Effects of Chronic Treatment with the c-*kit* Ligand, Stem Cell Factor, on Immunoglobulin E-dependent Anaphylaxis in Mice

Genetically Mast Cell-deficient *Sl/Sl*^{d} Mice Acquire Anaphylactic Responsiveness, but the Congenic Normal Mice Do Not Exhibit Augmented Responses

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Abstract

We treated genetically mast cell-deficient WCB6F$_1$-*Sl/Sl*^{d} mice and the congenic normal (WCB6F$_1$-+/+) mice with the c-*kit* ligand recombinant rat stem cell factor^{164} (rrSCF^{164}; 100 μg/kg per d, subcutaneously) or with vehicle for 21 d, then passively sensitized the mice with anti-dinitrophenol$_{30-40}$ immunoglobulin E (IgE) antibodies, and 1 d later measured the changes in heart rate, pulmonary dynamic compliance, and pulmonary conductance, and assessed the death rates associated with intravenous challenge of these animals with specific antigen. rrSCF^{164} treatment induced the development of mast cells in *Sl/Sl*^{d} mice, and these mice exhibited tachycardia, but not death, after challenge with IgE and antigen. rrSCF^{164} treatment induced mast cell hyperplasia in +/+ mice, but the cardiopulmonary changes associated with passive anaphylaxis in these mice were virtually indistinguishable from those observed in control +/+ mice treated with vehicle instead of rrSCF^{164}. Moreover, the highest dose of antigen challenge produced significantly fewer fatalities in rrSCF^{164}-treated than in vehicle-treated +/+ mice (1/11 vs. 8/11, respectively, $P < 0.01$). Thus, in normal mice, chronic treatment with rrSCF^{164} induces mast cell hyperplasia but does not increase, and in certain respects diminishes, the severity of IgE-dependent anaphylactic reactions. (*J. Clin. Invest.* 1993. 92:1639–1649.) Key words: allergy • asthma • c-*kit* • immunoglobulin E receptors • stem cell factor

Introduction

Three groups simultaneously reported that the *Sl* locus on mouse chromosome 10 encodes a new growth factor that represents a ligand for the c-*kit* receptor (1–7). This growth factor has been designated stem cell factor (SCF;[1] references 3–5), mast cell growth factor (MGF; references 1 and 2), kit ligand (KL; reference 6), and steel factor (SLF; references 8 and 9). The wild-type SCF is produced as a transmembrane protein (3, 4, 6, 7, 9–11), of which two major alternatively spliced forms have been described in the mouse (11). The longer of these consists of an ~ 189-amino acid extracellular ligand domain, an ~ 21-amino acid transmembrane domain, and an ~ 36-amino acid intracytoplasmic tail; proteolytic cleavage of the extracellular domain of the membrane-associated product releases an ~ 164-amino acid biologically active soluble form of SCF (11).

A large body of in vitro and in vivo data indicate that SCF can critically regulate mast cell development and secretory function (12, 13). WCB6F$_1$-*Sl/Sl*^{d} mice, which have mutations at the *Sl* locus that result in a failure of production of transmembrane SCF (2, 5, 6, 11), virtually lack tissue mast cells (14). However, daily subcutaneous injection of *Sl/Sl*^{d} mice with recombinant rat SCF^{164} (rrSCF^{164}) for 21 d results in the development of many mast cells in the skin at the injection site (5, 15). rrSCF^{164} can promote the tissue localization and/or survival of mouse mast cell precursors in vivo (5, 15), the proliferation of both immature and mature mouse mast cells in vitro (16), the maturation of immature mouse mast cells in vitro (16, 17), and mouse mast cell maturation and proliferation in vivo (5, 15, 16). In rats, intravenous treatment with rrSCF^{164} results in hyperplasia of both "connective tissue-type mast cells" (CTMC), such as those in the skin and peritoneal cavity, and "mucosal mast cells" (MMC), such as those in the mucosa of the ileum or glandular stomach (15). rrSCF^{164} also can directly induce c-*kit*-dependent mast cell activation when injected intradermally in mice (18), can directly stimulate serotonin release by purified mouse peritoneal mast cells in vitro (19), and, at even lower concentrations, can significantly augment serotonin release by mouse peritoneal mast cells stimulated via their surface Fc$_\epsilon$RI (19).

SCF not only influences mast cell development and function in rodents, but also in humans and other primates. Administration of recombinant human SCF (rhSCF) subcutaneously to baboons (*Papio* species) or cynomolgus monkeys (*Macaca fascicularis*) in vivo induces mast cell hyperplasia in many anatomical sites (20). rhSCF also can promote the development of human mast cells in vitro, particularly when rhSCF is used as the only exogenous cytokine (21–24). Finally, studies of isolated human mast cells demonstrate that concentrations of rhSCF in the 1–10-ng/ml range can induce low levels of mediator release from skin mast cells (25), and that brief preincubation with even lower concentrations of rhSCF can significantly augment Fc$_\epsilon$RI-dependent mediator release by skin (24) or lung (26) mast cells.

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1. *Abbreviations used in this paper:* ANOVA, analysis of variance; C_{dyn}, pulmonary dynamic compliance; CTMC, connective tissue-type mast cell; DNP-HSA, dinitrophenol$_{30-40}$ human serum albumin; G_L, pulmonary conductance; HR, heart rate; MMC, mucosal mast cell; rhSCF, recombinant human stem cell factor; rrSCF^{164}, recombinant rat stem cell factor^{164}.

Given the known spectrum of biological effects of SCF on mast cell development and function, we felt that it would be of some interest to determine whether prolonged administration of SCF influenced either the mast cell activation, the physiological changes, or the mortality associated with a systemic IgE-dependent biological response in vivo. We therefore administered rrSCF^{164} or vehicle daily for 21 d to mast cell–deficient WCB6F$_1$-*Sl/Sl^d* mice and to the congenic normal WCB6F$_1$-+/+ mice. We then attempted to elicit IgE-dependent passive anaphylaxis in these animals, and evaluated the characteristics of these responses. Some of these results have been presented in abstract form (27).

Methods

Animals. We studied male genetically mast cell–deficient WCB6F$_1$-*Sl/Sl^d* (*Sl/Sl^d*) mice and the congenic normal (WCB6F$_1$-+/+): (WC/ReJ-*Sl*/+ × C57BL/6J-*Sl^d*/+)F$_1$-(*Sl/Sl^d*, +/+) mice (The Jackson Laboratory, Bar Harbor, ME) that were 12–16 wk old (25–35 g body weight) at the beginning of the experiment. The *Sl/Sl^d* mice have mutations at the *Sl* locus on chromosome 10, which encodes a ligand (SCF) for the c-*kit* tyrosine kinase growth factor receptor (1–7). The *Sl* mutation is a deletion of all coding sequences (2, 5, 6), whereas *Sl^d* produces a truncated transcript (5, 11) encoding almost all of the extracellular domain of SCF but lacking the coding sequence for the transmembrane and intracellular domains of the wild-type protein (11). *Sl/Sl^d* mice are anemic, lack melanocytes in the skin, are sterile, and virtually lack tissue mast cells (4, 12, 14). The skin of adult *Sl/Sl^d* mice contains < 0.5% the number of mast cells present in the skin of the congenic +/+ mice, and no mast cells whatsoever are identifiable in the trachea, lungs, or multiple other organs or anatomical sites (4, 12, 14). However, apart from defects in responses significantly influenced by mast cells, the expression of immunological and inflammatory reactions in *Sl/Sl^d* mice is similar or identical to that in the congenic normal animals (12).

Reagents. rrSCF^{164}, representing essentially the entire extracellular portion of the transmembrane protein, was purified from *Escherichia coli* and modified by the covalent attachment of polyethylene glycol (5, 15); rrSCF^{164} was provided by Drs. K. Langley and K. Zsebo (Amgen, Inc., Thousand Oaks, CA). H 1 DNP-ε-26 hybridoma cells, which produce a mouse monoclonal IgE antibody with specificity for DNP (28), were generously provided by Drs. Fu-Tong Liu and David Katz. DNP$_{30-40}$ HSA was purchased from Sigma Chemical Co. (St. Louis, MO).

Physiological measurements. Heart rate (HR) and the pulmonary mechanical parameters, dynamic compliance (C_{dyn}), and lung conductance (G_L) were measured in mice anesthetized with 70–90 mg/kg i.p. of sodium pentobarbital using a plethysmographic method (29–31). C_{dyn} and pulmonary resistance were calculated from the recordings of volume, flow, and pressure using standard techniques (32). Baseline values of HR, C_{dyn}, and G_L were determined 20–30 min after preparation of the animals for recording pulmonary parameters.

Histologic studies. The presence of tissue mast cells and their state of activation were assessed in 1 μm, Epon-embedded, Giemsa-stained sections (30, 33, 34). Tissues were removed and fixed as previously described (31, 33, 34), either immediately after death induced by DNP-HSA challenge, or after death by cervical dislocation 60 min after challenge. Sections of dermis at subcutaneous injection sites (5, 15), dermis of ear skin (31, 34), trachea (30), and peribronchial tissue (30, 31) were evaluated for mast cell numbers as previously described. Mast cells also were quantified in sections of the mid right lower lobe of the lung, extending to the pleura, the mid free wall of the left ventricle of the heart, the middle of a hepatic lobe, the middle of the spleen, the mid portion of the forestomach (mast cells were quantified in the submucosa), and the mid glandular stomach (mast cells were quantified separately in the mucosa, submucosa, and muscularis propria). Sections were coded so that the observer was not aware of the identity of individual specimens, and examined at ×400 by light microscopy. Mast cells were classified as "extensively degranulated" (> 50% of the cytoplasmic granules exhibiting fusion, staining alterations, and/or extrusion from the cell), "moderately degranulated" (10–50% of the granules exhibiting fusion or discharge), or "normal" (31, 33, 34).

Protocols. To induce the development of mast cells in genetically mast cell–deficient *Sl/Sl^d* mice, and to induce mast cell hyperplasia in the congenic +/+ mice, groups of 9–10 *Sl/Sl^d* or congenic +/+ mice received for 21 d a daily subcutaneous injection of rrSCF^{164} (100 μg/kg per d) in 150–200 μl of sterile saline containing 0.1% BSA (fraction V, fatty acid free; ICN Immunobiologicals, Lisle, IL) (15). Injections of rrSCF^{164}, or in control *Sl/Sl^d* or congenic +/+ mice, vehicle alone, were performed with the mice under light ether anesthesia and were delivered to approximately the same site on the dorsal back skin (15). Approximately 4 h after the last of the 21 injections of rrSCF^{164} or vehicle, which were performed at ~ 8:00–9:00 a.m., the mice were injected intravenously with ~ 20 μg of monoclonal mouse anti-DNP IgE antibodies, given as 0.1 ml of a 1:20 dilution of ascites in sterile 0.9% NaCl. Preliminary experiments demonstrated that this amount of anti-DNP IgE adequately sensitized normal mice for passive anaphylaxis elicited by intravenous injection of 200 or 1,000 μg of DNP-HSA 24 h after injection of the anti-DNP IgE, but that identically sensitized and challenged genetically mast cell–deficient WBB6F$_1$-*W/W^v* mice exhibited no physiological evidence of an anaphylactic response (data not shown). Approximately 24 h after passive sensitization with anti-DNP IgE, the mice were prepared for physiological measurements as described above. When stable values of HR, C_{dyn}, and G_L had been obtained, mice received a rapid intravenous infusion of 50 μl of 0.9% NaCl (control challenge) or 0.9% NaCl containing 5, 200, or 1,000 μg of DNP-HSA. HR, C_{dyn}, and G_L were measured at multiple intervals until death due to challenge or, after a period of 60 min, cervical dislocation. Additional groups of five to six rrSCF^{164}-treated *Sl/Sl^d* or +/+ mice, or vehicle-treated +/+ mice, which were not used for physiological measurements, were challenged with 0.9% NaCl and then killed by cervical dislocation for histological analysis 60 min later.

To assess the effects of administering rrSCF^{164} immediately before antigen challenge, some mice received a series of 22 daily subcutaneous injections of rrSCF^{164} (100 μg/kg per d) or vehicle, with the last injection administered ~ 1 d after the mice received anti-DNP IgE (on day 21, as above). The last injection of rrSCF^{164} or vehicle was given immediately after the administration of sodium pentobarbital (as described above), ~ 1 h before the administration of additional sodium pentobarbital and then challenge with 1,000 μg of DNP-HSA.

Statistical analysis. Differences among the various groups of mice in the time courses of HR, C_{dyn}, or G_L responses were examined for statistical significance by analysis of variance (ANOVA). Differences in the maximum diminution in C_{dyn} or G_L, or the maximum HR responses, and differences in the numbers of mast cells in the tissues of different groups of mice, were examined by the Student's *t* test (two-tailed). Differences in the extent of mast cell degranulation in various groups of mice were examined for statistical significance by the χ^2 test. $P < 0.05$ was regarded as significant. Differences in the death rates between different experimental groups were examined for statistical significance by Fisher's exact test. Unless otherwise specified, results are expressed as the mean±SEM.

Results

Chronic treatment with rrSCF^{164} induces the development of mast cells in Sl/Sl^d mice and mast cell hyperplasia in the congenic +/+ mice. The numbers of mast cells in various tissues of rrSCF^{164}- or vehicle-treated mice were assessed histologically in specimens that had been obtained and fixed immediately upon completion of the physiological studies in these animals. Even though extensively degranulated mast cells can occasionally be difficult to identify, this problem occurs primarily when counts are performed at long intervals after mast cell activation

Table I. Numbers of Mast Cells in Various Tissues of Genetically Mast Cell-deficient WCB6F$_1$-Sl/Sl^d (Sl/Sl^d) Mice or Congenic Normal WCB6F$_1$-+/+ (+/+) Mice That Had Been Treated for 21 d with rrSCF (100 μg/kg per d) or with Vehicle

Anatomical site	+/+				Sl/Sl^d		
	(A) SCF	(B) Vehicle	A vs. B	A/B	(C) SCF	A vs. C	A/C
	No./mm^2	*No./mm^2*	*P*		*No./mm^2*	*P*	
Skin at injection site	2,370±171	49±5	0.0001	48	611±132	0.0001	3.9
Ear skin	420±25	275±11	0.0001	1.5	0.8±0.6	0.0001	525
Trachea	96±9	48±5	0.0001	2.0	0.1±0.1	0.0001	960
Bronchus	106±4	58±3	0.0001	1.8	0.06±0.06	0.0001	1,770
Lung	0.43±0.12	0.0±0.0	0.0005	—	1.6±0.4	0.0023	0.3
Heart	1.9±0.4	0.7±0.1	0.0111	2.7	0.02±0.02	0.0001	95
Liver	2.2±0.4	0.0±0.0	0.0001	—	16±3	0.0001	0.1
Spleen	48±8	1.0±0.2	0.0001	48	100±22	0.0289	0.5
Forestomach	137±9	83±7	0.0001	1.7	1.2±0.8	0.0001	11
Glandular stomach							
Mucosa	50±7	27±5	0.0089	1.9	4.5±1.2	0.0001	11
Submucosa	207±25	140±18	0.0339	1.5	38±9	0.0001	5.4
Muscularis propria	65±7	59±6	0.5037	1.1	1.7±0.6	0.0001	38

Data, which are expressed as mean±SEM for 26–30 mice per group, are from the same mice used for the experiments shown in the figures (i.e., data shown in columns A, B, and C were pooled from mice of the same genotype and rrSCF^{164} or vehicle treatment status that had been challenged with 0–1,000 μg of DNP-HSA). The areas of tissue examined (mm^2, mean±SEM) were as follows: skin (dermis) at injection site, 0.57±0.10 or 1.40±0.07 for rrSCF^{164}-treated or vehicle-treated mice, respectively; ear skin, 0.29±0.01; trachea, 0.35±0.01; bronchus (peribronchial tissue), 0.43±0.02; lung parenchyma, 2.95±0.16; heart (left ventricle), 3.49±0.16; liver, 2.96±0.15; spleen, 2.33±0.06; forestomach (submucosa), 0.21±0.01; glandular stomach, mucosa, 0.99±0.04; submucosa, 0.071±0.004; muscularis propria, 0.23±0.02.

(34) rather than, as in this study, only 1 h after stimulation (31). In the present study, numbers of mast cells in the ears, trachea, lungs, heart, spleen, liver, or skin injection sites were not significantly lower in mice challenged with 1,000 μg of DNP-HSA than in corresponding mice challenged with saline alone. In rrSCF^{164}-treated +/+ mice, ~ 21% fewer mast cells were counted in the bronchi of mice challenged with 1,000 μg of DNP-HSA than in those challenged with saline ($P < 0.05$ by Student's two-tailed *t*-test). Because the data from mice of a given genotype and rrSCF^{164} or vehicle treatment status that had been challenged with 0.9% NaCl or with different doses of DNP-HSA were quite similar, data from mice challenged with 0–1000 μg DNP-HSA were pooled for presentation in Table I. Analysis of mast cell numbers in the tissues of 0.9% NaCl-challenged rrSCF^{164}- or vehicle-treated mice that had not been used for physiological measurements gave similar results (data not shown).

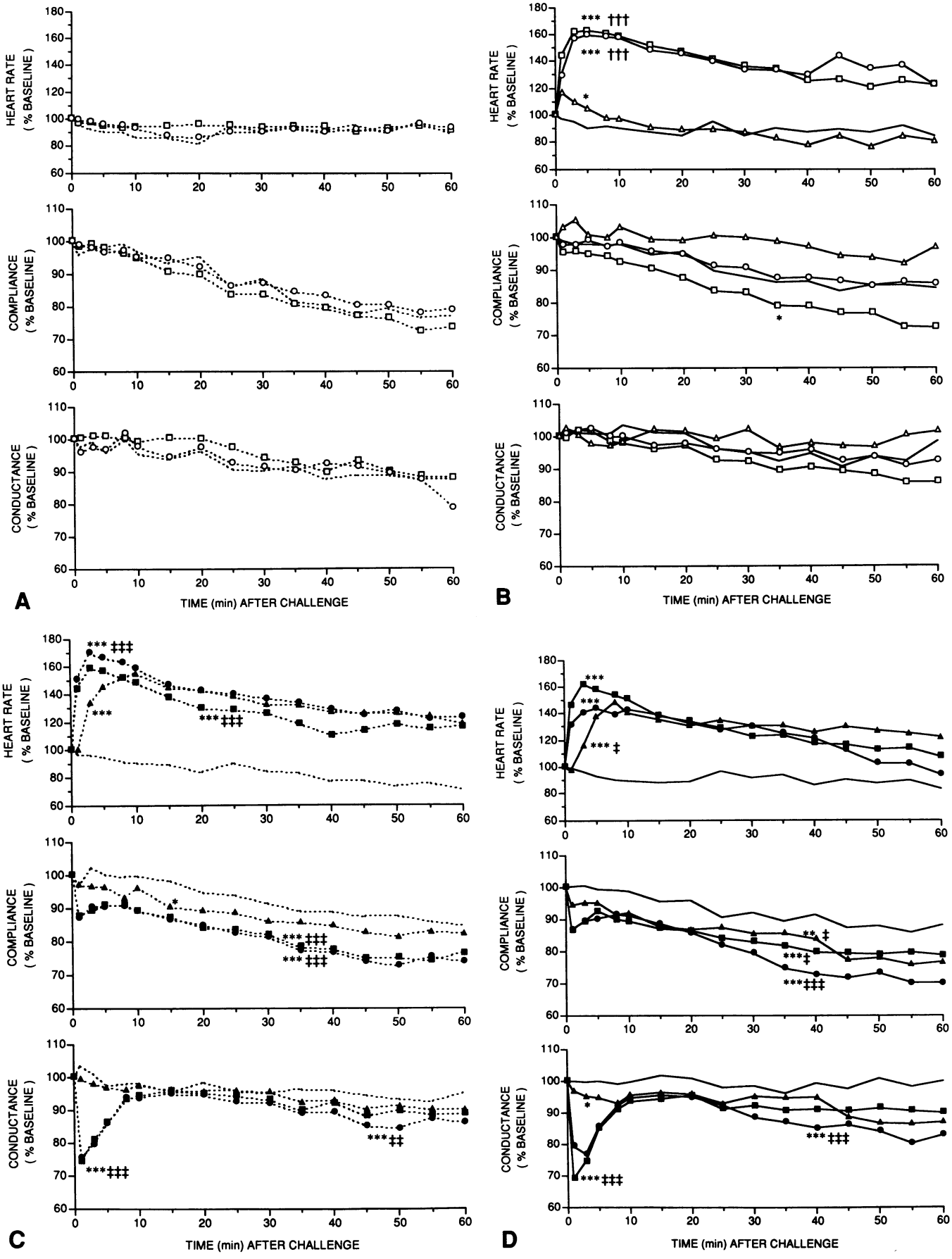
In confirmation of our previous study (15), +/+ mice treated with rrSCF^{164} exhibited 48-fold higher densities of mast cells in the skin at the subcutaneous injection site than did the vehicle-treated mice. rrSCF^{164} treatment of +/+ mice also resulted in an almost 50-fold elevation of mast cell densities in the spleen. The results were less striking in other anatomical sites. However, rrSCF^{164} treatment resulted in statistically significant increases in peribronchial, tracheal, and cardiac mast cells (increases of 80, 100, and 170%, respectively), 50–90% increases in mast cell densities in the ear skin, forestomach, and glandular stomach mucosa and submucosa, and the appearance of small numbers of mast cells in the liver and pulmonary parenchyma, two sites that lacked mast cells in the vehicle-treated +/+ mice. Of all the sites examined, only the muscularis propria of the stomach did not exhibit a statistically significant increase in mast cells in response to rrSCF^{164} treatment.

Vehicle-treated Sl/Sl^d mice lacked any histologically detectable mast cells (data not shown). By contrast, mast cells were detectable in all of the anatomical sites examined in rrSCF^{164}-treated Sl/Sl^d mice (Table I). As in rrSCF^{164}-treated +/+ mice, the most striking effects occurred in the skin at the injection site and in the spleen, which exhibited mast cell densities that were 26% or approximately two-fold, respectively, those in the corresponding sites in rrSCF^{164}-treated +/+ mice. rrSCF^{164}-treated Sl/Sl^d mice had 3.7-fold the density of pulmonary parenchymal mast cells and 7.3-fold the density of hepatic mast cells, as did rrSCF^{164}-treated +/+ mice. In contrast, in other anatomical sites, the densities of mast cells in the rrSCF^{164}-treated Sl/Sl^d mice were much less than those in the rrSCF^{164}-treated +/+ mice. For example, values in the ear skin, trachea, bronchus, or heart of rrSCF^{164}-treated Sl/Sl^d mice were 0.2, 0.1, 0.06, or 1.1% those in rrSCF^{164}-treated +/+ mice.

Table II. Baseline Values of HR, C_{dyn}, and G_L in WCB6F$_1$-Sl/Sl^d (Sl/Sl^d) Mice or WCB6F$_1$-+/+ (+/+) Mice That Had Been Treated for 21 d with rrSCF^{164} (100 μg/kg per d) or with Vehicle

Mice	Treatment	HR	C_{dyn}	G_L
		beats/min	*ml·cm H_2O^{-1}*	*ml·s^{-1}·cm H_2O^{-1}*
+/+	SCF	179±36*	0.037±0.006‡	1.25±0.18
+/+	Vehicle	181±27§	0.038±0.007*	1.22±0.18
Sl/Sl^d	SCF	155±26	0.034±0.007	1.21±0.20
Sl/Sl^d	Vehicle	154±27	0.033±0.004	1.24±0.23

Values are mean±SD (n = 27–33/group). * $P < 0.01$; ‡ $P < 0.06$; § $P < 0.001$ by Student's *t* test (two tailed), vs. data from Sl/Sl^d mice of the same rrSCF^{164} or vehicle treatment status.


HEART RATE (% BASELINE)
COMPLIANCE (% BASELINE)
CONDUCTANCE (% BASELINE)
TIME (min) AFTER CHALLENGE
A
B
C
D

Chronic treatment with rrSCF^{164} does not alter baseline values for HR, C_{dyn}, or G_L in Sl/Sl^d or +/+ mice. As noted in our previous study (30), the baseline values for HR and C_{dyn} in WCB6F$_1$-+/+ mice were higher (~ 15%) than those in the congenic *Sl/Sl^d* mice (Table II). However, there was no statistically significant difference in values for G_L between +/+ and *Sl/Sl^d* mice, nor were there any statistically significant differences in values for HR, C_{dyn}, or G_L in rrSCF^{164}- vs. vehicle-treated +/+ or *Sl/Sl^d* mice.

Vehicle-treated Sl/Sl^d mice fail to express IgE-dependent passive anaphylaxis. Whether judged by the entire time course of the changes in HR, C_{dyn}, or G_L observed after antigen challenge (Fig. 1 *A*), or by the maximum percent change in these measurements during the first 10 min after challenge (Table III), vehicle-treated *Sl/Sl^d* mice challenged with 200 or 1,000 μg of DNP-HSA gave responses that were little or no different than those of vehicle-treated *Sl/Sl^d* mice challenged with saline. And none of these mice died as a result of antigen challenge (Table IV).

Chronic treatment with rrSCF^{164} permits Sl/Sl^d mice to express IgE-dependent passive anaphylaxis. rrSCF^{164}-treated *Sl/Sl^d* mice developed significant tachycardia after challenge with 5, 200, or 1,000 μg of DNP-HSA (Fig. 1 *B* and Table III). Indeed, the HR responses in rrSCF^{164}-treated *Sl/Sl^d* mice challenged with 200 or 1,000 μg DNP-HSA were statistically indistinguishable from those in identically challenged rrSCF^{164}-treated +/+ mice (Fig. 1, *B* and *D*, and Table III). In contrast, the HR response to challenge with 5 μg of DNP-HSA, although significant when compared with the response in rrSCF^{164}-treated mice challenged with saline ($P = 0.0103$ when compared by ANOVA over the first 20 min after challenge), was substantially less in rrSCF^{164}-treated *Sl/Sl^d* mice than in the rrSCF^{164}-treated +/+ mice ($P = 0.0124$ when compared by ANOVA over the first 20 min after challenge). Notably, rrSCF^{164}-treated *Sl/Sl^d* mice developed little or no changes in C_{dyn} or G_L in response to antigen challenge, with a small, albeit statistically significant, response observed only in C_{dyn} and only in mice challenged with the highest dose of DNP-HSA (Fig. 1 *B* and Table III). Furthermore, none of the rrSCF^{164}-treated *Sl/Sl^d* mice died as a result of antigen challenge (Table IV).

Chronic treatment with rrSCF^{164} does not increase the severity of IgE-dependent passive anaphylaxis in WCB6F$_1$-+/+ mice. We showed previously that intradermal injection of rrSCF^{164} induces c-*kit*-dependent mast cell activation in mice (18), and that short-term incubation of mouse peritoneal mast cells with rrSCF^{164} both directly induces these cells to release serotonin and, at even lower concentrations, significantly augments IgE-dependent serotonin release by these cells (19). Yet WCB6F$_1$-+/+ mice that had approximately two-fold elevations of tracheal, peribronchial, and cardiac mast cells because of chronic subcutaneous treatment with rrSCF^{164} exhibited cardiopulmonary changes in association with IgE-dependent pas-

Table III. Maximum Percent Increase in HR or Percent Decrease in C_{dyn} or G_L during the First 10 min after DNP-HSA Challenge in Mice That Had Been Passively Sensitized with Mouse Monoclonal IgE Anti-DNP

Pretreatment	DNP-HSA	HR	C_{dyn}	G_L
	μg/mouse			
WCB6F$_1$-+/+ mice				
SCF	1,000	64±7*	−15±4*	−34±12*
SCF	200	50±28*	−14±6*	−27±14*
SCF	5	42±17*	−9±4‡	−6±6
SCF	0	−1±1	−3±3	−4±2
Vehicle	1,000	61±7*	−14±3*	−28±7*
Vehicle	200	72±6*	−14±4*	−27±5*
Vehicle	5	57±13*	−7±3	−5±5
Vehicle	0	−1±1	−5±3	−5±4
WCB6F$_1$-*Sl/Sl^d* mice				
SCF	1,000	65±32*§	−9±4‡**	−4±5¶
SCF	200	62±24*§	−5±3**	−2±4¶‖
SCF	5	17±20‡	−2±7	−4±6
SCF	0	−1±4	−4±3	−5±8
Vehicle	1,000	1±6¶	−6±2¶	−5±4¶
Vehicle	200	1±3‡¶	−6±5**	−8±5¶
Vehicle	0	−4±5	−5±2	−8±8

Data are mean±SD of the maximum percent change vs. the baseline values at time of challenge. * $P < 0.001$, ‡ $P < 0.05$ by Student's *t* test (two tailed), vs. data from mice of the same genotype and rrSCF^{164} or vehicle treatment status that had been challenged with 0 μg DNP-HSA. § $P < 0.001$, ‖ $P < 0.01$ by Student's *t* test (two tailed), vs. data from the vehicle-treated mice of the same genotype that had been challenged with the same dose of DNP-HSA. ¶ $P < 0.001$, ** $P < 0.01$ by Student's *t* test (two tailed), vs. data from WCB6F$_1$-+/+ mice of the same rrSCF^{164} or vehicle treatment status that had been challenged with the same dose of DNP-HSA.

Figure 1. Pulmonary dynamic compliance, pulmonary conductance, and heart rate in genetically mast cell–deficient *Sl/Sl^d* mice (*open symbols*) or congenic normal (+/+) mice (*filled symbols*) that had been passively sensitized with monoclonal mouse anti-DNP IgE antibodies, and then, 1 d later, challenged by rapid intravenous infusion of 0.9% NaCl (*lines without symbols*) or 0.9% NaCl containing 5 μg (*triangles*), 200 μg (*circles*), or 1,000 μg (*squares*) of DNP-HSA. (*A*) *Sl/Sl^d* mice that had been treated by daily subcutaneous injection of vehicle alone for 21 d before passive sensitization with IgE anti-DNP. (*B*) *Sl/Sl^d* mice that had been treated by daily subcutaneous injection of rrSCF^{164} for 21 d before passive sensitization with IgE anti-DNP. (*C*) +/+ mice that had been treated by daily subcutaneous injection of vehicle alone for 21 d before passive sensitization with IgE anti-DNP. (*D*) +/+ mice that had been treated by daily subcutaneous injection of rrSCF^{164} for 21 d before passive sensitization with IgE anti-DNP. In groups in which some mice died before the end of the 60-min period of observation, mean values were determined based on data for the surviving mice. For clarity, only mean values are shown. At all intervals in all groups, the SEM for measurements of C_{dyn}, G_L, or HR were always ≤ 25% and usually < 10% of the mean. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ by ANOVA over the first 20 min of the response vs. data from mice of the same genotype and rrSCF^{164} or vehicle treatment status, but that had been challenged with 0 μg of DNP-HSA. ††† $P < 0.001$ by ANOVA over the first 20 min of the response vs. data from the vehicle-treated mice of the same genotype that had been challenged with the same dose of DNP-HSA. ‡ $P < 0.5$, ‡‡ $P < 0.01$, ‡‡‡ $P < 0.001$ by ANOVA over the first 20 min of the response vs. data from the *Sl/Sl^d* mice of the same rrSCF^{164} or vehicle treatment status that had been challenged with the same dose of DNP-HSA.

Table IV. Death Rates in SCF- or Vehicle-treated WCB6F$_1$-Sl/Sl^d (Sl/Sl^d) or WCB6F$_1$-+/+ (+/+) Mice That Had Been Passively Sensitized with Mouse Monoclonal IgE Anti-DNP and then Challenged with Various Doses of DNP-HSA

Mice	Treatment	Death rate from DNP-HSA challenge			
		0 μg/mouse	5 μg/mouse	200 μg/mouse	1,000 μg/mouse
+/+	SCF	0/15	0/4	4/7*	1/11‖
+/+	Vehicle	0/14	0/4	3/8‡	8/11§
Sl/Sl^d	SCF	0/14	0/4	0/10	0/9
Sl/Sl^d	Vehicle	0/8	ND	0/9	0/9

DNA, does not apply; ND, not done. ‡ $P < 0.05$, * $P < 0.01$, or § $P < 0.001$ by Fisher's exact test vs. data from mice of the same genotype and rrSCF^{164} or vehicle treatment status that had been challenged with 0 μg DNP-HSA. ‖ $P < 0.01$ vs. data from vehicle-treated mice of the same genotype that had been challenged with the same dose of DNP-HSA.

sive anaphylaxis that were virtually indistinguishable from those in identically challenged WCB6F$_1$-+/+ mice that had been treated with vehicle instead of rrSCF^{164} (Fig. 1, *C* and *D*, and Table III). Indeed, when the responses in rrSCF^{164}- vs. vehicle-treated mice were compared by ANOVA over the first 5, 10, 20, or 60 min after antigen challenge, the only significant difference to emerge was a significantly ($P < 0.05$) lower HR response in rrSCF^{164}-treated vs. vehicle-treated mice over the first 5 min after challenge with 200 μg of DNP-HSA.

Analysis of death rates in antigen-challenged rrSCF^{164}- vs. vehicle-treated +/+ mice revealed an even more remarkable result (Table IV). None of the WCB6F$_1$-+/+ mice challenged with 5 μg of DNP-HSA died, and challenge with 200 μg DNP-HSA produced statistically indistinguishable death rates in rrSCF^{164}-treated mice (4/7) or vehicle-treated mice (3/8). However, challenge with 1,000 μg of DNP-HSA resulted in significantly ($P < 0.01$) fewer deaths in rrSCF^{164}-treated mice (1/11) than in vehicle-treated mice (8/11). The intervals until death in rrSCF^{164}- or vehicle-treated +/+ mice were ~ 40, 40, 50, and 60 min or ~ 40, 50, and 60 min, respectively, at the 200-μg dose of DNP-HSA, and ~ 50 min or ~ 20, 30, 40, 50, 50, 55, 60, and 60 min, respectively, at the 1,000-μg dose of DNP-HSA.

Comparison of the cardiopulmonary changes and death rates associated with passive anaphylaxis in rrSCF^{164}-treated WCB6F$_1$-+/+ vs. WCB6F$_1$-Sl/Sl^d mice. The HR responses of rrSCF^{164}-treated Sl/Sl^d or +/+ mice challenged with various doses of DNP-HSA were quite similar (Fig. 1, *B* and *D*, and Table III). Indeed, the only significant differences occurred at 5 μg DNP-HSA, with greater responses in the +/+ than in the Sl/Sl^d mice ($P = 0.0124$ or 0.0048 when compared by ANOVA over the first 20 or 60 min after challenge, respectively). In marked contrast, the pulmonary responses in the +/+ mice were significantly greater than those in the Sl/Sl^d mice at all three doses of antigen challenge (Fig. 1, *B* and *D*, and Table III). Moreover, challenge with 200 μg of DNP-HSA resulted in the death of 4 of 7 rrSCF^{164}-treated +/+ mice but 0 of 10 rrSCF^{164}-treated Sl/Sl^d mice ($P = 0.0147$ by Fisher's exact test). By contrast, challenge with 1,000 μg of DNP-HSA resulted in the deaths of only 1 of 11 rrSCF^{164}-treated +/+ mice and 0 of 9 rrSCF^{164}-treated Sl/Sl^d mice ($P = 0.55$ by Fisher's exact test).

Extent of mast cell activation associated with IgE-dependent passive anaphylaxis in rrSCF^{164}-treated vs. vehicle-treated mice. The extent of mast cell activation associated with anaphylaxis was assessed in WCB6F$_1$-+/+ mice (Figs. 2 and 3) or in rrSCF^{164}-treated Sl/Sl^d mice (Fig. 4). Figs. 2–4 present the results obtained in those anatomical sites containing > 10 mast cells/mm^2 (see Table I). This analysis established several points. First, in the absence of challenge with IgE and antigen, rrSCF^{164}-treated mice did not exhibit histological evidence of significant mast cell activation. Thus, in +/+ mice challenged with saline (0 μg DNP-HSA), there were no statistically significant differences in the extent of mast cell activation in rrSCF^{164}- vs. vehicle-treated mice, even at the skin sites that had been repeatedly injected with rrSCF^{164} (Figs. 2 and 3), nor was there significant mast cell activation in the tissues of rrSCF^{164}-treated Sl/Sl^d mice challenged with 0 μg DNP-HSA (Fig. 4). Similar results were obtained in 0.9% NaCl-challenged +/+ or Sl/Sl^d mice that had not been used for physiological measurements (data not shown).

Second, an antigen dose-response effect on mast cell activa-

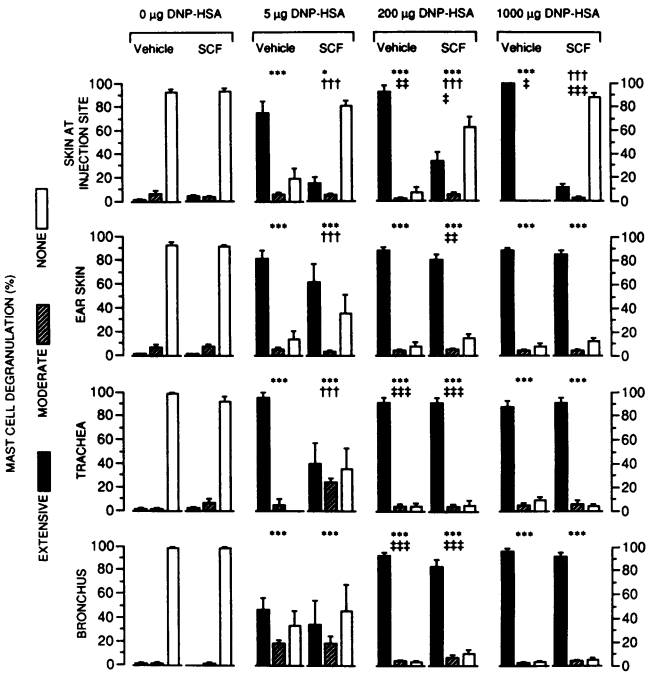


Figure 2. Extent of activation of mast cell populations in the skin at the rrSCF^{164} or vehicle injection site, ear skin, trachea, or peribronchial tissue in rrSCF^{164}- or vehicle-treated WCB6F$_1$-+/+ mice that had been challenged with various doses of DNP-HSA. 1-μm thick, Epon-embedded, Giemsa-stained sections were examined as described in the text to assess the extent of mast cell activation in the tissues. Data, which are expressed as mean±SEM, are from the same mice shown in Fig. 1, *C* and *D*. * $P < 0.05$ and *** $P < 0.001$ by the χ^2 test vs. data from the same anatomical site in mice of the same rrSCF^{164} or vehicle treatment status that had been challenged with 0 μg DNP-HSA. ††† $P < 0.001$ by the χ^2 test vs. data from the same anatomical site in vehicle-treated mice that had been challenged with the same dose of DNP-HSA. ‡ $P < 0.05$, ‡‡ $P < 0.01$, and $P < 0.001$ by the χ^2 test vs. data from the same anatomical site in mice of the same genotype and rrSCF^{164} or vehicle treatment status that had been challenged with the next lower dose of DNP-HSA.

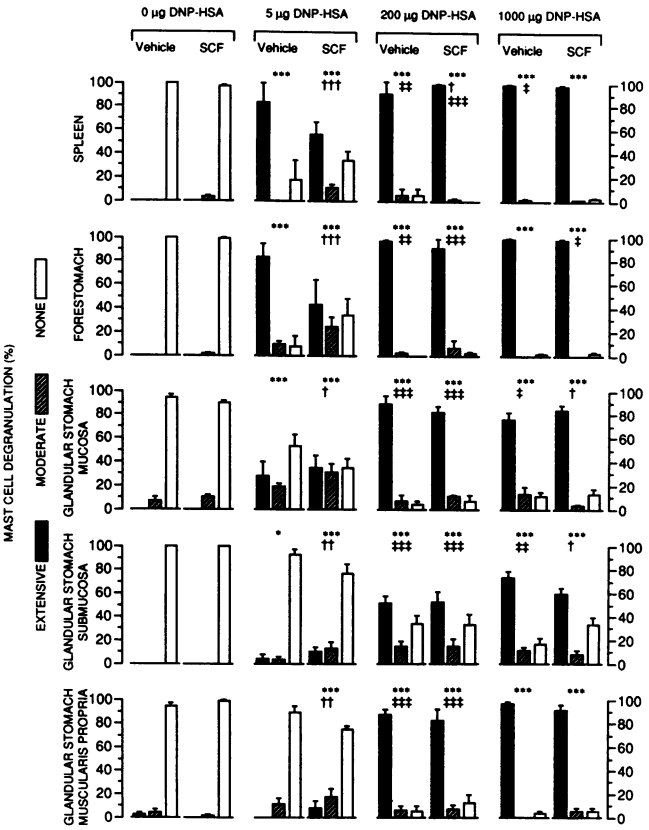


Figure 3. Extent of activation of mast cell populations in the spleen, forestomach, and glandular stomach mucosa, submucosa, or muscularis propria in rrSCF^{164}- or vehicle-treated WCB6F$_1$-+/+ mice that had been challenged with various doses of DNP-HSA. 1-μm-thick, Epon-embedded, Giemsa-stained sections were examined as described in the text to assess the extent of mast cell activation in the tissues. Data, which are expressed as mean±SEM, are from the same mice shown in Fig. 1, *C* and *D*. *$P < 0.05$ and ***$P < 0.001$ by the χ^2 test vs. the data from the same anatomical site in mice of the same rrSCF^{164} or vehicle treatment status that had been challenged with 0 μg DNP-HSA. †$P < 0.05$, ††$P < 0.01$, and †††$P < 0.001$ by the χ^2 test vs. the data from the same anatomical site in vehicle-treated mice that had been challenged with the same dose of DNP-HSA. ‡$P < 0.05$, ‡‡$P < 0.01$, and $P < 0.001$ by the χ^2 test vs. data from the same anatomical site in mice of the same genotype and rrSCF^{164} or vehicle treatment status that had been challenged with the next lower dose of DNP-HSA.

tion was observed in all rrSCF^{164} or vehicle treatment and genotype groups. Thus, in many anatomical sites, challenge with 5 μg of DNP-HSA produced substantially less mast cell activation than did challenge with the higher doses of antigen (see particularly the results for tracheal or peribronchial mast cells in Fig. 2, for glandular stomach mast cells in Fig. 3, and for spleen or glandular stomach mast cells in Fig. 4). By contrast, challenge with 200 or 1,000 μg DNP-HSA produced similar, and in most cases extensive, mast cell activation in virtually all anatomical sites. One striking exception to this generalization was a significantly ($P = 0.0002$) lower level of mast cell activation at rrSCF^{164} injection sites in WCB6F$_1$-+/+ mice challenged with 1,000 μg of DNP-HSA than in those challenged with 200 μg of DNP-HSA (Fig. 2). This result, while unexplained, may be of some interest, particularly since the death rate in rrSCF^{164}-treated WCB6F$_1$-+/+ mice challenged with 200 μg of DNP-HSA was significantly higher than that in rrSCF^{164}-treated +/+ mice challenged with 1,000 μg of DNP-HSA (Table IV).

Third, chronic treatment with rrSCF^{164} appeared to alter the ability of some mast cell populations to respond to IgE-dependent activation, but the nature of this effect differed in different anatomical sites. For example, the extent of mast cell activation at the cutaneous injection site was substantially and significantly ($P < 0.001$) less in rrSCF^{164}-treated than in vehicle-treated +/+ mice at every dose of antigen challenge. And at the 5-μg dose of antigen challenge, rrSCF^{164}-treated +/+ mice also exhibited significantly less mast cell activation than did vehicle-treated +/+ mice in ear skin, trachea, and spleen (Figs. 2 and 3). However, challenge with 5 μg of DNP-HSA produced slightly, but significantly, more gastric mast cell activation in rrSCF^{164}-treated +/+ mice than in the corresponding vehicle-treated animals (Fig. 3).

Finally, the pattern of mast cell activation observed in the tissues of rrSCF^{164}-treated *Sl/Sl^d* mice was different than that seen in the rrSCF^{164}-treated congenic normal mice. Compared with rrSCF^{164}-treated +/+ mice, rrSCF^{164}-treated *Sl/Sl^d* mice exhibited at rrSCF^{164} injection sites somewhat less mast cell activation at 5 μg of DNP-HSA challenge but somewhat more activation at 1,000 μg of DNP-HSA (Fig. 4). Similarly, in the submucosa of the glandular stomach, rrSCF^{164}-treated *Sl/Sl^d* mice exhibited slightly less mast cell activation than did the corresponding +/+ mice at 5 μg of DNP-HSA challenge, but exhibited significantly more activation than did the +/+ mice after challenge with 200 or 1,000 μg of DNP-HSA (Fig. 4). The reasons for the differences in the extent of IgE-dependent activation of mast cells in the tissues of *Sl/Sl^d* or congenic +/+ mice after chronic treatment with rrSCF^{164} are not clear.

Cardiopulmonary changes and death rates associated with IgE-dependent passive anaphylaxis in rrSCF^{164}-treated WCB6F$_1$-+/+ or -Sl/Sl^d mice are similar in mice that had received the last injection of rrSCF^{164} 1 d or 1 h before antigen challenge. We considered the possibility that providing the last dose of rrSCF^{164} 1 h before antigen challenge might have a greater effect on the severity of IgE-dependent anaphylaxis than giving the last dose of the cytokine 1 d before elicitation of the response. Baseline values of HR, C_{dyn}, and G_L were not significantly different in groups of four to five +/+ or *Sl/Sl^d* mice treated for 22 d with rrSCF^{164} or vehicle (data not shown). After challenge with 1,000 μg of DNP-HSA, rrSCF^{164}- or vehicle-treated *Sl/Sl^d* mice exhibited maximum changes in HR, C_{dyn}, or G_L (Table V) that were not significantly different than those observed in the corresponding groups of *Sl/Sl^d* mice that had been treated with rrSCF^{164} or vehicle for 21 d (Table III). In +/+ mice, the maximum changes in HR, C_{dyn}, or G_L observed after challenge with 1,000 μg of DNP-HSA were virtually identical in mice treated for 22 d with rrSCF^{164} or vehicle (Table V). However, the maximum intensity of the pulmonary responses in the +/+ mice that had been treated with rrSCF^{164} or vehicle for 22 d was slightly but significantly greater than that in the corresponding groups of +/+ mice that had been treated with rrSCF^{164} or vehicle for 21 d (compare data in Tables III and V). Death rates in +/+ mice were 1 of 4 or 3 of 5 in mice treated for 22 d with rrSCF^{164} or vehicle, respectively, and in the corresponding groups of *Sl/Sl^d* mice were 0 of 5 or 0 of 4, respectively. These results are very similar to those obtained in mice treated with rrSCF^{164} or vehicle for 21 d (Table IV, last column). Histological analysis revealed

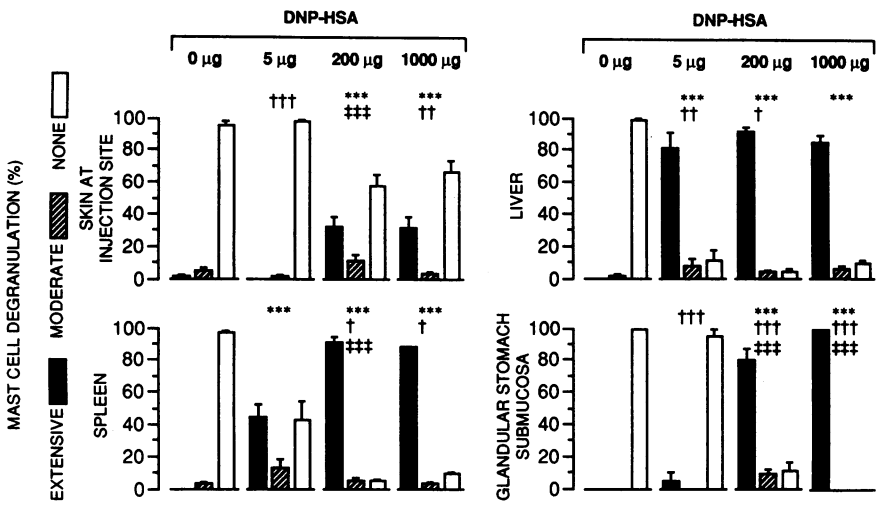


Figure 4. Extent of activation of mast cell populations in the skin at the rrSCF^{164} or vehicle injection site, spleen, liver, and glandular stomach mucosa in rrSCF^{164}-treated WCB6F$_1$-*Sl/Sl^d* mice that had been challenged with various doses of DNP-HSA. 1-μm-thick, Epon-embedded, Giemsa-stained sections were examined as described in the text to assess the extent of mast cell activation in the tissues. Data, which are expressed as mean±SEM, are from the same mice shown in Fig. 1 *B*. ***$P < 0.001$ by the χ^2 test vs. data from the same anatomical site in mice that had been challenged with 0 μg DNP-HSA. †$P < 0.05$, ††$P < 0.01$, and †††$P < 0.001$ by the χ^2 test vs. data from the same anatomical site in rrSCF^{164}-treated WCB6F$_1$-+/+ mice that had been challenged with the same dose of DNP-HSA. ‡$P < 0.05$, ‡‡$P < 0.01$, and ‡‡‡$P < 0.001$ by the χ^2 test vs. data from the same anatomical site in mice of the same genotype and rrSCF^{164} or vehicle treatment status that had been challenged with the next lower dose of DNP-HSA.

that numbers of mast cells and patterns of mast cell activation in the tissues of rrSCF^{164}-treated +/+ or *Sl/Sl^d* mice or vehicle-treated +/+ mice were quite similar in mice that had received 21 or 22 d of treatment. However, in some organs, +/+ mice treated with rrSCF^{164} for 22 d exhibited less extensive mast cell activation than did +/+ mice treated with vehicle for 22 d (e.g., 64±8 vs. 79±3% tracheal mast cells with extensive activation, $P < 0.05$) or treated with rrSCF^{164} for 21 d (e.g., 64±8 vs. 91±4% tracheal mast cells with extensive activation in +/+ mice treated with rrSCF^{164} for 22 vs. 21 d, respectively, $P < 0.001$). Taken together, all of these findings indicate that chronic treatment with rrSCF^{164} does not increase the severity of IgE-dependent passive anaphylaxis, whether the last administration of cytokine is given 1 d or 1 h before antigen challenge.

Table V. Maximum Percent Increase in HR or Percent Decrease in C_{dyn} or G_L during the First 10 min after Challenge with 1,000 μg of DNP-HSA in Mice That Had Been Passively Sensitized with Mouse Monoclonal IgE Anti-DNP and That Had Received the Last Injection of rrSCF^{164} or Vehicle 1 h before DNP-HSA Challenge

Pretreatment (22-d injection)	DNP-HSA	HR	C_{dyn}	G_L
	μg/mouse			
WCB6F$_1$-+/+ mice				
SCF	1,000	80±33	−23±3*	−45±10
Vehicle	1,000	78±48	−20±3*	−42±10*
WCB6F$_1$-*Sl/Sl^d* mice				
SCF	1,000	73±48‡	−9±5§	−4±2‖
Vehicle	1,000	−1±4	−7±3‖	−6±5¶

Data are mean±SD of the maximum percent change vs. the baseline values at time of challenge. * $P < 0.01$ by Student's *t* test (two tailed), vs. data from mice of the corresponding genotype and rrSCF^{164} or vehicle and DNP-HSA treatment status that had been injected with the last dose of rrSCF^{164} or vehicle 1 d before DNP-HSA challenge (Table III). ‡ $P < 0.01$ by Student's *t* test (two tailed), vs. the vehicle-treated group challenged with the same dose of DNP-HSA. § $P < 0.01$, ‖ $P < 0.001$ by Student's *t* test (two tailed), vs. the corresponding WCB6F$_1$-+/+ group of the same rrSCF^{164} or vehicle treatment status.

Discussion

Chronic treatment with rrSCF^{164} induces mast cell development in WCB6F$_1$-Sl/Sl^d mice, and these mice express cardiopulmonary changes in response to challenge for passive anaphylaxis. Vehicle-treated *Sl/Sl^d* mice were essentially devoid of tissue mast cells, and these animals did not exhibit detectable alterations in HR, C_{dyn}, or G_L in response to passive sensitization with anti-DNP IgE and challenge with DNP-HSA. In contrast, rrSCF^{164}-treated *Sl/Sl^d* mice developed mast cell populations in many anatomical sites. These mice also developed tachycardia after passive sensitization and challenge with DNP-HSA. Indeed, rrSCF^{164}-treated *Sl/Sl^d* mice challenged with 200 or 1,000 μg of DNP-HSA exhibited HR responses that were statistically indistinguishable from those in the identically challenged rrSCF^{164}-treated congenic normal (+/+) mice.

However, rrSCF^{164}-treated *Sl/Sl^d* mice did not exhibit striking changes in pulmonary function in association with passive anaphylaxis. None of the G_L responses in mice challenged with 5, 200, or 100 μg of DNP-HSA was significantly different than those in mice challenged with saline, and only the 1,000-μg dose of antigen challenge produced a C_{dyn} response that was significantly different than the values in mice challenged with saline alone. And at all three doses of antigen challenge, rrSCF^{164}-treated +/+ mice exhibited diminutions in C_{dyn} and/or G_L that were significantly greater than those in the identically challenged rrSCF^{164}-treated *Sl/Sl^d* mice.

The inability of rrSCF^{164}-treated *Sl/Sl^d* mice to develop a significant pulmonary component of passive anaphylaxis may have reflected the fact that even though these mice had numbers of mast cells in the pulmonary parenchyma that were 3.7-fold those in rrSCF^{164}-treated +/+ mice, the density of mast cells in the trachea or peribronchial tissues of rrSCF^{164}-treated

Sl/Sl^d mice was only 0.1 or 0.06%, respectively, that in the same anatomical site in the rrSCF^{164}-treated +/+ mice. On the other hand, in a section of heart, rrSCF^{164}-treated *Sl/Sl*^{d} mice had only 1.1% the density of mast cells observed in rrSCF^{164}-treated +/+ mice. Yet the magnitude of the HR responses associated with passive anaphylaxis induced by challenge with 200 or 100 μg of DNP-HSA were quite similar in rrSCF^{164}-treated *Sl/Sl*^{d} or +/+ mice. These findings suggest that the tachycardia associated with passive anaphylaxis in this model system may largely reflect factors other than the production of mediators by activated cardiac mast cells. Alternatively, elevation in HR may represent an especially sensitive indicator of mast cell mediator release, such that significant responses can be produced by the activation of relatively small numbers of cardiac mast cells.

Chronic treatment with rrSCF^{164} induces mast cell hyperplasia in WCB6F$_1$-+/+ mice, but these mice do not exhibit passive anaphylaxis responses that are increased in severity compared with those in the vehicle-treated +/+ mice. The demonstration that induction of mast cell development in *Sl/Sl*^{d} mice by treatment with rrSCF^{164} confers on these animals the ability to express some of the cardiopulmonary changes that are associated with passive anaphylaxis in normal mice provides further evidence that mast cells can be important in some, but perhaps not all, IgE-dependent anaphylactic reactions (30, 31, 35–37). Yet this was not a surprising result. In contrast, we did not expect that rrSCF^{164}-treated WCB6F$_1$-+/+ mice, which developed statistically significant increases in the numbers of mast cells in the heart, trachea, peribronchial tissues, and many other anatomical sites, would exhibit cardiopulmonary changes associated with IgE-dependent passive anaphylaxis that were virtually identical to those in vehicle-treated +/+ mice. Given evidence indicating that short-term incubation of mouse (19) or human (25, 26) mast cells with SCF in vitro augments IgE-dependent mediator release from these cells, our observation that challenge with the highest dose of antigen produced significantly fewer deaths during the first 60 min after administration of DNP-HSA in rrSCF^{164}-treated +/+ mice (1/11) than in vehicle-treated +/+ mice (8/11, $P < 0.01$) was even more unexpected.

On the other hand, the living organism is so complex in comparison with in vitro systems that it is not uncommon for in vivo experiments to produce findings that on first glance appear to be inconsistent with the results of work in vitro. We have no data that can explain why the cardiopulmonary changes associated with IgE-dependent passive anaphylaxis in rrSCF^{164}-treated +/+ mice were not significantly different than those in identically challenged vehicle-treated mice. And we can not explain the lower fatality rate associated with passive anaphylaxis in rrSCF^{164}-treated, as opposed to vehicle-treated, +/+ mice. However, it is possible to speculate concerning some of the factors that may have contributed to these findings.

Even though a single intradermal administration of rrSCF^{164} induces c-*kit*-dependent activation of dermal mast cells in mice (18), neither dermal mast cells at rrSCF^{164} injection sites, nor any of the other mast cell populations we assessed, exhibited morphological evidence of significant activation in the absence of challenge with specific antigen, nor did rrSCF^{164}-treated +/+ mice exhibit baseline values for HR, C_{dyn}, or G_L that were significantly different than those in the vehicle-treated +/+ mice. Taken together, these findings indicate that daily subcutaneous administration of rrSCF^{164} for 21 d does not result in a persistent state of extensive mast cell activation. The extent to which this reflects the biodistribution of rrSCF^{164} administered by subcutaneous injection, as opposed to desensitization of mast cell populations to some of the effects of this cytokine, remains to be determined.

In mouse peritoneal mast cells, preincubation with rrSCF^{164} for up to 2 d before antigen challenge augmented IgE-dependent serotonin release by these cells (19). However, in isolated human skin mast cells, 24-h preincubation with rhSCF, in contrast to short-term (10 min to 1 h) preincubation with the cytokine, did not augment the ability of these cells to release mediators in response to IgE-dependent activation (25). In the present study, morphological assessment indicated that the effects of chronic treatment with rrSCF^{164} on the extent of IgE-dependent mast cell activation in vivo may vary both in magnitude and in sign in anatomically distinct populations of mast cells. Thus, in +/+ mice challenged with 5 μg of DNP-HSA, rrSCF^{164} treatment was associated with greatly reduced IgE-dependent mast cell activation in the skin, trachea, peribronchial tissue, spleen, and forestomach, but with slightly enhanced IgE-dependent mast cell activation in the glandular stomach.

We also found that the dermal mast cells that had been chronically exposed to the highest concentrations of rrSCF^{164}, i.e., those at the actual rrSCF^{164} injection sites, exhibited a more dramatic reduction in responsiveness to IgE-dependent activation than did dermal mast cells at sites not directly injected with the cytokine (i.e., in the ear skin). Thus, the percent of mast cells that exhibited extensive activation at injection sites treated with vehicle vs. rrSCF^{164} for 21 d was 75±10 vs. 15±5% at 5 μg of DNP-HSA, 92±4 vs. 33±8% at 200 μg of DNP-HSA, and 99±0.3 vs. 11±3% at 1,000 μg of DNP-HSA. In contrast, there were no statistically significant differences in the extent of dermal mast cell activation in the ear skin of vehicle- vs. rrSCF^{164}-treated +/+ mice challenged with 200 or 1,000 μg of DNP-HSA. A number of factors may have contributed to these findings besides differences in the local concentrations of rrSCF^{164}. One of these is the possibility that the extraordinarily high densities of mast cells at rrSCF^{164} injection sites influence the biodistribution or biological activity of the passively administered anti-DNP IgE or DNP-HSA used to induce mast cell activation in this system.

It should be noted that the diminished IgE-dependent activation exhibited by mast cell populations in rrSCF^{164}-treated vs. vehicle-treated +/+ mice may not necessarily indicate that the net amount of mast cell–associated mediators released in association with passive anaphylaxis in rrSCF^{164}-treated mice was less than that in the vehicle-treated animals. The largest difference in mast cell activation in animals treated with vehicle vs. rrSCF^{164} for 21 d was at subcutaneous injection sites in mice challenged with 1,000 μg of DNP-HSA (99±0.3 vs. 11±3% extensively activated mast cells in vehicle- vs. rrSCF^{164}-treated mice, a nine-fold difference). However, the mast cell density at these sites in rrSCF^{164}-treated mice was 48-fold that in vehicle-treated animals. As a result, it is possible that the amount of mast cell–associated mediators released at the rrSCF^{164}-treated sites actually exceeded that at the vehicle-treated sites.

The concept that the large mast cell populations at the rrSCF^{164} injection sites may have represented a source of very large quantities of mast cell–associated mediators also may

provide one explanation for the unusual dose-response relationship between the amount of antigen challenge and the death rate in rrSCF^{164}-treated mice. In all anatomical sites other than the subcutaneous injection sites, challenge with 200 or 1,000 μg of DNP-HSA produced very similar amounts of mast cell activation in both rrSCF^{164}-treated and vehicle-treated +/+ mice. And in vehicle-treated +/+ mice, challenge with 1,000 μg of DNP-HSA produced slightly more mast cell activation at subcutaneous injection sites than did challenge with 200 μg of antigen (99±0.3 vs. 92±4% extensively activated mast cells, respectively). However, challenge with 1,000 μg of DNP-HSA produced significantly less mast cell activation at subcutaneous injection sites in rrSCF^{164}-treated +/+ mice than did challenge with 200 μg of DNP-HSA (11±3 vs. 33±8% extensively activated mast cells, respectively). Thus, in rrSCF^{164}-treated +/+ mice, the highest mortality rate due to passive anaphylaxis occurred in mice challenged with the dose of antigen that produced the most extensive activation of the large mast cell population at the rrSCF^{164} injection sites. Unfortunately, we have no data that can explain why 200 μg of DNP-HSA produced more activation of this mast cell population than did 1,000 μg of DNP-HSA.

Furthermore, we do not know why fatalities due to passive anaphylaxis in rrSCF^{164}-treated +/+ mice challenged with 1,000 μg of DNP-HSA were so low compared with those in identically challenged vehicle-injected +/+ mice. Several lines of evidence (30, 31), including the present results, indicate that the death associated with at least some IgE-dependent passive anaphylaxis reactions in mice is mast cell dependent. Yet the rrSCF^{164}-treated mice had significantly higher mast cell densities than the vehicle-treated mice in almost all anatomical sites tested. Moreover, at all anatomical sites other than the subcutaneous injection sites, the extent of mast cell activation that was produced by challenge with 1,000 μg of DNP-HSA was virtually identical in both the rrSCF^{164} and the vehicle treatment groups. And as suggested above, the low level of IgE-dependent activation of the large population of mast cells at the rrSCF^{164} injection sites may have resulted in the release of the same or greater amounts of mediators, as did the more extensive degree of activation of the much smaller mast cell population at the corresponding vehicle-injected sites.

On the other hand, it is not known whether the spectrum and amounts of mediators produced upon IgE-dependent mast cell activation are identical in rrSCF^{164}- or vehicle-treated +/+ mice, nor do we understand the mechanism of death in mice experiencing IgE-dependent passive anaphylaxis. But whatever the explanation for the lower death rates in rrSCF^{164}- vs. vehicle-injected mice, our analysis of the extent of mast cell activation, the cardiopulmonary changes, and the mortality associated with IgE-dependent passive anaphylaxis in rrSCF^{164}- or vehicle-treated normal mice indicates that prolonged treatment with this cytokine does not augment, and in certain respects may diminish, the severity of the reaction.

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