W^{-sh}</i> , C57BL/6 background	47-day long model, sensitization w/o <i>I</i> adjuvant	Possible contributory role for MCs (but no studies in MC knock-in mice)	[84]	<ul style="list-style-type: none"> Differences in genetic backgrounds (C57BL/6 vs. BALB/c) can influence importance of roles of MCs in this model in <i>Ki^lW^{-sh}W^{-sh}</i> mice.
	C.B6- <i>Ki^lW^{-sh}W^{-sh}</i> , BALB/c background		Full development of ‘asthmatic’ features in the MC-deficient mice		
	Mas-TRECK	28-day long model, sensitization w/o <i>I</i> adjuvant	Inducing MC depletion prior to challenge reduced AHR ² , but had no detectable effect on eosinophil infiltration	[50]	<ul style="list-style-type: none"> Differences in roles of MCs as revealed by MC engraftment into <i>Ki^l</i> mutant mice prior to sensitization (by other groups) vs. by MC-depletion in DT treated Mas-TRECK mice after sensitization (this study) Roles of adoptively transferred MCs in <i>Ki^l</i> mutant mice may differ from those of MCs in Mas-TRECK mice.
	MC knock-in <i>Ki^lW^{+/+}</i>	K/BxN [83] serum transfer model	Important role for MCs in enhancing	[85]	<ul style="list-style-type: none"> Important role for neutrophils in the model in WT mice may result in “redundant” contributions of MCs being masked in presence of neutrophilia (in <i>Ki^lW^{-sh}W^{-sh}</i> mice) or normal levels of neutrophils (in Cre-Master mice) but being revealed in presence of neutropenia (in <i>Ki^lW^{+/+}</i> mice)
	<i>Ki^lW^{+/+}</i>	Anti-collagen antibodies		[12]	
	<i>Ki^lW^{-sh}W^{-sh}</i>	K/BxN [83] serum transfer model	Full development of features of arthritis in MC-deficient mice	[86, 87]	
		Anti-collagen antibodies		[12]	
	Cre-Master	K/BxN [83] serum transfer model		[19]	
Antibody-dependent arthritis					

Model/disease	Mutant mice used	Test response	Main findings/conclusions	Refs.	Possible reason(s) for any discrepancies in results*
					<ul style="list-style-type: none"> Differences in genetic backgrounds of WBB6F₁-<i>Kit^{W/W-v}</i> mice vs. that (C57BL/6) in other types of MC-deficient mice MCs have redundant or no roles in this model.
Experimental allergic encephalomyelitis (EAE)	MC knock-in <i>Kit^{W/W-v}</i>	MOG ₃₅₋₅₅ ³ in CFA ⁴ containing <i>M. tuberculosis</i> (s.c.) and PTX ⁵ (i.v.)	Important role for MCs in exacerbating pathology	[88]	<ul style="list-style-type: none"> Different doses of peptide and/or adjuvant (in some experiments) or effects of other factors that might influence these responses* (as possible explanations for differences in results obtained by different groups using <i>Kit^{W/W-v}</i> mice or among different types of MC-deficient mice) Differences in genetic backgrounds of WBB6F₁-<i>Kit^{W/W-v}</i> mice vs. that (C57BL/6) in other types of MC-deficient mice Important roles for other immune cells in the models in WT mice (and perhaps enhancement of certain T cell functions in <i>Kit^{W-sh/W-sh}</i> mice [85–87]) may result in “redundant” contributions of MCs only being revealed (in some but not all [85] studies) in the context of the immune cell defects present in <i>Kit^{W/W-v}</i> mice. In EAE models, MCs engrafted into <i>Kit</i> mutant mice exhibit different roles than do native MCs in WT mice (i.e., MCs may have redundant or no roles in these models in WT mice).
	<i>Kit^{W/W-v}</i> and <i>Kit^{W-sh/W-sh}</i>		Full development of the disease in MC-deficient mice	[19, 89]	
	<i>Kit^{W-sh/W-sh}</i>		Exacerbation of the disease in MC-deficient mice	[90, 91]	
	Cre-Master		Full development of the disease in MC-deficient mice	[19]	
Cutaneous contact hypersensitivity (CHS)	<i>Kit^{W/W-v}</i>	Sheep red blood cells or picryl chloride-induced CHS	Reduced development of CHS responses in MC-deficient mice	[92]	<ul style="list-style-type: none"> Differences in details of CHS protocols (e.g., types and/or concentrations of haptens,

Model/disease	Mutant mice used	Test response	Main findings/conclusions	Refs.	Possible reason(s) for any discrepancies in results*
		Pieryl chloride- or Ox δ -induced CHS	Redundant roles or no roles for MCs in the CHS responses	[58]	<div>types of vehicles [in some experiments]) or effects of other factors that might influence these responses*</div> <div>Different times of assessment and types of features analyzed to quantify responses (in some experiments).</div> <div>Differences in details of CHS protocols or effects of other factors that might influence these responses*</div> <div>MCs engrafted into <i>kit</i> mutant mice, and IL-10 derived from such engrafted MCs, exhibit different roles in CHS in kit mutant mice than do native MCs in WT mice (i.e., MCs and MC-derived IL-10 may suppress the responses in <i>Kit^{W-W-sh}</i> mice (e.g., perhaps because certain T cell functions are abnormal in these mice [85–87] but have redundant or no roles in the responses analyzed in WT mice)</div>
		TNCB γ -induced CHS	MCs can enhance elicitation of the CHS response (included MC knock-in experiments)	[93]	
		Ox δ -induced CHS, sensitization with low-dose Ox δ	Reduced development of active or passive models of CHS responses in MC-deficient mice	[59]	
		Ox δ -induced CHS, sensitization with low-dose Ox δ	MCs can facilitate elicitation of the CHS response	[94]	
	Ox δ -induced CHS, sensitization with high-dose Ox δ	MCs can down regulate the CHS response			
	Mas-TRECK	DNFB δ -induced CHS	Reduced CHS response when MCs are absent or depleted by DT treatment	[51]	
	MC knock-in <i>kit</i> mutant mice	DNFB δ and unshiol-induced CHS	Increased CHS responses and skin pathology when MCs or MC-derived IL-10 are absent	[11]	
		DNFB δ -induced CHS	<i>kit</i> -mutant MC-deficient mice have increased CHS responses	[30]	
	<i>Mcpt5-Cre; R-DTA, Mcpt5-Cre; iDTR, Mcpt5-Cre; Il10^{fl/fl}</i>		Reduced swelling in first 2 days of CHS response when MCs are absent or depleted by DT treatment; no detectable role for MC-derived IL-10 in the CHS response	[30]	

¹ w/o; without

² AHR; airway hyperreactivity

³MOG; myelin oligodendrocyte glycoprotein

⁴CFA; complete Freund's adjuvant

⁵PTX; pertussis toxin

⁶Ox; oxazolone

⁷TNFB; trinitrochlorobenzene

⁸DNFB; 1-fluoro-2,4-dinitrobenzene

* In light of recent work indicating that immune responses in mice can be influenced by differences in the host's endogenous microflora [95–97], it is possible that this factor (or other differences affecting animals housed in different institutions) can influence the importance of the MC's role in innate or adaptive immune responses, and thereby contribute to the differences in results obtained when similar or identical protocols for inducing such responses are tested in different laboratories.