The c-kit Ligand, Stem Cell Factor, Can Enhance Innate Immunity Through Effects on Mast Cells

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Summary

Mast cells are thought to contribute significantly to the pathology and mortality associated with anaphylaxis and other allergic disorders. However, studies using genetically mast cell–deficient WBB6F1-KitW/KitW-v and congenic wild-type (WBB6F1+/+) mice indicate that mast cells can also promote health, by participating in natural immune responses to bacterial infection. We previously reported that repetitive administration of the c-kit ligand, stem cell factor (SCF), can increase mast cell numbers in normal mice in vivo. In vitro studies have indicated that SCF can also modulate mast cell effector function. We now report that treatment with SCF can significantly improve the survival of normal C57BL/6 mice in a model of acute bacterial peritonitis, cecal ligation and puncture (CLP). Experiments in mast cell–reconstituted WBB6F1-KitW/KitW-v mice indicate that this effect of SCF treatment reflects, at least in part, the actions of SCF on mast cells. Repetitive administration of SCF also can enhance survival in mice that genetically lack tumor necrosis factor (TNF-α), demonstrating that the ability of SCF treatment to improve survival after CLP does not solely reflect effects of SCF on mast cell–dependent (or –independent) production of TNF-α. These findings identify c-kit and mast cells as potential therapeutic targets for enhancing innate immune responses.

Key words: c-kit • innate immunity • mast cells • stem cell factor • tumor necrosis factor-α

Mast cells are thought of primarily as key effector cells in IgE-dependent immune responses, such as those involved in the pathogenesis of allergic disorders or in certain examples of immunity to parasites (1). However, recent work has identified another facet of mast cell effector function, the promotion of innate, or “natural”, immunity to bacterial infection (2, 3). For example, Echtenacher et al. (2) reported that genetically mast cell–deficient KitW/KitW-v mice exhibited greatly increased mortality after cecal ligation and puncture (CLP) compared with wild-type mice, and that the survival of KitW/KitW-v mice in this model of septic peritonitis was improved if the KItW/KitW-v mice had undergone adoptive repair of their peritoneal mast cell deficiency before CLP. The same study also showed that the mast cell–dependent protective response to CLP could be greatly diminished in mice treated with antibodies to TNF-α (2). Prodeus et al. (4) later reported that normal levels of mast cell activation and TNF-α production in this CLP model required an intact complement system, and that complement C3 or C4 knockout mice had greatly increased mortality after CLP compared with wild-type mice.

These findings indicated that a lack of mast cells, or deficits in other components of innate defense mechanisms (e.g., TNF-α, complement), can result in impaired natural immunity to bacterial infection (2–5). However, these studies did not evaluate whether, in normal animals, manipulations that can increase mast cell numbers and/or enhance mast cell function might improve the animals’ ability to express innate immunity.
We therefore investigated whether repetitive administration of the c-kit ligand, stem cell factor (SCF) [references 6, 7; also known as kit ligand [reference 8], mast cell growth factor [MGF, reference 9], or steel factor [reference 10]], could influence the survival of mice subjected to CLP. By acting synergistically with other growth factors, SCF can promote the proliferation and further differentiation of hematopoietic progenitor cells; SCF is also critical for the normal development of germ cells, melanocytes, and interstitial cells of Cajal (6, 7). However, interactions between SCF and c-kit are especially important in promoting mast cell survival (11–13), proliferation (14, 15), and maturation (14, 15), and can also enhance certain mast cell effector functions (16–19). We previously reported that the daily subcutaneous administration of E. coli-derived recombinant rat SCF (rrSCF) to normal mice or rats can increase mast cell numbers in many anatomical sites, including the peritoneal cavity (13, 15, 20). We now report that repetitive treatment of mice with SCF can markedly improve their survival after CLP, and that this effect of SCF treatment reflects, at least in part, its actions on mast cells.

Materials and Methods

Animals. C57BL/6 mice, genetically mast cell–deficient WBB6F1-Kit<sup>W–</sup>/Kit<sup>W+</sup> (Kit<sup>W–</sup>/Kit<sup>W+</sup>) mice, and the congenic normal WBB6F1-Kit<sup>+/+</sup>/Kit<sup>+/+</sup> (Kit<sup>+/+</sup>/Kit<sup>+/+</sup>) mice were purchased from The Jackson Laboratory, Bar Harbor, ME. Adult Kit<sup>W–</sup>/Kit<sup>W+</sup> mice ordinarily contain <1.0% of the number of dermal mast cells present in the skin of the congenic normal (+/+ ) mice, and have no detectable mature mast cells in the gastrointestinal tract or peritoneal cavity (21–23). TNF-α<sup>−/−</sup> mice were generated by gene targeting, and TNF-α<sup>−/−</sup> and Kit<sup>W–/−</sup> mice were maintained on a mixed 129/Sv × C57BL/6 genetic background (24). Mice were kept in community cages at the Animal Care Facilities of the Beth Israel Deaconess Medical Center or, for TNF-α<sup>−/−</sup> and Kit<sup>W–/−</sup> mice, the GSF-Forschungszentrum, at light periods of 12 h and 64% relative humidity. Animals were maintained on a diet of standard chow and water ad libitum. All animal care and experimentation was conducted in accord with current National Institutes of Health and Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee guidelines or under official permission from the Regierung der Oberpfalz.

Treatment with SCF. Mice received 21 daily subcutaneous injections into the same area of back skin of vehicle (sterile 0.9% NaCl containing 0.1% BSA, fraction V, fatty acid-free [ICN Immunobiologicals, Lisle, IL]), E. coli–derived recombinant rat SCF (rrSCF) at 50, 100, or 200 μg/kg per day in 150–250 μl of vehicle, or rrSCF that had been modified by the covalent attachment of polyethylene glycol (rrSCF-peg) to increase the biological half-life of the cytokine, at 30 or 100 μg/kg per day in 150–250 μl of vehicle (13, 15, 25). rrSCF and rrSCF-peg were from AMGEN Inc. (Thousand Oaks, CA).

Peritoneal Lavage. Mice were killed by CO<sub>2</sub> inhalation, then the abdominal skin was washed with 70% ethanol, the peritoneum was exposed by a 1–2-cm midline abdominal incision, and 2.0 ml of sterile, pyrogen-free 0.9% NaCl and 8.0 ml of air were injected into the peritoneal cavity via a 25-gauge needle. The abdomen was massaged gently for ~3 min and the peritoneal fluid was recovered via a 22-gauge needle, stained for mast cells by Kimura stain, and counted in a N eubauer chamber; the lavage fluid was then cytospun and stained by May Grünwald-Giemsa stain (26).

Results and Discussion

Effective Treatment with SCF Increases Peritoneal Mast Cell Numbers and Enhances Survival after CLP in C57BL/6 Mice. We first administered various doses of non–peg–derivatized rrSCF (rrSCF) or peg–derivatized rrSCF (rrSCF-peg), or vehicle alone subcutaneously to C57BL/6 mice daily for 21 d, then killed some of the mice for quantification of mast cells in the peritoneal cavity and in the rrSCF or vehicle cutaneous injection sites, whereas other, identically treated, mice underwent CLP; the CLP-treated mice continued to receive daily subcutaneous injections of rrSCF or rrSCF-peg, or vehicle for as long as they survived.

We found that rrSCF-peg was more effective than rrSCF in increasing numbers of mast cells at the cutaneous injection sites (Fig. 1 A) or in the peritoneal cavity (Fig. 1 B). Both rrSCF-peg and rrSCF exhibited a positive dose–response effect on mast cell numbers at the skin injection site (27). In brief, mice were deeply anesthetized and the cecum was exposed by a 1–2-cm midline incision on the anterior abdomen and subjected to ligation of the distal half followed by a single puncture with a 0.7–or 0.9-mm (for TNF-α<sup>−/−</sup> or Kit<sup>W–/−</sup> mice) needle. The cecum was then replaced into the abdomen and the wound was closed using 9-0 mm steel wound clips. Mice were observed for mortality at least four times daily over a period of 14 d. Mice that clearly were moribund were killed by CO<sub>2</sub> inhalation. Some TNF-α<sup>−/−</sup> and Kit<sup>W–/−</sup> mice were subjected to ligation of 80% of the distal cecum followed by two punctures with a 0.9-mm needle in order to induce a more severe bacterial peritonitis.

Selective Mice of C57BL/6 mice, genetically mast cell–deficient Kit<sup>W+</sup>/Kit<sup>W+</sup> Mice. Kit<sup>W+</sup>/Kit<sup>W+</sup> mice (male, 4–6 wk old) were repaired of their mast cell deficiency selectively and locally by the injection of growth factor–dependent bone marrow–derived cultured mast cells (BM CM Cs) into the peritoneal cavity (2, 23). In brief, femoral bone marrow cells from Kit<sup>W+</sup>/Kit<sup>W+</sup> mice were maintained in vitro for ~4 wk in IL–3–containing, Con A–stimulated mouse spleen cell–conditioned medium until mast cells represented ~95% of the total cells according to staining by Giemsa (6, 16, 22). Mice receiving the BM CM Cs from BM CMCs failed to improve the recipients’ anemia (6, 22, 23). Histological Studies. After the mice had been killed, biopsy specimens of the back skin at SCF or vehicle injection sites were fixed in Carnoy’s fixative, processed into paraffin-embedded, Alcian blue–stained sections, coded so that the observer was not aware of the identity of the individual specimens, and then examined at 400× by light microscopy to quantify mast cells per squared millimeter of dermis (15).

Statistical Analysis. The significance of differences in the survival rates after CLP was assessed using the Mantel-Cox Logrank test. All other data were tested for statistical significance using the unpaired two-tailed Student’s t test. Unless otherwise specified, all data are presented as the mean ± SEM.
Survival after CLP was significantly better in all SCF treatment groups (compared with that in the vehicle-treated group) except for the one that had been treated with rrSCF at 50 μg/kg per day (Fig. 1 C). Even mice treated with rrSCF at 100 μg/kg per day, a dose that had little or no effect on numbers (Fig. 1 A) or percentages (data not shown) of PMCs, exhibited survival in mice treated with rrSCF-peg at 30 μg/kg per day (Fig. 1 C). This was also the treatment protocol that had the greatest effect on numbers of PMCs (Fig. 1 B).

Because the repair of the mast cell deficiency in +/− BM CMC → KITW/KITW−/− mice is selective (2, 22, 23), the
notypic/functional differences between the endogenous tors may have contributed to this finding, including phe-

having higher numbers of PMCs (Fig. 2

B

To assess the extent to which the effects of SCF treatment on CLP survival might be TNF-α dependent, we performed two experiments in which survival after CLP was compared in vehicle- or rrSCF-peg- (30 μg/kg per day) treated TNF-α/−/− or +/+ mice. Both experiments gave very similar results, which are pooled in Fig. 3, B–D.

These experiments used a more severe CLP procedure (80% ligation, two punctures with a 0.9-mm needle) in order to observe better any favorable effect of SCF treatment on survival. In these experiments, in contrast to those shown in Fig. 3 A, vehicle-treated TNF-α/−/+ mice exhibited only marginally enhanced overall survival (P = 0.0655) compared with vehicle-treated TNF-α/−/− mice (Fig. 3 B). However, SCF treatment resulted in improved survival after CLP in both TNF-α/−/+ mice (P < 0.0001 versus vehicle-treated TNF-α/−/− mice) and TNF-α/−/+ mice (P = 0.0119 versus vehicle-treated TNF-α/−/+ mice). Indeed, SCF treatment had an even more striking effect on survival after CLP in TNF-α/−/− mice than in the wild-type controls (Fig. 3 B).

Notably, treatment with rrSCF-peg at 30 μg/kg per day did not significantly increase numbers of PMCs in TNF-α/−/− or +/+ mice, possibly in part because “baseline” levels of PMCs (e.g., in vehicle-treated mice) were already substantially higher in TNF-α/−/− or +/+ mice than in C57BL/6 mice (compare Fig. 3 C with Fig. 1 B). On the other hand, SCF treatment did greatly increase mast cell numbers at skin injection sites in TNF-α/−/− and +/+ mice (Fig. 3 D).

Conclusions. In C57BL/6 mice, repetitive treatment with SCF significantly enhanced survival after CLP roughly in parallel with the ability of such treatment to increase numbers of PMCs. However, improved survival after CLP was also seen in C57BL/6 mice treated with 100 μg/kg per day of non-peg-derivated rrSCF, and in TNF-α/−/− or TNF-α/−/+ mice treated with 30 μg/kg per day of rrSCF-peg, even though these SCF-treated mice did not exhibit significantly increased numbers of PMCs. The latter findings strongly suggest that actions of SCF treatment other than simply the expansion of PMC numbers can contribute to the ability of this agent to enhance survival in CLP. These alternative consequences of SCF treatment in this model of innate immunity may include effects on mast cell

adoptionally transferred mast cells are the only cellular lineage in these mice that express the wild-type kit. Accordingly, the ability of SCF treatment to enhance survival after CLP in +/+ BMCMC → Kitw/Kitw mice must reflect actions of SCF treatment on mast cells. Indeed, CLP survival in SCF-treated +/+ BMCMC → Kitw/Kitw mice was not significantly different (albeit somewhat lower) than that in SCF-treated wild-type mice (Fig. 2 B and Table 1). However, compared with results in C57BL/6 mice, treatment of Kit+/+ mice with rrSCF-peg at 30 μg/kg per day had more modest effects on both PMC numbers (compare Fig. 2 A with Fig. 1 B) and survival after CLP (compare Fig. 2 B with Fig. 1 C), perhaps reflecting strain differences in these responses to SCF treatment. Note also that despite having higher numbers of PMCs (Fig. 2 A), vehicle-treated +/+ BMCMC → Kitw/Kitw mice had significantly poorer survival 14 d after CLP than did vehicle-treated Kit+/+ mice (=4 vs. 30%, P < 0.0001 for overall survival and P = 0.0046 for after day 3 survival). A number of factors may have contributed to this finding, including phenotypic/functional differences between the endogenous PMC s in Kit+/+ mice and the adoptively transplanted, in vitro-derived mast cells in the +/+ BMCMC → Kitw/Kitw mice (15, 23).

Repetitive Treatment with SCF Improves Survival after CLP in TNF-α/−/− Mice. Several lines of evidence indicate that TNF-α represents one important mediator of mast cell-dependent host resistance in CLP and other models of innate immunity to bacteria (2–4). In support of this hypothesis, we found that TNF-α/−/− mice exhibited significantly impaired survival after the standard CLP procedure (50% ligation, one needle puncture), in comparison to the corresponding wild-type mice (Fig. 3 A). To assess the extent to which the effects of SCF treatment on CLP survival might be TNF-α dependent, we performed two experiments in which survival after CLP was compared in vehicle- or rrSCF-peg- (30 μg/kg per day) treated TNF-α/−/− or +/+ mice. Both experiments gave very similar results, which are pooled in Fig. 3, B–D.

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Table 1. Significance (P values, Mantel-Cox Logrank Test) of Differences in Overall or Late-phase (>3 d after CLP) Survival in the Mice Shown in Fig. 2 B

| Kitw/Kitw (vehicle) | Kitw/Kitw (SCF) | Kitw/Kitw + M Cs (vehicle) | Kitw/Kitw + M Cs (SCF) | Kit+/+ (vehicle) | Kit+/+ (SCF) |
|---------------------|----------------|---------------------------|------------------------|----------------|-------------|
| K itw/Kitw (vehicle) | -              | 0.7113                    | 0.0325                 | 0.0015        | 0.0001      |
| K itw/Kitw (SCF)    | 0.6104         | -                         | 0.0634                 | 0.0076        | 0.0005      |
| K it+/+ (vehicle)   | 0.5151         | 0.0397                    | -                      | 0.0811        | 0.0124      |
| K it+/+ (SCF)       | 0.0084         | 0.0018                    | 0.0169                 | -             | 0.4274      |

M Cs, mast cells.

*Late-phase survival (>3 d after CLP).

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effector function (16–20); they also may include actions of SCF on c-kit+ lineages other than mast cells. For example, the CD56bright subset of human natural killer cells expresses c-kit and can exhibit enhanced IFN-γ production in response to stimulation with SCF (29, 30); however, we have no data that would permit us to speculate about the relevance of these in vitro findings in human cells to our in vivo study in mice.

On the other hand, the experiments with +/- BM CMC KIt+/Kit+/+ mice indicate that at least some of the critical effects of SCF on CLP survival can reflect actions of SCF on mast cells. Thus, KIt+/Kit+/+ mice exhibited no protective effect of SCF treatment on survival after CLP unless the animals had first been repaired of their PMC deficiency; in this setting, only the adoptively transferred mast cells of wild-type origin expressed normal kit, and therefore could have responded normally to SCF treatment.

Our studies also indicate that treatment with SCF can enhance survival after CLP even in mice that genetically lack TNF-α, indicating that SCF treatment must be able to augment mechanisms of host defense in innate immunity that can be mobilized independently of TNF-α. Finally, in confirmation of the results of our earlier experiments with WCB6F1-/+/+ mice (20), we found that mice treated with rrSCF-peg (30 μg/kg per day for 21 d) did not appear to be at substantially increased risk (versus vehicle-treated mice) for death when IgE-dependent systemic anaphylaxis was induced by intraperitoneal challenge with specific antigen (our unpublished data).

These findings are the first to show that survival in a model of innate immunity can be enhanced by treatment with SCF, a cytokine with diverse effects on mast cells, as well as many other cell types. These data are also the first to show that normal animals that have been treated to develop higher than baseline levels of mast cells can exhibit enhanced resistance to bacterial infection. Although great caution must be exercised when extrapolating from mouse studies to human medicine, our findings suggest a new approach for attempting to manage patients at risk for bacterial infection. It may be of particular interest to evaluate SCF treatment in patients with congenital or acquired immunodeficiency disorders, since such individuals have been reported to have greatly decreased numbers of mast cells in the gastrointestinal mucosa (31).

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