Lessons From Genetically Engineered Animal Models
IX. Mast cell-deficient mice and intestinal biology

BARRY K. WERSHIL
Department of Pediatrics, State University of New York Health Science Center at Brooklyn, Brooklyn, New York 11203

Wershil, Barry K. Lessons From Genetically Engineered Animal Models. IX. Mast cell-deficient mice and intestinal biology. Am. J. Physiol. Gastrointest. Liver Physiol. 278: G343–G348, 2000.—Mutant mice that express abnormalities in mast cell development represent a powerful tool for the investigation of multiple aspects of mast cell biology. In addition, the identification of the genes affected by these mutations has not only increased our knowledge about the mast cell but has opened new areas of investigation as to the role of these gene products in gastrointestinal pathology, immunology, and physiology.

mast cells; stem cell factor; c-kit; neutrophils; neuropeptides

FOR ALMOST HALF A CENTURY, mice with naturally occurring mutations at the Dominant Spotting (W) locus on chromosome 5 or the Steel (Sl) locus on chromosome 10 have been studied as models to understand several important developmental programs. Homozygous mutant animals are sterile, devoid of coat color, and anemic as a result of defects in germ cells, melanocytes, and erythrocyte development (reviewed in Ref. 25). In the late 1970s, Kitamura and associates (14, 15) reported that these mice also have a profound deficiency in the numbers of tissue mast cells. The fact that these mutant mice exhibit a variety of defects in addition to a lack of mast cells makes it somewhat of a misnomer to refer to them as mast cell-deficient mice. However, at least for mutations at the W locus, it is clear that mast cells are more affected than any other cell lineage (11). Studies using mast cell-deficient mice have provided new insights into the origin, differentiation, heterogeneity, and function of the mast cell in multiple organ systems. This article reviews the mast cell-deficient mouse model from a historical perspective and discusses the utility and limitations of the model for the study of mast cell biology in the gastrointestinal (GI) tract.

MAST CELL-DEFICIENT MICE

Early transplantation and embryo fusion studies employing W or Sl mutants indicated that W mutants had a defect in precursor cells of affected lineages, whereas the Sl mutants had a defect in microenvironmental elements that support the development of the affected lineages (25). In their original description of the mast cell deficiency of these mice, Kitamura and co-workers (15) reported that bone marrow transplantation of W/Wv mice corrected the mast cell deficiency, suggesting that the defect in mast cell development was in the early progenitor cells of the mast cell lineage. In contrast, bone marrow transplant did not correct the mast cell deficiency of Sl/Slv mice, supporting the concept of a microenvironmental defect in Sl mutants (14). These studies provided the first direct evidence that mast cell precursors reside in the bone marrow and demonstrated the importance of a yet-unidentified microenvironmental factor that was necessary for mast cell development.

As reported by Kitamura and colleagues (reviewed in Refs. 8 and 9), mast cell-deficient W/Wv and Sl/Slv mice are virtually devoid of mature, morphologically identifiable mast cells in all organs and anatomical sites examined. This includes both connective tissue-type mast cells characteristically found in the skin, peritoneal cavity, and the submucosa and muscularis propria of the gut, and mucosal-type mast cells found in the mucosa of GI tissues. Otherwise, W/Wv and Sl/Slv mice have normal leukocyte counts (including basophils and eosinophils) and platelet counts and express unimpaired peripheral T and B lymphocyte function in vivo and in vitro. However, it is important to emphasize that the skin of adult W/Wv and Sl/Slv mice has rare morphologically identifiable mature mast cells (~0.3% of the number present in the skin of the congenic normal mice; see Table 1).

Before the identification of the mast cell deficiency in W/Wv and Sl/Slv mice, the involvement of mast cells in GI responses was based largely on one or more lines of evidence. Mast cell participation was implied by studies showing an effect of individual mediators produced
suggested that mast cells played a significant role in cell-deficient KitW/KitW-v and MgfSl/MgfSl-d mice. Characteristics of genetically mast cell-deficient KitW/KitW-v and MgfSl/MgfSl-d mice

<table>
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<th>KitW/KitW-v</th>
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*The ligand for c-kit has been named stem cell factor, kit ligand, mast cell growth factor, and Steel factor. The skin of adult KitW/KitW-v and MgfSl/MgfSl-d mice contains rare mast cells (~0.3% of the number present in the respective congenic +/- mice). No mast cells are seen in the bone marrow, spleen, thymus, brain, heart, lung, kidney, urinary bladder, liver, stomach, ileum, cecum, mesentery, peritoneal cavity, hindlimb skeletal muscle, or uterus. Modified from Galli et al. (8).

by mast cells, histological evidence of mast cell degranulation or changes in the numbers of mast cells, and/or pharmacological studies using mediator inhibitors or mast cell stabilizing agents. These studies generated a long list of biological processes that were thought to involve mast cells, but, for the most part, the information gained through these experimental approaches was indirect and problematic. A few examples of the problems that arise from such studies are as follows: few mediators are exclusively produced by mast cells, histological evidence of degranulation does not necessarily mean that mast cells are involved in a biologically significant way, and mast cell stabilizing agents can affect other cell types in addition to the mast cell (29).

Mast cell-deficient mice provided a new approach to investigate the function of mast cells in vivo (29). A given reaction could be elicited in mast cell-deficient and the respective normal (+/-) mice. If differences in the reaction were observed comparing mutant and normal mice, and if the observed differences were abolished in bone marrow-repaired W/Wv mice, this suggested that mast cells played a significant role in the response. However, caution was necessary in the interpretation of these experiments because bone marrow transplantation corrected other abnormalities in these mice, such as the anemia. A major advancement in the experimental model occurred with the report that transplantation of immature, bone marrow-derived mast cells could selectively repair the mast cell deficiency of W/Wv mice (18). The mast cell populations that developed in the tissues of these mast cell-reconstituted mice exhibited multiple anatomical, ultrastructural, biochemical, histochemical, and functional characteristics of the native mast cell populations present in the corresponding anatomical sites in the congenic +/- mice (18, 30). Accordingly, W/Wv mice selectively reconstituted with in vitro-derived mast cells of congenic +/- origin (so-called mast cell “knock-in” mice; see Ref. 10) could now be used as powerful probes for the analysis of mast cell function in vivo. Thus, although early studies using bone marrow reconstitution of W/Wv mice still left some doubt as to the precise role of mast cells in the processes examined, the ability to selectively reconstitute W/Wv mice with mast cell populations alone permitted more definitive conclusions to be drawn.

IDENTIFICATION OF THE GENES ENCODED AT THE W AND Sl LOCi IN MICE

As in many instances in modern science, biological studies were taking place using W/Wv and Sl/Sld mouse before the knowledge of the actual genes involved, but in the late 1980s and early 1990s the genes encoded at the W and Sl loci were identified. This information not only had a major impact on the mast cell field but also resulted in the finding of new defects in W/Wv and Sl/Sld mice that were not obvious. First, the W locus was found to be allelic with the c-kit protooncogene (4, 12), which encodes a member of the type III family of tyrosine kinase receptors, now called the stem cell factor (SCF) receptor (SCFR) or the kit receptor. The Sl locus was subsequently found to encode the ligand for c-kit, known as SCF, mast cell growth factor (Mgf), or kit ligand (4). SCF is produced as a transmembrane protein that retains biological activity after the extracellular ligand domain is released from the cell by proteolytic cleavage (11). Thus the proper designation of W/Wv and Sl/Sld mutations is now KitW/KitWv and MgfSl/MgfSl-d, respectively.

The KitWv mutation is a point mutation at an exon/intron junction that causes exon skipping and the production of a truncated receptor lacking the transmembrane domain, resulting in its failure to be expressed on the cell surface (11). The KitWv mutation is a point mutation (Thr(589)→Met) that maps to the tyrosine kinase domain. Although this receptor is expressed on the cell surface, it exhibits markedly reduced intrinsic kinase activity (19). MgfSl is a deletion that encompasses the entire SCF coding sequence (11), whereas MgfSl-d is an intragenic insertion/deletion that removes 242 bp at the 3’ end of the coding sequence; the truncated transcript encodes almost the entire extracellular domain of SCF but lacks coding sequences for...
both the transmembrane and intracellular domains of the wild-type protein. Transfection studies demonstrated that the MgfsL-md transcript cannot produce a transmembrane protein but instead results in the secretion of a truncated SCF molecule that retains biological activity (3). It is the expression of some SCF activity by the MgfsL-md product and the expression of limited SCFR kinase activity by the KitW-V product that permits MgfsL/ MgfSl-md and KitW/ KitW-v mice to survive into adulthood, despite exhibiting certain phenotypic abnormalities as a result of their respective mutations. In contrast, MgfsL/ MgfSl and KitW/ KitW mice typically die in late gestation or shortly after birth.

**DISCOVERY OF ADDITIONAL PHENOTYPIC ABNORMALITIES IN KitW/KitW AND MgfSl/MgfSl MICE**

The identification of the gene products of W and Sl resulted in the development of the experimental reagents necessary to search for additional sites of expression of SCF or its receptor that were not predictable based on the more obvious phenotypic abnormalities of Kit or Mgf mutant mice. For example, certain cells in the nervous system and some tumor cells were found to express SCFR (reviewed in Ref. 11). Defects in KitW were found to affect the development of the interstitial cells of Cajal that are necessary for intestinal pacemaker activity (13, 16), and SCF/SCFR interactions were found to influence intraepithelial lymphocyte populations in the gut (23). The functional consequences of these new abnormalities in KitW/KitW and MgfSl/MgfSl mice have not been fully delineated. However, these defects and potentially yet undiscovered defects must be taken into consideration when using these mice for in vivo studies and emphasizes the importance of the mast cell knock-in model for isolating and precisely defining the contribution of the mast cell in various biological responses.

**KNOWLEDGE GAINED THROUGH THE USE OF KitW/KitW AND MgfSl/MgfSl MICE IN INVESTIGATION OF MAST CELL FUNCTION IN THE GI TRACT**

Mast cell-deficient mice were initially used for studies of mast cell function in the skin, since this is a very convenient site for experimental manipulations. Now the model has been developed for study of a variety of pathological, immunological, and physiological processes in the GI tract. Although a comprehensive presentation of the literature is beyond the scope of this article, there are several important examples that illustrate the utility (and the limitations) of using mast cell-deficient mice to examine mast cell function in the GI tract.

The role of mast cells in parasite rejection. Several lines of evidence indicated that mast cells may have an important role in the immune response to certain types of parasites and, in particular, helminthic infection. These parasitic infections are associated with an increase in the levels of parasite-specific IgE, mast cell hyperplasia, and mast cell degranulation (reviewed in Ref. 2). Also, certain mediators produced by mast cells, including histamine and serotonin (in rodent species), have physiological effects on vascular permeability, intestinal fluid and mucus secretion, and intestinal motility that may enhance local host defense against parasites (2). However, studies using mast cell-deficient mice raised significant questions as to the importance of mast cells in the host response to certain parasites.

For example, most studies of experimental parasite infection using either Nippostrongylus brasiliensis, Trichinella spiralis, or Strongyloides ratti showed that the duration of these experimental parasite infections was prolonged in mast cell-deficient mice compared with normal animals (reviewed in Ref. 24). However, the impairment of parasite rejection in mast cell-deficient mice was relatively minor, unlike athymic nude mice, and eventual resolution of the infection did occur. The importance of mast cells in the rejection of N. brasiliensis was further brought into question by the observation that the prolongation of the infection in KitW/ KitW-v mice did not improve after bone marrow transplantation (19). Furthermore, although bone marrow reconstitution of KitW/ KitW-v mice did improve the course of rejection in T. spiralis or S. ratti infection, this was not necessarily the result of mast cell reconstitution but could be due to some other element derived from the bone marrow. Thus, although mast cells were thought to play an important role in intestinal parasite rejection, these studies suggest that, at least with certain parasites, mast cells are not a necessary component of the host response.

Mast cells and immediate-type hypersensitivity reactions in the GI tract. As in the case of intestinal parasite infections, strong circumstantial evidence implicated the mast cell in immediate-type hypersensitivity reactions in the GI tract. In several experimental models, the elicitation of intestinal anaphylaxis was associated with fluid and electrolyte secretion by intestinal epithelial cells (reviewed in Ref. 5). This prompted a study examining the involvement of mast cells in ion secretion associated with intestinal anaphylaxis using mast cell-deficient and normal mice. It was found that mast cell-deficient mice exhibited a significantly diminished secretory response compared with normal mice (21). However, a reproducible secretory response that was 10–15% of the response in normal mice was seen in mast cell-deficient mice (21). Bone marrow transplantation of KitW/ KitW-v mice completely corrected the abnormality in antigen-driven fluid secretion. The authors concluded that most, but not all, of the ion secretion induced by intestinal anaphylaxis involved the participation of mast cells (21).

Another interesting finding of this study involved the pathogenesis of the secretory response elicited by direct nerve stimulation (transmural electrical stimulation). They reported that this response was markedly diminished in mast cell-deficient KitW/ KitW-v mice and was normalized after bone marrow transplantation (21). Thus this observation provided strong evidence of a functional communication between mast cells, nerves, and intestinal epithelial cells.
Mast cells and late phase reactions in the stomach. Immediate-type hypersensitivity responses in the skin, lung, and other sites are frequently followed by recurrence of the symptoms 6–8 h later, which is characterized by the infiltration of leukocytes. This latter reaction has been termed the late phase response (LPR), but LPRs were not well described in the GI tract. This led to a study of the occurrence of GI LPRs using mast cell-deficient and normal mice. IgE-dependent neutrophil and mononuclear cell infiltration was found in normal mice but was completely absent in mast cell-deficient KitW/KitW-v mice (28). The selective and local repair of the mast cell deficiency in the stomach of KitW/KitW-v mice resulted in the ability to express both neutrophil and mononuclear cell infiltration in association with the reaction (28). A subsequent study demonstrated that mast cell-derived tumor necrosis factor (TNF)-α was an important mediator involved in the recruitment of neutrophils into sites of IgE-dependent gastric inflammation (23).

Mast Cells and Neuropeptide Interactions in the GI Tract

The enteric nervous system is rich in a variety of neuropeptides that act as neurotransmitters, but they may also influence immune and inflammatory responses in the GI tract (20). A particularly well-studied aspect of the relationship between enteric nerves and the immune system is the interaction between mast cells and the neuropeptide substance P. Several lines of evidence suggested that there was a functional link between mast cells and nerves in the GI tract and that this link was mediated, at least in part, by substance P (22). However, there was little direct evidence demonstrating that the interaction of mast cells and substance P significantly contributed to immune or physiological responses in the GI tract until the study of Wang et al. (26), examining the participation of mast cells in substance P-induced intestinal ion secretion. They reported that substance P elicited significant fluid and electrolyte secretion in normal mice, but mast cell-deficient KitW/KitW-v mice exhibited a markedly diminished secretory response (~50% that of +/+ mice) (26). The secretory response to substance P was normalized in bone marrow-transplanted KitW/KitW-v mice (26), suggesting that mast cells accounted for the difference in the response between the normal mice and mast cell-deficient KitW/KitW-v mice.

The pathophysiological link between mast cells and substance P was also examined using an experimental inflammatory reaction induced by Clostridium difficile toxin A. This exotoxin elicits epithelial fluid secretion and leukocyte infiltration into the gut that is mediated in some part by substance P and occurs in association with mast cell degranulation. The role of mast cells in the inflammatory response to C. difficile toxin A was examined in more detail using mast cell-deficient mice, normal mice, and mast cell-deficient KitW/KitW-v mice that were selectively reconstituted by the systemic administration of in vitro-derived mast cells. This modification of the model permitted the reconstitution of mast cells in all mast cell compartments, making these animals more amenable to the study of mast cell function in a large, diffuse organ system like the GI tract (27).

This study reported that substance P participated in toxin A-induced fluid secretion through both a mast cell-dependent and a mast cell-independent pathway, whereas substance P contributed to neutrophil recruitment by a mechanism involving predominantly mast cells (27).

The Role of Mast Cells in Host Defense Against Bacterial Infections

Although a century of research has examined the deleterious effects of mast cells in allergic phenomena, an exciting and unexpected role for mast cells in the host response to bacterial infections was recently found using mast cell-deficient mice. In two studies using an experimental model of bacterial peritonitis, mast cell-deficient KitW/KitW-v mice exhibited significantly greater mortality than normal mice (6, 17). Mast cells accounted for this difference in mortality, as demonstrated by improved mortality rates after reconstitution of mast cells in the peritoneal cavity of KitW/KitW-v mice. Furthermore, it was found that TNF-α, either from mast cells, stimulated by mast cells, or both, mediated this protective response (6, 17).

Potential Pitfalls to Consider When Using Mast Cell-Deficient Mice to Study Mast Cell Function in Vivo

New information regarding the defects in these mutant mice has identified certain potential problems in using them for the study of mast cell function. However, once understood, these problems can usually be addressed, either in experimental design or in the interpretation of experimental data. As mentioned, mast cell-deficient mice can have identifiable mast cells in the skin. KitW/KitW-v mice can also develop mast cells in the setting of chronic cutaneous inflammation, either induced by phorbol esters or naturally occurring in the form of idiopathic dermatitis (8). The development of mast cells in the GI tract of KitW/KitW-v mice has also been reported in T. spiralis infection (1). The precise etiology of the development of mast cells in these inflammatory responses is not known but may reflect increased levels of cytokines that affect mast cell growth, such as interleukin-3.

However, many other biological responses examined in KitW/KitW-v mice that involve chronic inflammation are not associated with the development of mast cells. Nonetheless, it is important to perform appropriate histological studies to assess whether mast cells have developed in the mutants.

As previously noted, Kit or Mgf mutant mice exhibit an expanded list of phenotypic abnormalities that are apparently unrelated to the effect on the mast cell lineage. Accordingly, the discovery of a difference in the expression of a biological response in KitW/KitW-v or MgfSt/KitW-v mice and the respective congenic +/+...
mice does not prove that this difference is the result of the mast cell deficiency. The most definitive approach for assessing the specific contribution of mast cells in a response is to selectively repair the mast cell deficiency in KitW/ KitW mice with bone marrow-derived mast cells from the congenic +/− mice, creating the above-mentioned mast cell knock-in mice (10). However, it is important to appreciate some limitations of this approach. There may be differences in the anatomical distribution and density of mast cells in normal mice compared with mast cell knock-in mice, depending on the number of mast cells transferred, the method of administration, and the length of time between adoptive transfer and the use of the animals in experiments. These variables must be taken into consideration when using these mice.

FUTURE DIRECTIONS

Once mast cell knock-in mice have been used to demonstrate the contribution of mast cells in the expression of a particular biological response, a variety of approaches can be taken to examine the specific function of mast cells in these reactions in greater detail. Several examples were presented in which it was possible to identify the particular mast cell mediators that contributed importantly to the reaction. In fact, it has been hypothesized that mast cells may be involved in the initiation and/or perpetuation of inflammatory reactions by directly producing certain mediators and by downstream events such as stimulating mediator production by other resident cells or by the recruitment of leukocytes. A new direction for the refinement of the mast cell-deficient mouse model will be to develop various mast cell populations deficient in a single mediator that can then be used for adoptive transfer into KitW/ KitW mice. This will permit the precise analysis of the contribution of that mediator derived only from mast cells, as opposed to other cells.

An exciting outgrowth of the identification of the gene products of W and SI has been the ability to use these mice to study new, previously unappreciated functions that may be dependent on and/or independent of mast cells, such as intraepithelial lymphocyte function, motility issues, and the role of SCF in a variety of pathological processes. Thus mast cell-deficient mice are not only useful for the study of mast cell function in vivo but also offer an exciting opportunity to explore new areas of GI biology and pathophysiology.

Address for reprint requests and other correspondence: B. K. Wershil, Dept. of Pediatrics, SUNY Health Sciences Center at Brooklyn, Box 49, Brooklyn, NY 11203 (E-mail: bkwershil@aol.com).

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