MINIREVIEW

Microenvironmental Factors that Influence Mast Cell Phenotype and Function (43323C)

MARK SWIETER,[1] STEPHAN E. MERGENHAGEN, AND REUBEN P. SIRAGANIAN
Laboratory of Immunology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892

Mast cells play a central role in immediate hypersensitivity responses. They produce and store in their numerous cytoplasmic granules, and synthesize *de novo* when activated, an array of biologically potent substances whose combined effects account for the many symptoms of allergic reactions. It is, however, difficult to believe that mast cells evolved to serve in such pathological processes. Yet, despite having first been described over a century ago (1), we still know little about the physiologic functions of these cells.

Complicating efforts to understand the biology of mast cells is their heterogeneous nature (see below). In rats, mice, and humans, two phenotypically distinct subsets of mast cells have been identified (2–4), although it is likely that these represent merely two points in a spectrum of diversity (e.g., see Ref. 5). Sorting out the causes of this diversity should lead to a better understanding of the importance of mast cells in health and disease.

Recent evidence suggests that mast cell heterogeneity may largely be due to the site-specific blending of factors produced within the local microenvironment (2). Therefore, it is critical to identify these factors and determine precisely how they affect mast cell phenotype. Accordingly, in this review we will examine the most recent findings which suggest that factors released or expressed by other cells in the vicinity of mast cells may regulate their form and function.

Mast Cell Heterogeneity and Plasticity

Mast cells in the peritoneal cavity of rats and mice are easily obtained by lavage. In contrast, other mast cell populations require enzymatic digestions to disperse them from the tissues in which they are found. Since peritoneal mast cells share many similarities with those in most other parts of the body, they are widely used and have by default come to represent the prototypical mast cell. However, their appropriateness as a model needs to be assessed when new characteristics are found.

The intestinal mucosa of rodents contains a population of mast cells that increases dramatically in number in response to infection with intestinal nematodes such as *Nippostrongylus brasiliensis* (3). Compared with peritoneal mast cells, the intestinal mucosal mast cells are smaller, have less histamine, and synthesize unique neutral serine proteases (Table I). Due to the presence of chondroitin sulfate proteoglycans instead of heparin in their cytoplasmic granules, the intestinal mucosal mast cells also stain with Alcian blue, but not with safranin or berberine sulfate (3, 4). Importantly, and in sharp contrast to peritoneal mast cells that are reactive to a wide range of substances, including neuropeptides, the intestinal mucosal mast cells are unresponsive to most nonimmunologic secretagogues. As intestinal mucosal mast cells do have functional high affinity IgE receptors on their surfaces (6), it is likely that the primary mode of activation of these cells *in vivo* is through their IgE receptors.

Not long after Kitamura and colleagues demonstrated that mast cells in mice were of hematopoietic origin (7–9), several groups showed that nearly pure populations of mast cells could be generated by long-term (2–4 weeks) culture of bone marrow cells in interleukin (IL)-3-containing media (reviewed in Ref. 2). In contrast to other mast cell populations, the bone marrow-derived mast cells resemble those in the intestinal mucosa morphologically, histochemically, biochemically, and in their lack of responsiveness to nonimmunologic stimuli (2–4). However, at the ultrastructural

[1] To whom correspondence and requests for reprints should be addressed at Bldg 10, Rm 1A28, National Institutes of Health, Bethesda, MD 20892.

0037-9727/92/1991-0022$03.00/0

level, the bone marrow-derived mast cells appear immature (2). Because they can be easily obtained as pure populations, the culture-derived mast cells are frequently used as a prototype for mucosal mast cells.

In humans, mast cells have been found to fall into two populations based on their immunoreactivity with antibodies to two neutral serine proteases. MC^T are identified by their reactivity exclusively with antitryptase antibodies, whereas MC^{TC} react with antibodies to both tryptase and chymase (10). MC^T predominate in the alveoli of the lung (93%) and small intestinal mucosa (81%), while MC^{TC} are the most abundant phenotype in the skin (99%) and small intestinal submucosa (77%). However, human skin mast cells respond to a variety of neuropeptides that do not activate the histochemically analogous mast cells in the small intestinal submucosa (11). This shows that the functional phenotype of a mast cell is not always predictable from its histochemical characteristics.

Like lymphocytes, macrophages, and neurons, mast cells comprise distinctly different phenotypes within the same host. However, where mast cells may differ from the others is in the remarkable plasticity of their apparently differentiated states. Experimental evidence suggests that when the microenvironment in which they find themselves changes, what seem to be mature cells can in fact alter their granule contents by making new constituents of a different class (12–15). Thus, *in vitro* cultured, bone marrow-derived mast cells from mice transplanted into the peritoneal cavity convert from making chondroitin sulfate proteoglycans to producing heparin proteoglycans (12–14), and peritoneal mast cells placed into liquid culture with cytokines switch from synthesizing heparin to making chondroitin sulfate proteoglycans (15). Hence, the concept of mast cell heterogeneity now needs to include the possibility that during immune and inflammatory responses, mast cells may be induced to alter their form and function as a host defense mechanism.

Secretory Products that Regulate Mast Cell Phenotype

A number of cytokines and other secreted products have been shown to maintain mast cells in culture and affect their characteristics (Fig. 1).

Interleukin 3. IL-3 is a 28-kDa glycoprotein that promotes the survival, proliferation, and differentiation of hematopoietic progenitor cells of several lineages, including erythrocytes, lymphocytes, monocytes, granulocytes, megakaryocytes, and mast cells (reviewed in Refs. 16 and 17). It is produced predominantly by T cells undergoing antigenic or mitogenic stimulation (16–19), but can also be made by activated mast cells (20–23). That mast cells can make IL-3 (and numerous other cytokines as well; see Ref. 24) suggests that they may be capable of influencing their own proliferation in certain situations. IL-3-like activities have also been reported from keratinocytes (25) and astrocytes (26) as well.

As discussed above, IL-3 *in vitro* is an inducer of mast cell differentiation and proliferation from mouse and rat bone marrow precursors. *In vivo* IL-3 plays a role in the mast cell response to parasitic infection. Unlike normal murine rodents infected with nematodes such as *N. brasiliensis* (3), athymic (nude) mice do not undergo a T cell-dependent hyperplasia of mast cells in the intestinal mucosa (27, 28). However, when given repeated intraperitoneal injections of purified IL-3, nude mice do respond with a mucosal mastocytosis similar in degree to that of parasite-infected normal animals (29). Furthermore, mice given bolus injections of anti-IL-3 antibodies prior to and during nematode infection display a 40–50% reduction in mucosal mast cell responses (30). Taken together, these results show that IL-3 *in vivo* is important for the proliferation of mast cells in the small intestinal mucosa of mice and rats during parasitic infestations.

Given its role as a mast cell growth factor in the rapid and massive build up of intestinal mucosal mast cells during parasitic infections, IL-3 is often looked upon as functioning exclusively in immune and inflammatory responses. However, it is conceivable that under normal conditions, IL-3 plays a role in the routine maintenance of mast cell numbers throughout the body.

Interleukin 4. IL-4 is a 20-kDa glycoprotein product of T cells that induces B cells to switch from producing IgM to synthesizing IgE (31, 32). IL-4 has also been shown in mice to be a costimulator with IL-3 in the *in vitro* proliferation of both bone marrow-derived (33–35) and peritoneal mast cells (36–40). With peritoneal mast cells *in vitro*, IL-3 appears to function primarily to promote cell survival, while IL-4 acts as a cofactor stimulating cell proliferation (40). *In vivo*, anti-IL-4 antibodies administered to mice on Days 0 and 7 of infection with *N. brasiliensis* cause an approximately 50% reduction in the ensuing intestinal mastocytosis (30). When both anti-IL-3 and anti-IL-4 antibodies are given, upward of 85–90% of the mastocytosis is inhibited. Thus, IL-4 is a significant modulator of mast cell proliferation *in vivo* as well as *in vitro*.

Interestingly, there is no evidence that, alone or in combination, IL-3 and IL-4 can induce mast cells or their progenitors to differentiate into a heparin-producing phenotype. Indeed, although precursors for both the peritoneal and intestinal mucosal mast cells are found in the bone marrow, only chondroitin sulfate proteoglycan-containing mast cells develop in long-term bone marrow cultures in cytokine-rich media. Moreover, although a portion of peritoneal mast cells that are put into methylcellulose cultures with IL-3 and IL-4 give rise to berberine sulfate-positive colonies,

Table I. Mast Cell Heterogeneity in Rodents

	Mast cell source		
	Peritoneal cavity	Intestinal mucosa	*In vitro*-derived mast cells from hematopoietic progenitors
Histochemistry			
Alcian blue	+	+	+
Safranin	+	−	−
Berberine sulfate	+	−	−
Granule contents			
Major proteoglycan	Heparin	Chondroitin sulfate	Chondroitin sulfate
Serine protease[a]	RMCP I	RMCP II	RMCP II
Histamine (pg/cell)	10–30	1–2	1–2
Releasability			
IgE/anti-IgE	+	+	+
Compound 48/80	+	−	−

[a] Rat mast cell proteases (RMCP) I and II are found only in rats.

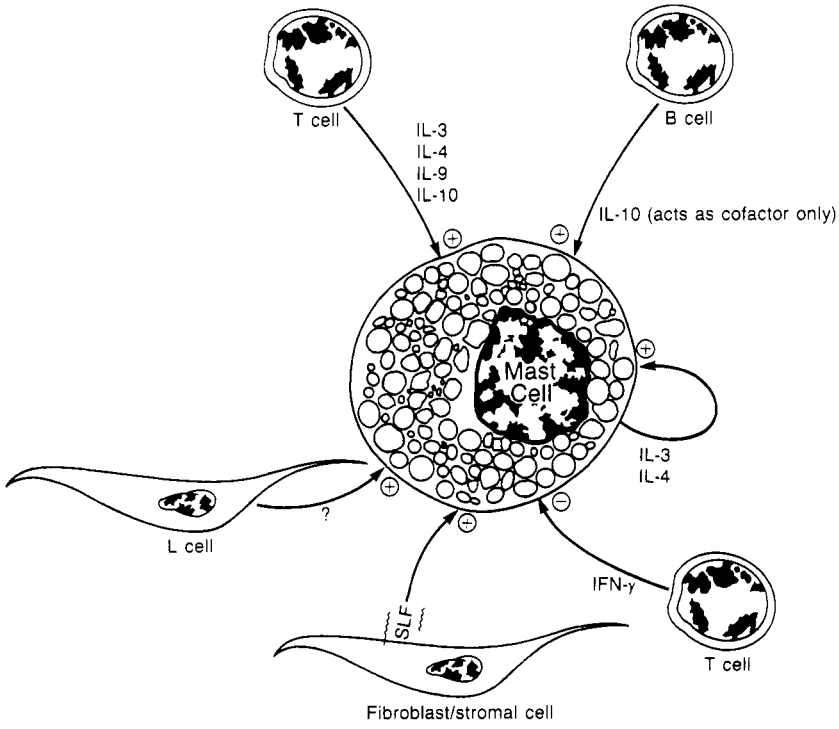

Figure 1. Microenvironmental factors that promote (+) or inhibit (−) the growth and differentiation of mast cells.

when these are then transferred to suspension culture, all mast cells that develop are berberine sulfate negative (15). This indicates that not only are the cytokines incapable of inducing heparin synthesis in mast cells, they cannot even maintain the phenotype.

Human bone marrow or umbilical vein cells cultured for up to 4 weeks in the presence of either IL-3 alone, IL-4 alone, or IL-3 and IL-4 in combination failed to yield any identifiable mast cells (41, 42). Cells with the morphology of basophils did, however, develop. The greatest percentage of basophils was present when IL-3 was used alone. Thus, IL-4 in these cultures actually appeared to down-regulate the basophil-promoting activity of IL-3.

Interleukin 9. IL-9 is a 35-kDa glycoprotein produced by T helper cells and mitogen-activated spleen cells that enhances the proliferation of IL-3-dependent mouse mast cell lines (43–45). When used in conjunction with IL-3, IL-9 also synergistically enhances the proliferation of primary mouse bone marrow-derived mast cell cultures (45). However, when used alone, IL-9 has no growth-promoting activity on primary mast cell cultures, although it does prolong their survival (45). Inasmuch as IL-3, IL-4, and IL-9 are all produced

by activated cloned T lymphocytes of the helper 2 class (34, 46, 47) and by spleen cells stimulated with mitogens (43–45), it seems likely that they act together to induce mast cell proliferation during inflammatory responses.

Mitogen-stimulated spleen cell supernatants have also been shown to support the survival and proliferation of mouse peritoneal mast cells in methylcellulose cultures (36, 37). Although purified IL-3 plus IL-4 were found to essentially mimic these results, there was no indication that the amounts of purified IL-3 and IL-4 used were similar to those present in the conditioned media. Hence, it may be possible that IL-9 assists other interleukins in supporting the survival and proliferation of peritoneal mast cells.

Interleukin 10. IL-10 is produced by activated T cells (48, 49) and normal B cells (50). Used alone, IL-10 fails to support the growth of either an IL-3-dependent mouse mast cell line or mast cell progenitors in mesenteric lymph nodes of *N. brasiliensis*-infected mice (51). However, when used in combination with IL-3 or IL-4, it enhances mast cell proliferation. That mast cell growth can be engendered by IL-4 and IL-10 in the complete absence of IL-3 suggests another pathway for mast cell production in circumstances when IL-3 would be unavailable. Importantly, the most potent growth-promoting stimulus for either primary cultured mast cells or IL-3-dependent mouse mast cells is the combination of all three cytokines together. As these three cytokines are coordinately synthesized by the same T cell populations (49) and appear to be produced during *N. brasiliensis* infections, in which intestinal mastocytosis develops (51), they may constitute a network of overlapping mast cell growth-promoting activities needed to ensure appropriate levels of mast cells as a host response to infection. The role of IL-9 in such a network awaits future studies. Likewise, although IL-3 and IL-4 have been shown to cooperate in stimulating mouse peritoneal mast cell proliferation in methylcellulose cultures (36), it remains to be determined whether IL-9 and/or IL-10 also function in this capacity.

L Cell Supernatants. Czarnetzki and co-workers have reported generating mast cells in liquid suspension cultures from either peritoneal (52, 53) or spleen and bone marrow cells of rats (54), or peripheral blood cells of humans (55). Mouse L cell supernatants were critical and horse serum worked better than fetal calf serum. After about 2 weeks, the rat peritoneal cell cultures contained around 90% mast cells, though viability and cell number decreased beyond this time point. The mast cells in the cultures bound ^{125}I-IgE, stained positively with safranin, and released up to 60% of their total cellular histamine in response to Compound 48/80. After 3 weeks in culture, approximately half of the human peripheral blood cells stained metachromatically with toluidine blue. Their cytoplasmic granules contained homogeneous, electron dense material and they were positive for the chloroacetate-AS-D-esterase reaction. Although it is not clear whether these cells are more like mast cells or basophils, a similar 3-week time frame for differentiation of mast cells from human umbilical cord blood cells induced by soluble fibroblast factors has recently been reported (56). Since L cells are fibroblasts, they may be capable of secreting soluble Steel factor (see below), a recently identified mast cell growth factor.

γ-Interferon. Mouse bone marrow-derived mast cells have been shown to express a small number of high affinity binding sites for γ-interferon (IFN) (57). The continuous supply of recombinant murine IFN-γ to mouse bone marrow cells cultured in cytokine-laden media brings about a marked reduction in the number of mast cells that develop. Similarly, recombinant murine IFN-γ added to IL-3- and IL-4-containing clonal cultures of mouse peritoneal mast cells also inhibits proliferation of these cells (58). However, replacement of IL-4 with another costimulant of peritoneal mast cell proliferation, such as the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate, renders IFN-γ ineffective as an inhibitor of IL-3-dependent cell division. Thus, at least with respect to mouse peritoneal mast cells, IFN-γ seems to interfere with the IL-4-dependent proliferation pathway.

Inhibitory Factors. Mouse peritoneal mast cells appear to be very sensitive to negative feedback signals for growth and differentiation. Intravenous injection of bone marrow cells from congenic normal littermates into mast cell-deficient W/W^v mice results in the appearance of mast cell precursors in the peritoneal cavity within weeks (59, 60). If, however, either mature peritoneal mast cells (59) or bone marrow-derived mast cells (60) from normal mice are transferred into the peritoneal cavity prior to transfusion of normal mouse bone marrow cells, the recruitment of mast cell precursors into the peritoneal cavity is inhibited. This suggests that peritoneal mast cells produce a contact-dependent or concentration-dependent factor that interferes with cell survival. Dissecting the mechanism of such inhibition could lead to a means of controlling mast cell proliferation in mastocytotic diseases.

Steel Factor and Its Receptor

Much of what is presently understood about the natural history of mast cells stems from experiments using mice with mutant genotypes. In particular, the W/W^v and Sl/Sl^d mutants have been invaluable. The search for the molecular basis of their defects has led recently to the identification of yet another mast cell growth factor.

Mice with two mutant alleles at either the dominant white spotting locus on Chromosome 5 (W) or at

the Steel locus on Chromosome 10 (*Sl*) display the same range of seemingly unrelated defects (61–63). They have aberrations in pigmentation that include coat color, deficiencies in sex cells often leading to sterility, and macrocytic anemia. Although some combinations of alleles are lethal, the W/W^v and Sl/Sl^d genotypes are sparing. Kitamura and colleagues showed that W/W^v and Sl/Sl^d mice also have a mast cell defect, with <1% the normal number in the skin and essentially no mast cells in any of the other tissues (64–66).

The mast cell deficiency in *W* mutants has been linked to a defect in hematopoietic progenitors, because bone marrow from normal (+/+) congenic littermates transplanted into W/W^v mice cures the mast cell defect (64). The nature of the problem with the bone marrow does not appear to involve a lack of mast cell precursors, since mast cells can be generated *in vitro* from W/W^v mouse bone marrow cells provided that either IL-3 or activated lymphocyte supernatant is present (67–69). Moreover, the problem does not seem to involve trafficking of the precursors, since normal numbers of mast cell precursors are present in both peripheral blood and spleens of the mutants (67–69). There does, however, appear to be a defect in the homing of mast cell precursors to the tissues (70). Therefore, the mast cell deficiency in W/W^v mice is likely due to a defect inherent in the mast cell precursor itself, affecting both its presence and differentiation in the tissues.

In contrast, the mast cell deficiency in *Sl* mutant mice has been linked, not to the mast cell or its antecedents, but rather to a defect in the tissue microenvironment. Thus, mast cells can be generated *in vitro* from Sl/Sl^d bone marrow (71), and Sl/Sl^d bone marrow transplanted into W/W^v recipients cures both the anemia and the mast cell deficiency of the W/W^v mice (65). However, normal +/+ donor bone marrow is ineffective at curing the Sl/Sl^d phenotype. Therefore, the *Sl* defect lies not in the production of hematopoietic precursors, but instead in their differentiation and maturation. Because mast cells appear to develop normally when placed in the proper microenvironment, these results show that it is the microenvironment in Sl/Sl^d mice that is deficient. To demonstrate this point further, when mast cell-deficient W/W^v skin is grafted onto Sl/Sl^d recipients, mast cells develop in the grafts; when Sl/Sl^d mouse skin is grafted onto congenic +/+ littermates, no mast cells arise (65). Hence, Sl/Sl^d mouse skin provides a microenvironment unsuitable for mast cell differentiation. Furthermore, the number of mast cell precursors in the skin of these animals is only a fraction of that in normal mice (72). Thus, the defect in Sl/Sl^d mutant mice appears to affect both the homing of mast cell progenitors to the tissues as well as their differentiation.

Using molecular genetic approaches, genes mapping to the *W* and *Sl* loci were recently shown to encode a cell surface growth factor receptor and its ligand (73–79). Distinct mutations in these genes were identified in a number of the mutant alleles (80–84), and the corresponding mRNA (80, 81, 83, 85, 86) and protein products (75, 80, 81) were found to be expressed only in those cells and tissues affected by the mutations. Thus, the mast cell deficiencies in *W* and *Sl* mutant mice appear to be due, at least in part, to mutations in single genes at these loci.

The *W* locus was found to be allelic with the proto-oncogene c-*kit* (73, 74), which encodes a 145- to 160-kDa cell surface receptor with tyrosine kinase activity (75, 87). This protein is a member of the receptor tyrosine kinase family that includes the platelet-derived growth factor receptor and the receptor for colony-stimulating factor-1 on macrophages (88). Its ligand is encoded by a gene that maps near or to the Steel locus (76, 77, 79), thereby accounting for the similarities in the defects caused by mutations at the *W* and *Sl* loci.

The growth factor encoded by the Steel locus gene has been referred to by many names, including mast cell growth factor (78), stem cell factor (89), and *kit* ligand (90). Steel factor (SLF; 91) is the one we will use. Furthermore, because growth factor receptors are commonly named after the ligand they bind, the c-*kit* protein will hereafter be referred to as the Steel factor receptor (SLFR).

Structurally, the SLFR has three domains: an N terminal, extracellular, ligand-binding domain with five immunoglobulin-like loops, a small transmembrane segment, and a C terminal intracellular tyrosine kinase region (75, 88). Mutations in the kinase region in many of the mutant *W* alleles resulted in a diminution in autophosphorylating activity in *in vitro* kinase assays (80–83). The amount of reduction in kinase activity correlated well with the severity of the developmental defects of the mutants (80, 81, 83). Hence, the mast cell deficiency in mice containing a double dose of mutant *W* alleles stems from the inability of the SLFR to autophosphorylate and probably to phosphorylate other cellular proteins as well.

The SLFR and its ligand, SLF, are both expressed at the earliest stages of embryonic development (92, 93). However, their presence on hematopoietic progenitors in postnatal animals is uncertain. Considering that precursors of both mast cells and erythrocytes are present in Sl/Sl^d mouse bone marrow (65, 71), if SLFR are present on them, then either signaling through the SLFR is not needed to produce these early stage cells or the sparing effect of the Sl^d allele involves the production of sufficient SLF to generate the precursors that are found in the bone marrow, but not enough to bring about their further differentiation (see below for further discussion of this issue).

The SLFR is also found on mature mast cells (80, 81, 90). It is possible that this expression reflects a

potential for expansion of the mast cell population when the need for additional mast cells arises. It is also possible that the presence of the SLFR on mature mast cells reflects a requirement by these cells for continuous signaling through the receptor in order to survive. In support of this idea, mast cells from the rat peritoneal cavity (94), human lung (95), and normal mouse bone marrow (96, 97) all survived for weeks in co-cultures with normal mouse 3T3 fibroblasts. Separation of the mast cells and fibroblasts with a 0.45-μm filter brought on cell death (94, 97). Likewise, co-culture of SLFR-deficient W/W^v mouse bone marrow-derived mast cells with normal mouse fibroblasts results in rapid death of the mast cells (97), due to an inability of the mast cells to transit the cell cycle (98). Inasmuch as normal mouse 3T3 cells have been shown to express surface membrane-associated SLF (99), but do not produce either IL-3 or IL-4 (97), it may be that SLFR-mediated signaling promotes cell survival, whereas the absence of receptor-ligand interactions leads to activation of genes for programmed cell death (100).

Expression of the SLFR can be regulated by various cytokines. Welham and Schrader (86) have found that mRNA for the SLFR in mast cells can be down-regulated by IL-3, granulocyte-macrophage colony-stimulating factor, and erythropoietin. These authors suggest that since there is evidence that the SLFR may serve as an adhesion molecule (84), its down-regulation by IL-3 may represent a mechanism by which mast cell precursors could be released from the hematopoietic tissues when needed (86). Ody *et al.* (101) found that 28 days after implanting miniosmotic pumps containing recombinant mouse IL-3 into W/W^v mice, safranin-positive mast cells were present in the skin. Their abundance was nearly identical to that of +/+ littermates. Galli *et al.* (102) also found heparin-containing mast cells in the ears of W/W^v mice suffering from chronic idiopathic dermatitis. Although the mechanism of mast cell formation in this case is not known, it too may involve IL-3. If IL-3 does down-regulate SLFR expression, then some mechanism for stimulating mast cell differentiation into heparin-containing mast cells other than through the SLFR is likely at work here.

As already noted above, the *Sl* locus appears to contain a gene that encodes Steel factor, the ligand of the SLFR (76, 77, 79). The nucleotide sequences of the cloned human (103), rat (103), and mouse (77, 104) SLF cDNA encode a predicted 245–248 amino acid transmembrane glycoprotein. There are apparently two forms of the protein, soluble and membrane attached, the result of alternative mRNA splicing (84, 89, 103, 104). Additionally, the soluble form of at least rat SLF may exist as noncovalently linked dimers (89). Although the tissue distribution of SLF has not been investigated, both the soluble and membrane-associated forms are produced by fibroblasts and bone marrow stromal cells (77, 99, 104). In addition, SLF activity has also been found in Schwann cell supernatants (105). Because rat osteosarcoma cells support the survival and maintenance of the phenotype of rat peritoneal mast cells in co-cultures (106), they too may express functional SLF.

Interestingly, bone marrow, brain, and spleen from Sl/Sl^d mice express normal or near normal levels of SLF mRNA (79, 84). Yet fibroblast lines derived from these animals fail to support the survival of normal, bone marrow-derived mast cells (71), and no SLF activity is detected in culture supernatants from these lines (107). The SLF mRNA from Sl/Sl^d mouse tissues does not encode the full-length, transmembrane form of SLF, but rather a truncated 183-amino acid form lacking the transmembrane and intracytoplasmic domains (84). Concordant with this, Sl/Sl^d fibroblasts do not express the surface form of SLF (84). Therefore, either the truncated soluble form of SLF in Sl/Sl^d mice is biologically inactive and insufficient to affect mast cell proliferation and differentiation or it is produced in quantities too limited to be effective.

In terms of biological activity, soluble rat (103) and mouse (104) SLF, whether purified from culture supernatants or in recombinant form, are capable of stimulating proliferation of mouse mast cell lines *in vitro*. In 10-day agar cultures of mouse bone marrow, both recombinant and natural soluble SLF stimulated formation of colonies containing monocytes, neutrophils, and blast cells, though no mention was made of mast cell development (89, 103). It is noteworthy that when purified soluble natural rat SLF was used on marrow cultures depleted of mature myeloid and lymphoid cells, no colonies developed (89). These results suggest that soluble SLF probably does not function to stimulate proliferation of immature progenitor cells, but they do not eliminate the possibility that membrane-associated SLF alone, or in conjunction with other agents, does. Furthermore, although mast cell precursors in the bone marrow may express the SLFR (92), the receptors may not become functional until the cells are ready to leave the bone marrow. Thus, these cells may be too few to recognize in *in vitro* assays.

Jarboe *et al.* (108) showed that concentrated culture supernatants from normal mouse 3T3 fibroblasts contained an activity that supported the differentiation of mast cells from precursors in mesenteric lymph nodes of *N. brasiliensis*-infected mice. Nocka *et al.* (90) followed up these experiments by purifying the activity to homogeneity and identifying it as SLF (77). Soluble SLF was shown to promote the survival and proliferation of peritoneal mast cells from normal mice, and to maintain the berberine sulfate/presumptive heparin-containing phenotype of these cells (90). SLF also promoted the survival and proliferation of cytokine-dependent bone marrow-derived mast cells, even in the

absence of cytokines. However, even though they proliferated, after 2 weeks in culture with soluble SLF, the bone marrow-derived mast cells did not become berberine sulfate positive. In contrast, Tsai *et al.* (109) reported recently that soluble rat SLF could induce normal mouse bone marrow-derived mast cells to become berberine sulfate positive. Although 4 weeks of culture with the growth factor were required before many of the mast cells had changed their histochemical phenotype, some berberine sulfate staining was apparent as early as 1 week in culture. Inasmuch as the mouse and rat soluble SLF cDNA share 95% identity (79), it is likely that soluble mouse SLF will also induce mast cell differentiation if given enough time.

Soluble rat SLF injected subcutaneously into *Sl/Sl*^{d} mice for 3 weeks resulted in the differentiation of heparin-containing mast cells at the injection sites, but not at any other locations examined (109, 110). That this mode of administration also reduced the severity of the anemia suggests that the growth factor reached the bone marrow. Therefore, given the widespread dissemination of SLF in this model in concentrations sufficient to bring about erythrocyte differentiation, it remains to be demonstrated why mast cell numbers at distant sites were not also increased.

Soluble recombinant human SLF was found to be about 800-fold less active at stimulating proliferation of mouse mast cells lines than its rat counterpart (103). In addition, human SLF was incapable of inducing *in vitro* colony formation from human bone marrow cells unless a cofactor, such as a human colony-stimulating factor or erythropoietin, was present (103). However, no mention was made of the presence of mast cells in the colonies. Therefore, precisely how important SLF is in humans is presently unclear.

In summary, aside from being a critical hemopoietin for erythrocytes, SLF also appears to be an important mast cell growth factor in the mouse, in which it promotes the survival and proliferation of naturally occurring peritoneal mast cells, mast cells developed *in vitro* from bone marrow in the presence of cytokines, and mast cell lines. It also appears to be involved in mast cell differentiation and the production of heparin, since when injected into the skin of mast cell deficient *Sl/Sl*^{d} mice, heparin-containing mast cells develop at the injection sites and, when added to the culture medium of bone marrow-derived mast cells, they become heparin producers. Yet we still have much to learn about it. For example, there is still no evidence that SLF can by itself directly activate mast cell differentiation from a genuine precursor. Indeed, the bone marrow data in all three species examined seems to argue that it is not an inducer of mast cell differentiation from this population of cells. Hence, other factors known or unknown are likely to be involved. Finally, the full extent of the mast cell phenotype induced by SLF needs to be determined, including granule constituents produced and functional capabilities of the differentiated cells.

Other Mutant Mice with Unusual Numbers of Mast Cells

The *W* and *Sl* loci of the mouse genome are not the only ones linked so far with alterations in mast cell numbers. The patch (*Ph*), microphthalmia (*mi*), and asebia (*ab*) loci have also been found to influence the abundance of mast cells in the tissues. Therefore, they may represent models deserving further attention.

Patch is a semidominant, white spotting locus on Chromosome 5 closely linked with *W* (111). Alone it has little effect on mast cell presence, but when combined as a double heterozygote with a *W* allele (*W*/+, +/*Ph*), it leads to substantial increases in mast cell numbers in the gut and spleen compared with heterozygotes (*W*/+, +/*Ph*) or wild-type homozygotes (+/+) (112). These results are intriguing because of the apparent preferential effects on mucosal type mast cells. They suggest the presence of microenvironmental regulatory factors within the gut and spleen.

The microphthalmia locus is semidominant, located on Chromosome 6, and influences the size and pigmentation of the eyes, the coat color, secondary resorption of bone leading to osteopetrosis, and numbers of mast cells at various sites (111). Both heterozygotes and homozygotes show unusual numbers of mast cells in the spleen, gut, and skin (112–114). The mast cell defect is cell autonomous and interferes with proliferative signals generated by receptor tyrosine kinases (115), thereby affecting signaling through the SLFR pathway. Thus, essentially pure populations of mast cells can be produced by culturing hematopoietic cells of *mi/mi* mice in a cytokine-rich medium. When these mast cells are then co-cultured with 3T3 fibroblasts in the absence of the conditioned medium, they fail to enter the S phase of the cell cycle and eventually die out (114). However, *mi/mi* fibroblast monolayers support the survival and proliferation of mast cells derived from normal littermates. Therefore, the defect is not with the fibroblasts, but rather with the mast cells themselves. It is intriguing that these results are similar to those found with *W*/*W*^{v} mouse mast cells and fibroblasts, even though there is only partial overlap in the range of defects caused by the two types of mutations. Presumably, bone marrow transplants from +/+ littermates should cure the *mi/mi* mast cell defect.

The c-*fms* gene is closely related to the c-*kit* proto-oncogene of the *W* locus and, therefore, like it, encodes a cell surface-associated receptor with an intracellular tyrosine kinase domain (115). Its ligand is colony-stimulating factor-1 (or macrophage colony-stimulating factor). Following transfection of the c-*fms* gene into cultured cytokine-dependent *mi/mi* and *W*/*W*^{v} mouse

mast cells (115), both kinds of cells proliferate in culture medium containing IL-3. However, when IL-3 is omitted and CSF-1 is present, only the transfected mast cells derived from W/W^v mice survive. Thus, the *mi* defect interferes with proliferative signals generated by receptor tyrosine kinases, but not those initiated by IL-3. How this happens is not yet clear. Perhaps the *mi* locus gene encodes another cell surface-associated protein important for transducing the signal to proliferate, or possibly the *mi* protein acts in the pathway leading to cell division at a point distal to the signal generated by ligand interaction with the receptor tyrosine kinases. In any event, this model should prove useful in identifying the critical initial steps involved in activating mast cells for survival and proliferation through the SLFR.

Asebia is a recessive gene localized to Chromosome 19 (111). A double dose of mutant alleles (*ab/ab*) results in mice nearly devoid of body hair, with dry, slightly scaly skin due to underdeveloped sebaceous glands, chronic epidermal hyperproliferation, dermal inflammation, and a 20-fold higher density of dermal mast cells than in normal mice (116, 117). The phenotype of these mast cells and the factors involved in their proliferation await future investigation.

Functional Capabilities of Mast Cells Are Regulated by Fibroblasts

Most of the information gathered so far about the effects of microenvironmental factors on mast cells is concerned with the acquisition or loss of synthetic capabilities, that is, the capacity of mast cells to produce their granule and cell surface constituents. Because a major focus of our work lies in dissecting the biochemical events involved in mast cell and basophil activation, we have become interested in determining whether microenvironmental factors could also affect the functional repertoire of mast cells. Over the years, we have identified a number of molecules in mast cells that play a role in cell signaling via the surface receptor with high affinity for IgE (118–120). The cell model for much of this work has been the rat basophilic leukemia (RBL-2H3) cell, a transformed line closely resembling intestinal mucosal mast cells (121). Thus, like intestinal mucosal mast cells, RBL-2H3 cells are unresponsive to most of the nonimmunologic stimuli tested so far (122, 123). Given our interests in the functional activation of mast cells, we wanted to see if we could determine whether the microenvironment, specifically fibroblasts, was capable of regulating the functional capabilities of mast cells.

Co-cultures of mouse 3T3 fibroblasts and RBL-2H3 cells were established. At various time points, the RBL-2H3 cells in co-culture (and control RBL-2H3 cells grown by themselves) were tested for responsiveness to the classic peritoneal mast cell secretagogue, Compound 48/80 (124; Swieter, Midura, Oliver, Nishikata, Berenstein, Hascall, Mergenhagen, and Siraganian, submitted for publication), and also to neuropeptides such as Substance P (unpublished). What we found was that not only did the co-cultured RBL-2H3 cells become sensitive to the agents, but the response was specific, noncytotoxic, dose-dependent, and, in the case of Compound 48/80, could even occur in the absence of extracellular Ca^{2+}. Moreover, cell to cell contact between the RBL-2H3 cells and fibroblasts was required and acquisition of the new functional capabilities were accompanied by a concomitant shift in proteoglycan synthesis from primary chondroitin sulfates toward more heparan sulfates.

Because the features of the fibroblast-induced alterations in functional phenotype of the RBL-2H3 cells suggest the involvement of SLF and its receptor, we are actively exploring this possibility. If SLF is found to be responsible, we will have shown that it induces not only changes in mediator content in mast cells, but changes in functional repertoire as well. If no link with SLF can be made, then we will be in search of another microenvironmental factor capable of influencing mast cell form and function.

1. Ehrlich P. Beiträge zur Theorie und Praxis der histologischen Färbung. Thesis. University of Leipzig. 1878. In: Himmelweit R, Marquardt M, Dale H, Eds. The Collected Papers of Paul Ehrlich. London: Pergamon Press, Vol 1: pp29–64 (original German) and 65–98 (English translation), 1956.
2. Galli SJ. New insights into "the riddle of the mast cells": Microenvironmental regulation of mast cell development and phenotypic heterogeneity. Lab Invest **62**:5–33, 1990.
3. Lee TDG, Swieter M, Befus AD. Mast cell responses to helminth infection. Parasitol Today **2**:186–191, 1986.
4. Lee TDG, Swieter M, Bienenstock J, Befus AD. Heterogeneity in mast cell populations. Clin Immunol Rev **4**:143–199, 1985.
5. Reynolds DS, Stevens RL, Lane WS, Carr MH, Austen KF, Serafin WE. Different mouse mast cell populations express various combinations of at least six distinct mast cell serine proteases. Proc Natl Acad Sci USA **87**:3230–3234, 1990.
6. Swieter M, Chan BMC, Rimmer C, McNeill K, Froese A, Befus D. Isolation and characterization of IgE receptors from rat intestinal mucosal mast cells. Eur J Immunol **19**:1879–1885, 1989.
7. Kitamura Y, Shimada M, Hatanaka K, Miyano Y. Development of mast cells from grafted bone marrow cells in irradiated mice. Nature **268**:442–443, 1977.
8. Kitamura Y, Shimada M, Go S. Presence of mast cell precursors in fetal liver. Dev Biol **70**:510–514, 1979.
9. Kitamura Y, Shimada M, Go S, Matsuda H, Hatanaka K, Seki M. Distribution of mast-cell precursors in hematopoietic and lymphopoietic tissues of mice. J Exp Med **150**:482–490, 1979.
10. Irani AMA, Bradford TR, Kepley CL, Schechter NM, Schwartz LB. Detection of MC^T and MC^{TC} types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. J Histochem Cytochem **17**:1509–1515, 1989.
11. Church MK, Lowman MA, Robinson C, Holgate ST, Benyon RC. Interaction of neuropeptides with human mast cells. Int Arch Allergy Appl Immunol **88**:70–78, 1989.
12. Nakano T, Sonoda T, Hayashi C, Yamatodani A, Kanayama

Y, Yamamura T, Asai H, Yonezawa T, Kitamura Y, Galli SJ. Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal, and intravenous transfer into genetically mast cell-deficient W/W^v mice. J Exp Med **162**:1085–1043, 1985.
13. Kobayashi T, Nakano T, Nakahata T, Asai H, Yagi Y, Tsuji K, Komiyama A, Akabane T, Kojima S, Kitamura Y. Formation of mast cell colonies in methylcellulose by mouse peritoneal cells and differentiation of these cloned cells in both the skin and the gastric mucosa of W/W^v mice: Evidence that a common precursor can give rise to both "connective tissue-type" and "mucosal" mast cells. J Immunol **136**:1378–1384, 1986.
14. Sonoda S, Sonoda T, Nakano T, Kanayama Y, Kanakura Y, Asai H, Yonezawa T, Kitamura Y. Development of mucosal mast cells after injection of a single connective tissue-type mast cell in the stomach mucosa of genetically mast cell-deficient W/W^v mice. J Immunol **137**:1319–1322, 1986.
15. Kanakura Y, Thompson H, Nakano T, Yamamura TI, Asai H, Kitamura Y, Metcalfe DD, Galli SJ. Multiple bidirectional alterations of phenotype and changes in proliferative potential during the in vitro and in vivo passage of clonal mast cell populations derived from mouse peritoneal mast cells. Blood **72**:877–885, 1989.
16. Schrader JW. The panspecific hemopoietin of activated lymphocytes (interleukin-3). Annu Rev Immunol **4**:205–230, 1986.
17. Ihle JN, Weinstein Y. Immunological regulation of hematopoietic/lymphoid stem cell differentiation by interleukin 3. Adv Immunol **39**:1–50, 1986.
18. Niemeyer CM, Sieff CA, Mathey-Prevot B, Wimperis JZ, Bierer BE, Clark SC, Nathan DG. Expression of human interleukin-3 (multi-CSF) is restricted to human lymphocytes and T-cell tumor lines. Blood **73**:945–951, 1989.
19. Oster W, Lindemann A, Merlelsmann R, Herrmann F. Production of macrophage-, granulocyte-, granulocyte-macrophage and multi-colony-stimulating factor by peripheral blood cells. Eur J Immunol **19**:543–547, 1989.
20. Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordan RP, Paul WE. Mast cell lines produce lymphokines in response to cross-linkage of FcERI or to calcium ionophores. Nature **339**:64–67, 1989.
21. Wodnar-Filipowicz A, Heusser CH, Moroni C. Production of the haemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. Nature **339**:150–152, 1989.
22. Burd PR, Rogers HW, Gordon JR, Martin CA, Jayaraman S, Wilson SD, Dvorak AM, Galli SJ, Dorf ME. Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. J Exp Med **170**:245–257, 1989.
23. Razin E, Leslie K, Schrader JW. Connective tissue mast cells in contact with fibroblasts express IL-3 mRNA. Analysis of single cells by polymerase chain reaction. J Immunol **146**:981–987, 1991.
24. Gordon JR, Burd PR, Galli SJ. Mast cells as a source of multifunctional cytokines. Immunol Today **11**:458–464, 1990.
25. Luger TA, Kock A, Kirnbauer R, Schwarz T, Ansel JC. Keratinocyte-derived interleukin 3. Ann NY Acad Sci **548**:253–261, 1988.
26. Farrar WL, Vinocour M, Hill JM. *In situ* hybridization histochemistry localization of interleukin-3 mRNA in mouse brain. Blood **73**:137–140, 1989.
27. Ruitenberg EJ, Elgersma A. Absence of intestinal mast cell response in congenitally athymic mice during a *Trichinella spiralis* infection. Nature **264**:258–260, 1976.
28. Olson CE, Levy DA. Thymus-dependency of the mast cell response to *Nippostrongylus brasiliensis* in mice. Fed Proc **35**:491, 1976.
29. Abe T, Ochiachi H, Minamishima Y, Nawa Y. Induction of intestinal mastocytosis in nude mice by repeated injection of interleukin-3. Int Arch Allergy Appl Immunol **86**:356–358, 1988.
30. Madden KB, Urban JF Jr, Ziltener HJ, Schrader JS, Finkelman FD, Katona IM. Antibodies to IL-3 and IL-4 suppress helminth-induced intestinal mastocytosis. FASEB J **5**:A1012, 1991.
31. Lebman DA, Coffman RL. Interleukin 4 causes isotype switching to IgE in T cell-stimulated clonal B cell cultures. J Exp Med **168**:853–862, 1988.
32. Snapper CM, Finkelman FD, Paul WE. Regulation of IgG1 and IgE production by interleukin 4. Immunol Rev **102**:51–75, 1988.
33. Smith CA, Rennick DM. Characterization of a murine lymphokine distinct from interleukin 2 and interleukin 3 (IL-3) possessing a T-cell growth factor activity and a mast-cell growth factor activity that synergizes with IL-3. Proc Natl Acad Sci USA **83**:1857–1861, 1986.
34. Lee F, Yokota T, Otsuka T, Meyerson P, Villaret D, Coffman R, Mosmann T, Rennick D, Roehm N, Smith C, Zlotnik A, Arai KI. Isolation and characterization of a mouse interleukin cDNA clone that expresses B-cell stimulatory factor 1 activities and T-cell- and mast-cell-stimulating activities. Proc Natl Acad Sci USA **83**:2061–2065, 1986.
35. Schmitt E, Fassbender B, Beyreuther K, Spaeth E, Schwarzkopf R, Rude E. Characterization of a T cell-derived lymphokine that acts synergistically with IL3 on the growth of murine mast cells and is identical with IL4. Immunobiology **174**:406–419, 1987.
36. Nakahata T, Kobayashi T, Ishiguro A, Tsuji K, Naganuma K, Ando O, Yagi Y, Tadokoro K, Akabane T. Extensive proliferation of mature connective-tissue type mast cells *in vitro*. Nature **324**:65–67, 1986.
37. Hamaguchi Y, Kanakura Y, Fujita J, Takeda SI, Nakano T, Tarui S, Honjo T, Kitamura Y. Interleukin 4 as an essential factor for in vitro clonal growth of murine connective tissue-type mast cells. J Exp Med **165**:268–273, 1987.
38. Takagi M, Nakahata T, Koike K, Kobayashi T, Tsuji K, Kojima S, Hirano T, Miyajima A, Arai KI, Akabane T. Stimulation of connective tissue-type mast cell proliferation by crosslinking of cell-bound IgE. J Exp Med **170**:233–244, 1989.
39. Tsuji K, Nakahata T, Takagi M, Kobayashi T, Ishiguro A, Kikuchi T, Naganuma K, Koike K, Miyajima A, Arai KI, Akabane T. Synergistic action of phorbol ester and IL-3 in the induction of "connective tissue-type" mast cell proliferation. J Immunol **144**:678–684, 1990.
40. Tsuji K, Nakahata T, Takagi M, Kobayashi T, Ishiguro A, Kikuchi T, Naganuma K, Koike K, Miyajima A, Arai KI, Akabane T. Effects of interleukin-3 and interleukin-4 on the development of "connective tissue-type" mast cells: Interleukin-3 supports their survival and interleukin-4 supports their proliferation synergistically with interleukin-3. Blood **75**:421–427, 1990.
41. Saito H, Hatake K, Dvorak AM, Leiferman KM, Donnenberg AD, Arai N, Ishizaka K, Ishizaka T. Selective differentiation and proliferation of hematopoietic cells induced by recombinant human interleukins. Proc Natl Acad Sci USA **85**:2288–2292, 1988.
42. Favre C, Saeland S, Caux C, Cuvert V, De Vries JE. Interleukin-4 has basophilic and eosinophilic cell growth-promoting activity on cord blood cells. Blood **75**:67–73, 1990.
43. Hultner L, Moeller J, Schmitt E, Jager G, Reisbach G, Ring J, Dormer P. Thiol-sensitive mast cell lines derived from mouse bone marrow respond to a mast cell growth-enhancing activity different for both IL-3 and IL-4. J Immunol **142**:3440–3446, 1989.
44. Moeller J, Hultner L, Schmitt E, Dormer P. Partial purification

of a mast cell growth-enhancing activity and its separation from IL-3 and IL-4. J Immunol **142**:3447–3451, 1989.
45. Hultner L, Druez C, Moiller J, Uyttenhove C, Schmitt E, Rude E, Dormer P, Van Snick J. Mast cell growth-enhancing activity (MEA) is structurally related and functionally identical to the novel mouse T cell growth factor P40/TCGFIII (interleukin 9). Eur J Immunol **20**:1413–1416, 1990.
46. Mosmann TR, Bond MW, Coffman RL, Ohara J, Paul WE. T-cell and mast cell lines respond to B-cell stimulatory factor 1. Proc Natl Acad Sci USA **83**:5654–5658, 1986.
47. Uyttenhove C, Simpson RJ, Van Snick J. Functional and structural characterization of P40, a mouse glycoprotein with T-cell growth factor activity. Proc Natl Acad Sci USA **85**:6934–6938, 1988.
48. Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV: Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med **170**:2081–2095, 1989.
49. Moore KW, Vieira P, Fiorentin D, Trounstine M, Khan T, Mosmann T. Homology of the cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRFI. Science **248**:1230–1234, 1990.
50. O'Garra A, Stapleton G, Dhar V, Pearce M, Schumacher J, Rugo H, Barbis D, Stall A, Cupp J, Moore K, Vieira P, Mosmann T, Whitmore A, Arnold L, Haughton G, Howard M. Production of cytokines by mouse B cells: B lymphomas and normal B cells produce interleukin 10. Int Immunol **2**:821–832, 1990.
51. Thompson-Snipes L, Dhar V, Bond MW, Mosmann TR, Moore KW, Rennick DM. Interleukin 10: A novel stimulatory factor for mast cells and their progenitors. J Exp Med **173**:507–510, 1991.
52. Czarnetzki BM, Hannich D, Niedorf H. In vitro studies on the development of rat peritoneal mast cells. Immunobiology **156**:470–476, 1979.
53. Czarnetzki BM, Behrendt H. Studies on the in vitro development of rat peritoneal mast cells. Immunobiology **159**:256–268, 1981.
54. Czarnetzki BM, Sterry W, Bazin H, Kalveram KJ. Evidence that tissue mast cells derive from mononuclear phagocytes. Int Arch Allergy Appl Immunol **67**:44–48, 1982.
55. Czarnetzki BM, Kruger G, Sterry W. In vitro generation of mast cell-like cells from human peripheral mononuclear phagocytes. Int Arch Allergy Appl Immunol **71**:161–167, 1983.
56. Furitsu T, Saito H, Dvorak AM, Schwartz LB, Irani AMA, Burdick JF, Ishizaka K, Ishizaka T. Development of human mast cells *in vitro*. Proc Natl Acad Sci USA **86**:10039–10043, 1989.
57. Nafziger J, Arock M, Guillosson JJ, Wietzerbin J. Specific high-affinity receptors for interferon-γ on mouse bone marrow-derived mast cells: Inhibitory effect of interferon-γ on mast cell precursors. Eur J Immunol **20**:113–117, 1990.
58. Takagi M, Koike K, Nakahata T. Antiproliferative effect of IFN-γ on proliferation of mouse connective tissue-type mast cells. J Immunol **145**:1880–1884, 1990.
59. Kanakura Y, Kuriu A, Waki N, Nakano T, Asai H, Yonezawa T, Kitamura Y. Changes in numbers and types of mast cell colony-forming cells in the peritoneal cavity of mice after injection of distilled water. Evidence that mast cells suppress differentiation of bone marrow-derived precursors. Blood **71**:573–580, 1988.
60. Waki N, Kitamura Y, Kanakura Y, Asai H, Nakano T. Intraperitoneally injected cultured mast cells suppress recruitment and differentiation of bone marrow-derived mast cell precursors in the peritoneal cavity of W/W^v mice. Exp Hematol **18**:243–247, 1990.
61. Russell ES. Hereditary anemias of the mouse: A review for geneticists. Adv Genet **20**:357–459, 1979.
62. Silvers WK. The Coat Colors of Mice: A Model for Gene Action and Interaction. New York: Springer-Verlag, 1979.
63. Gregory CJ, Eaves AC. Three stages of erythropoietic progenitor cell differentiation distinguished by a number of physical and biologic properties. Blood **51**:527–537, 1978.
64. Kitamura Y, Go S, Hatanaka K. Decrease of mast cells in W/W^v mice and their increase by bone marrow transplantation. Blood **52**:447–452, 1978.
65. Kitamura Y, Go S. Decreased production of mast cells in Sl/Sl^d anemic mice. Blood **53**:492–497, 1979.
66. Geissler EN, Russell ES. Analysis of the hematopoietic effects of new dominant spotting (W) mutations of the mouse. II. Effects on mast cell development. Exp Hematol **11**:461–466, 1983.
67. Crapper RM, Schrader JW. Frequency of mast cell precursors in normal tissues determined by an *in vitro* assay: Antigen induces parallel increases in the frequency of P cell precursors and mast cells. J Immunol **131**:923–928, 1983.
68. Suda T, Suda J, Spicer SS, Ogawa M. Proliferation and differentiation in culture of mast cell progenitors derived from mast cell-deficient mice of genotype W/W^v. J Cell Physiol **122**:187–192, 1985.
69. Sakoda H, Mori KJ. Distribution and differentiation of murine mast cell progenitors determined with soft agar culture. Exp Hematol **17**:791–794, 1989.
70. Kanakura Y, Sonoda S, Nakano T, Fujita J, Kuriu A, Asai H, Kitamura Y. Formation of mast-cell colonies in methylcellulose by mouse skin cells and development of mucosal-like mast cells from the cloned cells in the gastric mucosa of W/W^v mice. Am J Pathol **129**:168–176, 1987.
71. Fujita J, Onoue H, Ebi Y, Nakayama H, Kanakura Y. *In vitro* duplication and in vivo cure of mast-cell deficiency of Sl/Sl^d mutant mice by cloned 3T3 fibroblasts. Proc Natl Acad Sci USA **86**:2888–2891, 1989.
72. Hayashi C, Sonoda T, Nakano T, Nakayama H, Kitamura Y. Mast-cell precursors in the skin of mouse embryos and their deficiency in embryos of Sl/Sl^d genotype. Dev Biol **109**:234–241, 1985.
73. Chabot B, Stephenson DA, Chapman VM, Besmer P, Bernstein A. The proto-oncogene c-*kit* encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. Nature **335**:88–89, 1988.
74. Geissler EN, Ryan MA, Housman DE. The dominant-white spotting (W) locus of the mouse encodes the c-*kit* proto-oncogene. Cell **55**:185–192, 1988.
75. Majumder S, Brown K, Qiu FH, Besmer P. c-*kit* Protein, a transmembrane kinase: Identification in tissues and characterization. Mol Cell Biol **8**:4896–4903, 1988.
76. Copeland NG, Gilbert DJ, Cho BC, Donovan PJ, Jenkins NA, Cosman D, Anderson D, Lyman SD, Williams DE. Mast cell growth factor maps near the Steel locus on mouse chromosome 10 and is deleted in a number of Steel alleles. Cell **63**:175–183, 1990.
77. Huang E, Nocka K, Beier DR, Chu TY, Buck J, Lahm HW, Wellner D, Leder P, Besmer P. The hematopoietic growth factor KL is encoded by the Sl locus and is the ligand of the c-*kit* receptor, the gene product of the W locus. Cell **63**:225–233, 1990.
78. Williams DE, Eisenman J, Baird A, Rauch C, Van Ness K, March CJ, Park LS, Martin U, Mochizuki DY, Boswell HS, Burgess GS, Cosman D, Lyman SD. Identification of a ligand for the c-*kit* proto-oncogene. Cell **63**:167–174, 1990.
79. Zsebo KM, Williams DA, Geissler EN, Broudy VC, Martin FH, Atkins HL, Hsu RY, Birkett NC, Okino KH, Murdock DC,

Jacobsen FW, Langley KE, Smith KA, Takeishi T, Cattanacj BM, Galli SJ, Suggs SV. Stem cell factor is encoded at the *Sl* locus of the mouse and is the ligand for the c-*kit* tyrosine kinase receptor. Cell **63**:213–224, 1990.
80. Nocka K, Majumder S, Chabot B, Ray P, Cervone M, Bernstein A, Besmer P. Expression of c-*kit* gene products in known cellular targets of *W* mutations in normal and *W* mutant mice—evidence for an impaired c-*kit* kinase in mutant mice. Genes Dev **3**:816–826, 1989.
81. Nocka K, Tan JC, Chiu E, Chu TY, Ray P, Traktman P, Besmer P. Molecular bases of dominant negative and loss of function mutations at the murine c-*kit*/white spotting locus: W^{37}, W^{v}, W^{41} and *W*. EMBO J **9**:1805–813, 1990.
82. Tan JC, Nocka K, Ray P, Traktman P, Besmer P. The dominant W^{42} spotting phenotype results from a missense mutation in the c-kit receptor kinase. Science **247**:209–212, 1990.
83. Reith AD, Rottapel R, Giddens E, Brady C, Rorrester L, Bernstein A. *W* mutant mice with mild or severe developmental defects contain distinct point mutations in the kinase domain of the c-*kit* receptor. Genes Dev **4**:390–400, 1990.
84. Flanagan JG, Chan DC, Leder P. Transmembrane form of the *kit* ligand growth factor is determined by alternative splicing and is missing in the Sl^{d} mutant. Cell **64**:1025–1035, 1991.
85. Andre C, d'Auriol L, Lacombe C, Gisselbrecht S, Galibert F. c-*kit* Expression in human and murine hematopoietic cell lines. Oncogene **4**:1047–1049, 1989.
86. Welham MJ, Schrader JW. Modulation of c-*kit* mRNA and protein by hemopoietic growth factors. Mol Cell Biol **11**:2901–2904, 1991.
87. Yarden Y, Kuang WJ, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, Chen E, Schlessinger J, Francke U, Ullrich A. Human proto-oncogene c-*kit*: A new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J **6**:3341–3351, 1987.
88. Qiu F, Ray P, Brown K, Barker PE, Jhanwar S, Ruddle FH, Besmer P. Primary structure of c-*kit*: Relationship with the CSF-1/PDGF receptor kinase family—Oncogenic activation of v-*kit* involves deletion of extracellular domain and C terminus. EMBO J **7**:1003–1011, 1988.
89. Zsebo KM, Wypych J, McNiece IK, Lu HS, Smith KA, Karkare SB, Sachdev RK, Yuschenkoff VN, Birkett NC, Williams LR, Satyagal VN, Tung W, Bosselman RA, Mendiaz EA, Langley KE. Identification, purification, and biological characterization of hematopoietic stem cell factor from Buffalo rat liver-conditioned medium. Cell **63**:195–201, 1990.
90. Nocka K, Buck J, Levi E, Besmer P. Candidate ligand for the c-*kit* transmembrane kinase receptor: KL, a fibroblast derived growth factor stimulates mast cells and erythroid progenitors. EMBO J **9**:3287–3294, 1990.
91. Witte ON. Steel locus defines new multi-potent growth factor. Cell **63**:5–6, 1990.
92. Schmitt RM, Bruyns E, Snodgrass HR. Hematopoietic development of embryonic stem cells in vitro: Cytokine and receptor gene expression. Genes Dev **5**:728–740, 1991.
93. Matsui Y, Zsebo KM, Hogan BLM. Embryonic expression of a haematopoietic growth factor encoded by the *Sl* locus and the ligand for c-kit. Nature **347**:667–669, 1990.
94. Levi-Schaffer F, Austen KF, Caulfield JP, Hein A, Bloes WF, Stevens RL. Fibroblasts maintain the phenotype and viability of the rat heparin-containing mast cell in vitro. J Immunol **135**:3454–3462, 1985.
95. Levi-Schaffer F, Austen KF, Caulfield JP, Hein A, Gravallese PM, Stevens RL. Co-culture of human lung-derived mast cells with mouse 3T3 fibroblasts: Morphology and IgE-mediated release of histamine, prostaglandin D_2, and leukotrienes. J Immunol **139**:494–500, 1987.
96. Levi-Schaffer L, Austen KF, Gravallese PM, Stevens RL. Co-culture of interleukin 3-dependent mouse mast cells with fibroblasts results in a phenotypic change of the mast cells. Proc Natl Acad Sci USA **83**:6485–6488, 1986.
97. Fujita J, Nakayama H, Onoue H, Kanakura Y, Nakano T, Asai H, Takeda SI, Honjo T, Kitamura Y. Fibroblast-dependent growth of mouse mast cells in vitro: Depletion in mutant mice of W/W^{v} genotype. J Cell Physiol **134**:78–84, 1988.
98. Fujita J, Nakayama H, Onoue H, Ebi Y, Kanakura Y, Kuriu A, Kitamura Y. Failure of W/W^{v} mouse-derived cultured mast cells to enter S phase upon contact with NIH/3T3 fibroblasts. Blood **72**:463–468, 1988.
99. Flanagan JG, Leder P. The *kit* ligand: A cell surface molecule altered in Steel mutant fibroblasts. Cell **63**:185–194, 1990.
100. Duvall E, Wyllie AH. Death and the cell. Immunol Today **7**:115–119, 1986.
101. Ody C, Kindler V, Vassalli P. Interleukin 3 perfusion in W/W^{v} mice allows the development of macroscopic hematopoietic spleen colonies and restores cutaneous mast cell number. J Exp Med **172**:403–406, 1990.
102. Galli SJ, Arizono N, Murakami T, Dvorak AM, Fox JG. Development of large numbers of mast cells at sites of idiopathic chronic dermatitis in genetically mast cell-deficient WBB6F1-W/W^{v} mice. Blood **69**:1661–1666, 1987.
103. Martin FH, Suggs SV, Langley KE, Lu HS, Ting J, Okino KH, Morris CF, McNiece IK, Jacobsen FW, Mendiaz EA, Birkett NC, Smith KA, Johnson MJ, Parker VP, Flores JC, Patel AC, Fisher EF, Erjavec HO, Jerrera CJ, Wypych J, Sachdev RK, Pope JA, Leslie I, Wen D, Lin CH, Cupples RL, Zsebo KM. Primary structure and functional expression of rat and human stem cell factor DNAs. Cell **63**:203–211, 1990.
104. Anderson DM, Lyman SD, Baird A, Wignall JM, Eisenman J, Rauch C, March CJ, Boswell HS, Gimpel SD, Cosman D, Williams DE. Molecular cloning of mast cell growth factor, A hematopoietin that is active in both membrane bound and soluble forms. Cell **63**:235–243, 1990.
105. Ryan JJ, Huff TF. Schwann cell-conditioned medium supports development of mast cell-committed progenitors. FASEB J **5**:A1006, 1991.
106. Levi-Schaffer F, Bar-Shavit Z. Osteoblast-like cell line maintains *in vitro* rat peritoneal mast cell viability and functional activity. Immunology **69**:145–149, 1990.
107. Jarboe DL, Huff TF. The mast cell-committed progenitor. II: W/W^{v} mice do not make mast cell-committed progenitors and Sl/Sl^{d} fibroblasts do not support development of normal mast cell-committed progenitors. J Immunol **142**:2418–2423, 1989.
108. Jarboe DL, Marshall JS, Randolph TR, Kukolja A, Huff TF. The mast cell-committed progenitor. I: Description of a cell capable of IL-3-independent proliferation and differentiation without contact with fibroblasts. J Immunol **142**:2405–2417, 1989.
109. Tsai M, Takeishi T, Thompson H, Langley KE, Zsebo KM, Metcalfe DD, Geissler EN, Galli SJ. Induction of mast cell proliferation, maturation, and heparin synthesis by the rat c-kit ligand, stem cell factor. Proc Natl Acad Sci USA **88**:6382–6386, 1991.
110. Tsai M, Shih LS, Newlands GFJ, Takeishi T, Langley KE, Zsebo KM, Miller HRP, Geissler EN, Galli SJ. 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 **174**:125–131, 1991.
111. Lyon MF, Searle AG (Eds). Genetic Variants and Strains of the Laboratory Mouse, 2nd ed. Oxford: Oxford University Press, 1989.
112. Stevens J, Loutit JF. Mast cells in spotted mutant mice (*W Ph mi*). Proc R Soc Lond [Biol] **215**:405–409, 1982.

13. Stechschulte DJ, Sharma R, Dileepan KN, Simpson KM, Aggarwal N, Clancy J Jr, Jilka RL. Effect of the *mi* allele on mast cells, basophils, natural killer cells, and osteoclasts in C57B1/6J mice. J Cell Physiol **132**:565–570, 1987.
14. Ebi Y, Kasugai T, Seino Y, Onoue H, Kanemoto T, Kitamura Y. Mechanism of mast cell deficiency in mutant mice of *mi/mi* genotype: An analysis by co-culture of mast cells and fibroblasts. Blood **75**:1247–1251, 1990.
15. Dubreuil P, Forrester L, Rottapel R, Reeduk M, Fujita J, Bernstein A. The c-fms gene complements the mitogenic defect in mast cells derived from mutant *W* mice but not *mi* (microphthalmia) mice. Proc Natl Acad Sci USA **88**:2341–2345, 1991.
16. Brown WR, Hardy MH. A hypothesis on the cause of chronic epidermal hyperproliferation in *asebia* mice. Clin Exp Dermatol **13**:74–77, 1988.
17. Josefowicz WJ, Hardy MH. The expression of the gene *asebia* in the laboratory mouse. 3: Sebaceous glands. Genet Res **31**: 157–166, 1978.
18. Shimizu A, Tepler I, Benfey PN, Berenstein EH, Siraganian RP, Leder P. Human and rat mast cell high-affinity immunoglobulin E receptors: Characterization of putative α-chain gene products. Proc Natl Acad Sci USA **85**:1907–1911, 1988.
19. Benhamou M, Gutkind JS, Robbins KC, Siraganian RP. Tyrosine phosphorylation coupled to IgE receptor-mediated signal transduction and histamine release. Proc Natl Acad Sci USA **87**:5327–5330, 1990.
20. Kitani S, Berenstein E, Mergenhagen S, Tempst P, Siraganian RP. A cell surface glycoprotein of rat basophilic leukemia cells close to the high affinity IgE receptor (FcϵRI). Similarity to human melanoma differentiation antigen ME491. J Biol Chem **266**:1903–1909, 1991.
21. Seldin DC, Adelman S, Austen KF, Stevens RL, Hein A, Caulfield JP, Woodbury RG. Homology of the rat basophilic leukemia cell and the rat mucosal mast cell. Proc Natl Acad Sci USA **82**:3871–3875, 1985.
22. Shanahan F, Denburg JA, Fox J, Bienenstock J, Befus AD. Mast cell heterogeneity: Effects of neurenteric peptides on histamine release. J Immunol **135**:1331–1337, 1985.
23. Siraganian RP, Metzger H. Evidence that the "mouse mastocytoma" cell line (MCT-1) is of rat origin. J Immunol **121**:2584–2585, 1978.
24. Swieter M, Midura R, Oliver C, Berenstein E, Hascall V, Mergenhagen SE, Siraganian RP. Rat basophilic leukemia (RBL-2H3) cells acquire responsiveness to Compound 48/80 by coculture with 3T3 fibroblasts. FASEB J **5**:A1085, 1991.