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

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Summary

Mast cell development is a complex process that results in the appearance of phenotypically distinct populations of mast cells in different anatomical sites. Mice homozygous for mutations at the *W* or *Sl* locus exhibit several phenotypic abnormalities, including a virtual absence of mast cells in all organs and tissues. Recent work indicates that *W* encodes the c-*kit* tyrosine kinase receptor, whereas *Sl* encodes a c-*kit* ligand that we have designated stem cell factor (SCF). Recombinant or purified natural forms of the c-*kit* ligand induce proliferation of certain mast cell populations in vitro, and injection of recombinant SCF permits mast cells to develop in mast cell–deficient WCB6F$_1$-*Sl*/*Sl*^{d} mice. However, the effects of SCF on mast cell proliferation, maturation, and phenotype in normal mice in vivo were not investigated. We now report that local administration of SCF in vivo promotes the development of connective tissue–type mast cells (CTMC) in the skin of mice and that systemic administration of SCF induces the development of both CTMC and mucosal mast cells (MMC) in rats. Rats treated with SCF also develop significantly increased tissue levels of specific rat mast cell proteases (RMCP) characteristic of either CTMC (RMCP I) or MMC (RMCP II). These findings demonstrate that SCF can induce the expansion of both CTMC and MMC populations in vivo and show that SCF can regulate at least one cellular lineage that expresses c-*kit*, the mast cell, through complex effects on proliferation and maturation.

Studies in mice and rats indicate that mast cells are derived from hematopoietic precursors that arise in the bone marrow and circulate in the blood, but complete their program of differentiation and maturation within interstitial tissues, epithelia, or serosal cavities (reviewed in references 1–6). In murine rodents, this process results in the generation of at least two distinct mast cell populations that vary in many aspects of phenotype, the connective tissue–type mast cells (CTMC)[1], which occur in such sites as the skin and peritoneal cavity, and the mucosal mast cells (MMC), which occur in the mucosa of the gastrointestinal tract (reviewed in references 1–4). Evidence derived from both in vitro and in vivo studies indicates that IL-3 represents one major growth factor for MMC, whereas the regulation of CTMC proliferation requires additional and/or alternative factors (reviewed in references 1–6). For example, in vitro work indicates that one representative CTMC, peritoneal mast cells (PMC), do not proliferate in response to IL-3 alone but divide when IL-3 is provided with a second stimulus such as IL-4 (7) or PMA (8).

Several important insights into mast cell development have been derived from analyses of mice that virtually lack mast cells as a result of a double dose of mutant genes at either the *W* locus on chromosome 5 or the *Sl* locus on chromosome 10 (reviewed in references 1, 2, and 5). For example, *W* or *Sl* mutant mice lack both CTMC and MMC, indicating that the *W* or *Sl* gene products are important in the development of both of these mast cell populations in vivo (reviewed in references 1, 2, 5, and 6). Moreover, both in vitro and in vivo studies suggested that the mast cell deficiencies of these mutant mice reflected, in the case of *W* mutants, defects in the responsiveness of cells in the mast cell

[1] *Abbreviations used in this paper:* BrdU, 5-bromo-2'-deoxyuridine; CTMC, connective tissue–type mast cell; Hct, hematocrit; MMC, mucosal mast cell; PMC, peritoneal mast cell; RMCP, rat mast cell protease; rrSCF^{164}, recombinant rat stem cell factor^{164}.

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lineage to a stromal cell–derived growth factor and, in the case of *Sl* mutants, inadequate production of this growth factor by stromal cells (reviewed in references 1, 2, and 5).

The products encoded at *W* and *Sl* recently have been defined. *W* encodes the c-*kit* tyrosine kinase growth factor receptor (9, 10), whereas *Sl* encodes a newly recognized multifunctional growth factor that represents a ligand for c-*kit* (11–18). Several lines of evidence in addition to the lack of mast cells in *W* or *Sl* mutant mice indicate that one of the important biological activities of the c-*kit* ligand is to regulate mast cell development. The recombinant c-*kit* ligand can promote the proliferation of certain populations of immature, IL-3-dependent mast cells in vitro (11, 14, 15, 17, 18), and the c-*kit* ligand purified from the supernatants of BALB/3T3 fibroblasts (19), or the recombinant ligand (20), can induce proliferation of IL-3-independent mouse PMC in vitro. In addition, we demonstrated that daily subcutaneous injection of a recombinant c-*kit* ligand, recombinant rat stem cell factor^{164} (rrSCF^{164}), for 3 wk permitted mast cells to develop in the skin of genetically mast cell–deficient WCB6F$_1$-*Sl/Sl^d* mice (16). However, the mast cells that developed in WCB6F$_1$-*Sl/Sl^d* mice injected with rrSCF^{164} were not characterized according to phenotype. Nor was it determined whether rrSCF^{164} could influence mast cell development or phenotype in normal mice. In the present study, we therefore examined the effects of rrSCF^{164} on mast cell populations in normal mice and rats in vivo, using approaches that permitted the anatomical and phenotypic characterization of the responding cells as CTMC or MMC. Some of these results have been reported in abstract form (20).

Materials and Methods

Studies in Mice. Groups of five to seven female 8–12-wk-old WCB6F$_1$-+/+, WCB6F$_1$-*Sl/Sl^d*, WBB6F$_1$-+/+, and WBB6F$_1$-*W/W^v* mice (The Jackson Laboratory, Bar Harbor, ME) received for 3 wk a daily subcutaneous injection of rrSCF^{164} purified from *Escherichia coli* and modified by the covalent attachment of polyethylene glycol (16) or vehicle alone (0, 30, or 100 μg rrSCF^{164}/kg in 150–200 μl of sterile saline containing 0.1% BSA) (fraction V, fatty acid free; ICN Immunobiologicals, Lisle, IL). Injections were performed with the mice under light ether anesthesia and were delivered to approximately the same site on the dorsal back skin. Blood for determination of hematocrit (Hct) was obtained from the mice by retroorbital puncture under light ether anesthesia on the day before initiation of treatment and on the day of death. For labeling of tissue mast cells proliferating in vivo (21), 5-bromo-2'-deoxyuridine (BrdU) (Sigma Chemical Co., St. Louis, MO) was injected intraperitoneally (100 mg/kg in sterile saline) 1 h before death. After death by cervical dislocation, the cutaneous injection site was excised and fixed in Carnoy's fixative, and embedded in paraffin. 4-μm sections were cut, placed onto polylysine-coated slides, and processed as in reference 21 for staining of mast cells with 1.0% alcian blue, pH 1.0, and for immunohistochemical staining of BrdU-labeled nuclei, using an anti-BrdU mAb (Becton Dickinson & Co., Mountain View, CA). The slides were examined at 400× to quantify mast cells/mm^2 of dermis (22) and to determine the percent of mast cells positive for BrdU incorporation (21). Other slides of the Carnoy's-fixed specimens were stained with the heparin-binding fluorescent dye berberine sulfate (23) or with alcian blue/safranin, and were examined in an epifluorescent or light microscope, respectively, as previously described (1, 22).

Studies in Rats. Groups of five to eight female 16-wk-old Wistar rats received 14-d daily intravenous injections of either rrSCF^{164} (25 μg/kg/d in 1.0 ml of sterile, pyrogen-free 0.9% NaCl "saline") or 1.0 ml of saline alone. 24 h after the last injection, the rats were killed by exsanguination under deep ether anesthesia, and samples of tissues were fixed in 4.0% paraformaldehyde, processed into paraffin, and assessed in sections stained with 0.5% toluidine blue at pH 0.5 for 2 h (24). Mast cells were quantified at 400×, and the results were expressed as no. (mean ± SEM)/mm^2 of tissue (22). Other samples of the same tissues were processed as described (24) for determination of content of rat mast cell protease I (RMCP I) and RMCP II by ELISA, and the results were expressed as micrograms protease (mean ± SEM)/gram of tissue. The bone marrow cells were harvested from a single femur of each rat by flushing the marrow cavity with 0.9% NaCl and then washing the cells three times with HBSS/5% FCS (HBSS/FCS). The peritoneal lavage cells of each rat were obtained by lavaging each peritoneal cavity with 20 ml of HBSS/FCS and then washing the cells three times with HBSS/FCS. Aliquots of the total femoral bone marrow cells and peritoneal lavage cells were diluted 1:10 in methylene blue/propylene glycol for counting the numbers of total cells and mast cells in each preparation, the remainder of the femoral blue marrow or peritoneal cells were used for determination of RMCP I and II content by ELISA (24).

Results and Discussion

Local Administration of SCF Induces the c-kit-dependent Development of CTMC in the Skin of Mice. Recombinant rat SCF^{164} administered in daily subcutaneous injections for 3 wk induced a striking expansion of dermal mast cell populations in normal WBB6F$_1$-+/+ or WCB6F$_1$-+/+ mice, and in genetically mast cell–deficient WCB6F$_1$-*Sl/Sl^d* mice (Table 1). In WBB6F$_1$-+/+ mice, mast cell numbers at sites injected with 100 or 30 μg of rrSCF^{164}/kg were 166 or 15 times that in control sites injected with vehicle alone. In striking contrast to its effects on populations of dermal mast cells, rrSCF^{164} had little or no effect on the Hct of normal mice of either genotype tested (Table 1).

The majority of the mast cells at rrSCF^{164} injection sites, like dermal CTMC in the skin of untreated normal mice (1, 22), exhibited cytoplasmic reactivity with the heparin-binding fluorescent dye berberine sulfate, and many of them stained with safranin (Fig. 1, *A*, *B*, *D*, and *E*). Thus, injection of rrSCF^{164} as the sole exogenous cytokine resulted in the development of dermal mast cells with characteristics of CTMC. Colocalization of immunohistochemical staining for BrdU incorporated into nuclear DNA and mast cell cytoplasmic granule staining with alcian blue (21) indicated that morphologically identifiable mast cells were proliferating at sites of rrSCF^{164} injection (Table 1 and Fig. 1 *F*). This finding indicated that the increased numbers of mast cells at sites of rrSCF^{164} injection reflected at least in part the proliferation of differentiated dermal mast cells, not merely the proliferation and/or maturation of mast cell precursors.

However, the skin of *Sl/Sl^d* mice ordinarily contains virtually no dermal mast cells (Table 1 and reference 25). Thus, even though many differentiated mast cells were proliferating

Table 1. *C-kit-dependent Stimulation of Proliferation of Mouse Dermal Mast Cells in vivo by rrSCF¹⁶⁴*

Mouse genotype	rrSCF[164]	Hct		Mast cells	BrdU⁺ (percent of mast cells)
		Before treatment	After treatment		
	μg/kg/d	%		*no./mm² of dermis*	
WBB6F₁-+/+	100	48 ± 1.3	50 ± 0.7	4,710 ± 1,190*	16 ± 2.2*
	30	50 ± 0.8	51 ± 0.3	454 ± 155*	13 ± 2.7*
	0	51 ± 0.6	51 ± 0.5	31 ± 2	3.3 ± 1.2
WBB6F₁-*W/W*ᵛ	100	39 ± 0.8	36 ± 3.4	0	-
	30	40 ± 0.7	40 ± 2.5	0	-
	0	40 ± 0.6	39 ± 0.2	0	-
WCB6F₁-+/+	30	46 ± 0.8	44 ± 2.7	122 ± 17*	36 ± 3.5*
	0	47 ± 0.3	44 ± 0.4	29 ± 5	4.9 ± 0.7
WCB6F₁-*Sl/Sl*ᵈ	30	30 ± 1.1	38 ± 1.7*	61 ± 24*	29 ± 5.7
	0	30 ± 0.3	33 ± 0.6	0	-

Mice were killed 24 h after the last of 21 daily subcutaneous injections of rrSCF[164] or vehicle for assessment of the number of mast cells/mm² of dermis at the injection site and for quantification of the percent of these mast cells that were proliferating, based on nuclear incorporation of BrdU (See Materials and Methods). All results are expressed as mean ± SEM (n = 5–7).
* $p < 0.001$ vs. value for mice treated with 0 μg/kg/d by Student's t test (two tailed).

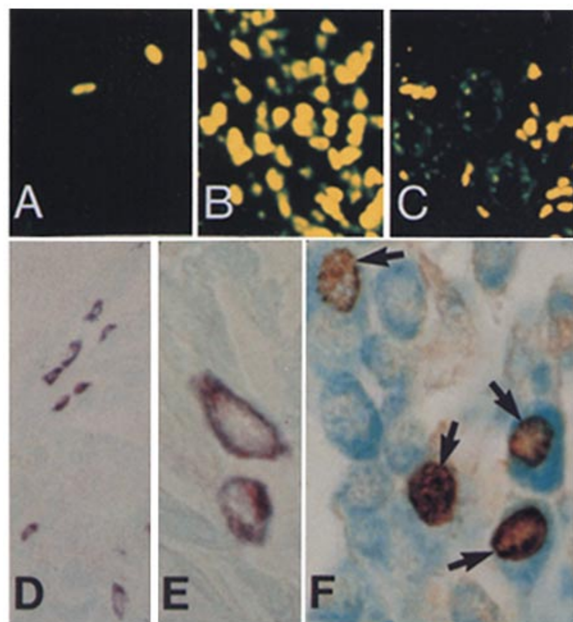

Figure 1. (*A–C*) Skin at sites injected daily for 3 wk with either rrSCF[164] or vehicle and then stained with berberine sulfate. (*A*) Vehicle injection site in a WBB6F₁-+/+ mouse exhibits only occasional brightly fluorescent mast cells. (*B*) Site in a WBB6F₁-+/+ mouse injected with rrSCF[164] (100 μg/kg) exhibits many mast cells. (*C*) Many berberine sulfate–positive mast cells in the skin of a WCB6F₁-*Sl/Sl*ᵈ mouse injected with rrSCF[164] (30 μg/kg). (*D* and *E*) Low (*D*) and high (*E*) power views of safranin-positive dermal mast cells at a rrSCF[164] injection site (30 μg/kg) in a WCB6F₁-+/+ mouse. (*F*) A rrSCF[164] (100 μg/kg) injection site in a WBB6F₁-+/+ mouse that had received BrdU to label proliferating cells. There are many mast cells (stained with alcian blue in this preparation); four of these mast cells have nuclei that are positive (brown immunoperoxidase reaction product) for BrdU (*arrows*) (*A–C*, *E*, and *F*; ×250; *D*: ×1,000).

at rrSCF[164] injection sites in *Sl/Sl*ᵈ mice 3 wk after initiation of treatment (Table 1), some of the effect of rrSCF[164] in these mice must have reflected the recruitment, proliferation, and/or induction of maturation of the mast cell precursors present in these animals (25). An effect of rrSCF[164] on mast cell maturation is also supported by the finding that many of the mast cells at rrSCF[164] injection sites in *Sl/Sl*ᵈ mice stained with berberine sulfate (Fig. 1 *C*) or safranin (data not shown), and by data indicating that rrSCF[164] can induce IL-3-dependent bone marrow–derived cultured mast cells to mature and acquire phenotypic characteristics of CTMC in vitro (20, 26).

In contrast to the effects of rrSCF[164] in *Sl/Sl*ᵈ mice, injection of rrSCF[164] into genetically mast cell–deficient WBB6F₁-*W/W*ᵛ mice influenced neither the profound mast cell deficiency, nor the anemia, of these animals (Table 1). This result, together with work indicating that the *W* and *W*ᵛ alleles encode c-*kit* products that express no (*W*) or markedly diminished (*W*ᵛ) tyrosine kinase activity (27), indicates that the ability of rrSCF[164] to induce the development of dermal CTMC in vivo requires that the rrSCF[164] interacts with a functionally active c-*kit* receptor. The complete failure of even very high doses of rrSCF[164] to induce cutaneous mast cell development in *W/W*ᵛ mice is also noteworthy in light of reports that this mutant can develop mature dermal mast cells in association with a chronic idiopathic dermatitis (22), in response to repeated epicutaneous applications of PMA (28), or as a result of treatment with IL-3 (29). The results reported here indicate that the appearance of mast cells in the skin of *W/W*ᵛ mice in these settings probably does not reflect increased local production of endogenous c-*kit* ligand, but instead may be due to other

effects such as the activation of either alternative signaling mechanisms or processes distal to the interaction between c-*kit* and its ligand.

Systemic Administration of SCF Expands Populations of CTMC and MMC in the Rat. Even though subcutaneous administration of $rrSCF^{164}$ increased the Hct of Sl/Sl^d mice (Table 1), the effects of $rrSCF^{164}$ on mast cell populations were most striking in the vicinity of the subcutaneous injection sites. For example, no mast cells appeared in the skin contralateral to $rrSCF^{164}$ injection sites in Sl/Sl^d mice or in the gastric tissues of these animals (data not shown). To assess the systemic effects of $rrSCF^{164}$ on mast cell development and phenotype, we turned to the rat. Daily intravenous injections are more readily performed in rats than in mice. More importantly, Huntley et al. (24) have reported highly sensitive and specific ELISA methods for quantifying tissue content of mast cell–specific proteases associated with CTMC and MMC (RMCP I and RMCP II, respectively) in this species.

We found that daily intravenous administration of $rrSCF^{164}$ to rats at a dose of 25 μg/kg/d for 14 d increased mast cell levels systemically and also produced striking increases in levels of both types of rat mast cell–associated proteases (Tables 2 and 3). In the skin, lung, and liver (Table 2), $rrSCF^{164}$ treatment resulted in marked increases in numbers of mast cells and in tissue content of the CTMC-associated protease, RMCP I, but no significant changes in levels of the MMC-associated protease, RMCP II. In the spleen, bone marrow, and peritoneal cavity (Tables 2 and 3), $rrSCF^{164}$ treatment resulted in striking expansion of mast cell populations, as well as marked increases in levels of both RMCP I and RMCP II. Note, however, that the total amounts of RMCP II in these sites in the $rrSCF^{164}$-treated animals were much less than the corresponding amounts of RMCP

Table 2. *Systemic Effects of $rrSCF^{164}$ on Mast Cell Populations and Tissue Mast Cell Protease Content in the Rat*

Tissue	$rrSCF^{164}$	Mast cells	RMCP I	RMCP II
	μg/kg/d	*no./mm² of tissue*	*μg/gm of tissue*	*μg/gm of tissue*
Skin	25	87 ± 7*	69 ± 7*	ND
	0	26 ± 3	27 ± 3	ND
Lung	25	48 ± 6*	13 ± 1.2*	10 ± 1.6
	0	7 ± 0.4	3.8 ± 0.8	11 ± 1.2
Liver	25	62 ± 10*	35 ± 8‡	1.2 ± 0.3
	0	3.6 ± 0.4	0.4 ± 0.06	0.8 ± 0.1
Spleen	25	204 ± 40*	92 ± 24‡	14.2 ± 5.0§
	0	0.07 ± 0.02	0.7 ± 0.08	0.10 ± 0.004
Glandular stomach:				
Mucosa	25	50 ± 9‡		
Submucosa	25	144 ± 36§	5.9 ± 1.2	19 ± 1.3§
Muscularis propria	25	32 ± 5‡		
Mucosa	0	12 ± 3		
Submucosa	0	45 ± 8	3.3 ± 0.4	13 ± 1.7
Muscularis propria	0	12 ± 2		
Ileum:				
Mucosa	25	80 ± 14§		
Submucosa	25	146 ± 51§	1.4 ± 0.5§	508 ± 83§
Muscularis propria	25	ND		
Mucosa	0	38 ± 12‡		
Submucosa	0	19 ± 4§	0.2 ± 0.1	272 ± 5
Muscularis propria	0	ND		

Rats were killed ~24 h after the last injection of 14 daily intravenous injections of $rrSCF^{164}$ or vehicle for assessment of mast cell numbers and content of RMCP I and RMCP II in various tissues (See Materials and Methods). All results are expressed as mean ± SEM (n = 5–8).
*, ‡, or § = $p < 0.001$, 0.01, or 0.05, respectively, vs. value for rats treated with 0 μg/kg/d by Student's t test (two tailed).

Table 3. *Systemic Effects of rrSCF^{164} on Peritoneal and Bone Marrow Mast Cells and Mast Cell Protease Content in the Rat*

Site	rrSCF^{164}	Cells/peritoneal cavity or femur		Protease content of total peritoneal cells or femoral bone marrow cells	
		Total cells (10^7)	Mast cells (10^6)	RMCP I	RMCP II
	μg/kg/d			*μg*	*ng*
Peritoneal cavity	25	2.3 ± 0.1	7.6 ± 0.5*	134 ± 7*	17 ± 5*
	0	2.1 ± 0.2	1.7 ± 0.3	49 ± 3	0.2 ± 0.2*
Femoral bone marrow	25	9.0 ± 0.3	3.9 ± 0.4*	34 ± 4*	42 ± 6*
	0	8.7 ± 0.6	0.3 ± 0.1	7 ± 1	8 ± 3

These data (mean ± SEM) are from the same rats shown in Table 2 (see Materials and Methods).
* $p < 0.001$ vs. value for rats treated with 0 μg/kg/d by Student's *t* test (two tailed).

I. rrSCF^{164} treatment significantly expanded populations of mast cells in the mucosa, submucosa, and muscularis propria of the glandular stomach, and in the mucosa and submucosa of the ileum (Table 2). MMC predominate in the mucosa of these organs, whereas CTMC predominate in the submucosa and muscularis propria (3, 4, 24). rrSCF^{164} treatment increased the content of both RMCP II ($p = 0.018$ by the two-tailed Student's *t* test) and RMCP I ($p = 0.045$ by the one-tailed Student's *t* test) in the glandular stomach, and also significantly increased the content of both proteases in the ileum (Table 2). Thus, according to anatomical distribution and mast cell protease phenotype (reviewed in references 3–5 and 24), intravenous administration of rrSCF^{164} to rats markedly expanded populations of CTMC and also increased levels of gastrointestinal MMC.

By immunohistochemistry (24), mast cells in rrSCF^{164}-treated rats exhibited a pattern of staining for RMCP I and II that was similar to that of mast cells in control rats (24). For example, dermal mast cells were exclusively RMCP I$^+$ and RMCP II$^-$, whereas mast cells in the mucosa of the ileum were predominantly RMCP II$^+$ and RMCP I$^-$. However, the effect of rrSCF^{164} treatment on the numbers of mast cells in various sites generally was more marked than the corresponding effect on RMCP content. For example, in the spleen, femoral bone marrow, and glandular stomach, mast cell numbers were ~3,000, 13, and 3 times those in control rats, whereas the corresponding ratios for total content of RMCP I and II in rrSCF^{164}-treated vs. control rats were ~130, 5, and 1.5. This finding may reflect any of a number of factors, including the recent expansion of mast cell populations in the rrSCF^{164}-treated animals. It is well established that the cytoplasmic granule–associated mediator content of rat mast cells increases progressively with the age of the cells (reviewed in references 30 and 31).

These data show that the local injection of SCF promoted the expansion of dermal CTMC populations in mice in vivo, and indicate that this effect required a functionally active c-*kit* receptor. In rats, intravenous administration of rrSCF^{164} resulted in striking expansions of mast cell populations in all of the anatomical sites examined. These included sites that usually contain large populations of CTMC, such as the skin and peritoneal cavity, and sites that ordinarily contain substantial numbers of MMC, such as the mucosa of the glandular stomach and ileum. These findings, taken together with the observation that *W/W^v* and *Sl/Sl^d* mice virtually lack both CTMC and MMC (1, 2, 6), indicate that interactions between c-*kit* and its ligand importantly regulate the normal development of both CTMC and MMC. However, administration of exogenous SCF not only produced significant expansions of populations of CTMC and MMC in sites ordinarily containing large numbers of mast cells, such as the skin and stomach, but also resulted in the appearance of many mast cells at sites that typically contain very few mast cells, such as the liver, spleen, and bone marrow. These findings raise the possibility that the mast cell proliferation associated with certain immunological or pathological responses may reflect, in part, excessive local or even systemic production of c-*kit* ligand.

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References

1. Nakano, T., C. Sonoda, A. Hayashi, Y. Yamatodani, T. Kanayama, H. Yamamura, Y. Asai, Y. Yonezawa, Y. Kitamura, and S.J. Galli. 1985. Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal, and intravenous transfer into genetically mast cell-deficient W/W^v mice. Evidence that cultured mast cells can gave rise to both connective tissue type and mucosal mast cells. *J. Exp. Med.* 162:1025.
2. Kitamura, Y., H. Nakayama, and J. Fujita. 1989. Mechanisms of mast cell deficiency in mutant mice of W/W^v and Sl/Sl^d genotype. *In* Mast Cell and Basophil Differentiation and Function in Health and Disease. S.J. Galli and K.F. Austen, editors. Raven Press, New York. 15–25.
3. Enerbäck, L. 1986. Mast cell heterogeneity: the evolution of the concept of a specific mucosal mast cell. *In* Mast Cell Differentiation and Heterogeneity. A.D. Befus, J. Bienenstock, and J.A. Denburg, editors. Raven Press, New York. 1–26.
4. Miller, H.R.P., J.F. Huntley, G.F.J. Newlands, S. Mackellar, J. Irvine, D.M. Haig, A. MacDonald, A.D. Lammas, D. Wakelin, and R.G. Woodbury. 1989. Mast cell granule proteases in mouse and rat: a guide to mast cell heterogeneity and activation in the gastrointestinal tract. *In* Mast Cell and Basophil Differentiation and Function in Health and Disease. S.J. Galli and K.F. Austen, editors. Raven Press, New York. 81–91.
5. Galli, S.J. 1990. New insights into "the riddle of the mast cells": microenvironmental regulation of mast cell development and phenotypic heterogeneity. *Lab. Invest.* 62:5.
6. Galli, S.J., E.N. Geissler, B.K. Wershil, J.R. Gordon, and M. Tsai. Insights into mast cell development and function derived from analysis of *W* or *Sl* mutant mice. *In* The Role of the Mast Cell in Health and Disease. M.A. Kaliner and D.D. Metcalfe, editors. Marcel Dekker, New York. In press.
7. Hamaguchi, Y., Y. Kanakura, J. Fujita, S.-I. Takeda, T. Nakano, S. Tarui, T. Honjo, and Y. Kitamura. 1987. Interleukin 4 as an essential factor for in vitro clonal growth of murine connective tissue-type mast cells. *J. Exp. Med.* 165:268.
8. Tsuji, K., T. Nakahata, M. Takagi, T. Kobayashi, A. Ishiguro, T. Kikuchi, K. Naganuma, K. Koike, A. Miyajima, K.-I. Arai, and T. Akabane. Synergistic action of phorbol ester and IL-3 in the induction of "connective tissue-type" mast cell proliferation. *J. Immunol.* 144:678.
9. Chabot, B., D.A. Stephenson, M. Chapman, P. Besmer, and A. Bernstein. 1988. The proto-oncogene c-*kit* encoding a transmembrane tyrosine kinase receptor maps to the mouse *W* locus. *Nature (Lond.).* 335:88.
10. Geissler, E.N., M.A. Ryan, and D.E. Housman. 1988. The dominant-white spotting (*W*) locus of the mouse encodes the c-*kit* proto-oncogene. *Cell.* 55:185.
11. Williams, D.E., J. Eisenman, A. Baird, C. Rauch, K. Van Ness, C.J. March, L.S. Park, U. Martin, D. Mochizuki, H.S. Boswell, G.S. Burgess, and S.D. Lyman. 1990. Identification of a ligand for the c-*kit* proto-oncogene. *Cell.* 63:167.
12. Copeland, N.G., D.J. Gilbert, B.C. Cho, P.J. Donovan, N.A. Jenkins, D. Cosman, D. Anderson, S.D. Lyman, and D.E. Williams. 1990. 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.
13. Flanagan, J.G., and P. Leder. 1990. The c-*kit* ligand: a cell surface molecule altered in steel mutant fibroblasts. *Cell.* 63:185.
14. Zsebo, K.M., J. Wypych, I.K. McNiece, H.S. Lu, K.A. Smith, S.B. Karkare, R.K. Sachdev, V.N. Yuschenkoff, N.C. Birkett, L.R. Williams, V.N. Satyagal, W. Tung, R.A. Bosselman, E.A. Mendiaz, and K.E. Langley. 1990. Identification, purification, and biological characterization of hematopoietic stem cell factor from buffalo rat liver-conditioned medium. *Cell.* 63:195.
15. Martin, F.H., S.V. Suggs, K.E. Langley, H.S. Lu, J. Ting, K.H. Okino, C.F. Morris, I.K. McNiece, F.W. Jacobsen, E.A. Mendiaz, N.C. Birkett, K.A. Smith, M.J. Johnson, V.P. Parker, J.C. Flores, A.C. Patel, E.F. Fisher, H.O. Erajavec, C.J. Herrera, J. Wypych, R.K. Sachdev, J.A. Pope, I. Leslie, D. Wen, C.-H. Lin, R.L. Cupples, and K.M. Zsebo. 1990. Primary structure and functional expression of rat and human stem cell factor DNAs. *Cell.* 63:203.
16. Zsebo, K.M., D.A. Williams, E.N. Geissler, V.C. Broudy, F.H. Martin, H.L. Atkins, R.-Y. Hsu, N.C. Birkett, K.H. Okino, D.C. Murdock, F.W. Jacobsen, K.E. Langley, K.A. Smith, T. Takeishi, B.M. Cattanach, S.J. Galli, and S.V. Suggs. 1990. 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.
17. Huang, E., K. Nocka, D.R. Beier, T.-Y. Chu, J. Buck, H.-W. Lahm, D. Wellner, P. Leder, and P. Besmer. 1990. 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.
18. Anderson, D.M., S.D. Lyman, A. Baird, J.M. Wignall, J. Elsenman, C. Rauch, C.J. March, H.S. Boswell, S.D. Gimpel, D. Cosman, and D.E. Williams. 1990. Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. *Cell.* 63:235.
19. Nocka, K., J. Buck, J. Levi, and P. Besmer. 1990. Candidate ligand for the c-*kit* transmembrane kinase receptor: KL, a fibroblast derived growth factor stimulates mast cells and erythroid progenitors. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:3287.
20. Tsai, M., T. Takeishi, E.N. Geissler, K.E. Langley, K.M. Zsebo, and S.J. Galli. 1991. Stem cell factor (SCF), a ligand for *c-kit*, promotes mast cell proliferation and maturation *in vitro* and *in vivo*. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 5:A1086 (abstr.)
21. Arizono, N., Y. Shiota, M. Yamada, Y. Matsumoto, H. Yoshikawa, S. Matsuda, and T. Tegoshi. 1990. Bromodeoxyuridine labeling studies on the proliferation of intestinal mucosal mast cells in normal and athymic rats. *Acta Pathol. Microbiol. Immunol. Scand.* 98:369.
22. Galli, S.J., N. Arizono, T. Murakami, A.M. Dvorak, and J.G. Fox. 1987. Development of large numbers of mast cells at sites of idiopathic chronic dermatitis in genetically mast cell-deficient $WBB6F_1$-W/W^v mice. *Blood.* 69:1661.
23. Enerbäck, L. 1974. Berberine sulphate binding to mast cell polyanions: a cytofluorometric method for the quantitation of

heparin. *Histochemistry.* 42:301.

24. Huntley, J.F., A. Mackellar, G.F.J. Newlands, J. Irvine, and H.R. Miller. 1990. Mapping of the rat mast cell granule proteinases RMCP I and II by enzyme linked immunosorbent assay and paired immunofluorescence. *Acta Pathol. Microbiol. Immunol. Scand.* 98:1933.
25. Kitamura, Y., and S. Go. 1979. Decreased production of mast cells in Sl/Sl^d mice. *Blood.* 53:492.
26. Tsai, M., T. Takeishi, H. Thompson, K.E. Langley, K.M. Zsebo, D.D. Metcalfe, E.N. Geissler, and S.J. Galli. Induction of mast cell proliferation, maturation and heparin synthesis by the rat c-*kit* ligand, stem cell factor. *Proc. Natl. Acad. Sci. USA.* In press.
27. Nocka, K., J.C. Tan, E. Chiu, T.Y. Chu, P. Ray, P. Traktman, and P. Besmer. 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 (Eur. Mol. Biol. Organ.) J.* 9:1805.
28. Gordon, J.R., and S.J. Galli. 1990. Phorbol 12-myristate 13-acetate-induced development of functionally active mast cells in W/W^v but not Sl/Sl^d genetically mast cell-deficient mice. *Blood.* 75:1637.
29. Ody, C., V. Kindler, and P. Vassali. 1990. 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.
30. Aldenborg, F., and L. Enerbäck. 1985. Allometric growth of the rat peritoneal mast cell mass and of the granular constituents: heparin, histamine and 5-hydroxytryptamine. *Growth.* 49:510.
31. Hammel, I., D. Lagunoff, and P.-G. Krüger. 1988. Studies on the growth of mast cells in rats. Changes in granule size between one and six months. *Lab. Invest.* 59:549.