Review

New models for analyzing mast cell functions *in vivo*

Laurent L. Reber[1*], Thomas Marichal[1*], and Stephen J. Galli[1,2]

[1] Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305-5324, USA
[2] Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305-5324, USA

In addition to their well-accepted role as critical effector cells in anaphylaxis and other acute IgE-mediated allergic reactions, mast cells (MCs) have been implicated in a wide variety of processes that contribute to disease or help to maintain health. Although some of these roles were first suggested by analyses of MC products or functions *in vitro*, it is critical to determine whether, and under which circumstances, such potential roles actually can be performed by MCs *in vivo*. This review discusses recent advances in the development and analysis of mouse models to investigate the roles of MCs and MC-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 MC deficiency.

The spectrum of potential MC functions: an embarrassment of riches

Trying to figure out what 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.

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*, for example, 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*. Although progress has been made in devising genetic approaches that address that goal, particularly over the past 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 compare and contrast the advantages and potential disadvantages of both older and newer approaches to investigate the roles of MCs *in vivo*. We 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 using newer models in which the MC deficiency is not dependent on mutations affecting c-*kit* structure or expression. We also 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 using the new 'KIT-independent' models of MC deficiency. Finally, we 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 with abnormalities affecting 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 WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ and C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice [5–12]. *Kit*^{W} is a point

Corresponding author: Galli, S.J. (sgalli@stanford.edu)
Keywords: basophils; c-*kit*; mouse model; Cre recombinase.
[*] These authors contributed equally to this work.

Box 1. Some general observations about 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, 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 [63–66].
- 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 [67]) but by a long list of other stimuli including physical agents, products of diverse pathogens [68], many innate danger signals [69], certain endogenous peptides and structurally similar peptides found in invertebrate and vertebrate venoms [62,70,71], and products of innate and adaptive immune responses including immune complexes of IgG, certain chemokines and cytokines (including IL-33 [72,73]), and products of complement activation [74].
- The ability of MCs to secrete biologically active mediators can be enhanced or suppressed by many factors, including interactions with other granulocytes [75], regulatory T cells [76], or other lymphocytes [77], and certain cytokines, including the main MC development and survival growth factor, the KIT ligand SCF [5,78–80], IL-33 [81], and interferon-γ [82].
- 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 [83,84].
- 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].

mutation that produces a truncated KIT that is not expressed on the cell surface [13]; $Kit^{W\text{-}v}$ is a mutation in the c-*kit* tyrosine kinase domain that substantially reduces the kinase activity of the receptor [14], and $Kit^{W\text{-}sh}$ 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 WBB6F$_1$-$Kit^{W/W\text{-}v}$ and C57BL/6-$Kit^{W\text{-}sh/W\text{-}sh}$ 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, WBB6F$_1$-$Kit^{W/W\text{-}v}$ mice are anemic, have reduced numbers of neutrophils [8,12,16,18] and basophils [8,19,20], and are sterile [5,6]. By contrast, C57BL/6-$Kit^{W\text{-}sh/W\text{-}sh}$ mice are neither anemic 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 used 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 ear skin or peritoneal cavity of WBB6F$_1$-$Kit^{W/W\text{-}v}$ or C57BL/6-$Kit^{W\text{-}sh/W\text{-}sh}$ mice, the numbers and anatomical 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 WBB6F$_1$-$Kit^{W/W\text{-}v}$ or C57BL/6-$Kit^{W\text{-}sh/W\text{-}sh}$ 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 resemble closely 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 cannot 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