A Role for Stem Cell Factor (SCF): c-kit Interaction(s) in the Intestinal Tract Response to Salmonella typhimurium Infection

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Summary

Cholera toxin (CT) has been shown to induce stem cell factor (SCF) production in mouse ligated intestinal loops. Further, SCF interaction(s) with its receptor (c-kit) was shown to be important for the intestinal tract secretory response after CT exposure. In this study, we have investigated whether SCF production is induced in the intestinal tract after exposure to Salmonella typhimurium and whether this production could be an important intestinal tract response to Salmonella infection. Using a mouse ligated intestinal loop model, increased levels of SCF mRNA were detected at 2-4 h post-Salmonella challenge. Intestinal fluid obtained from Salmonella-challenged loops contained high levels of SCF by ELISA. Human and murine intestinal epithelial cell lines were also shown to have increased levels of SCF mRNA after exposure to Salmonella. Inhibition of Salmonella invasion of epithelial cells was shown to be one potentially important role for SCF:c-kit interactions in host defense to Salmonella infection. Pretreatment of human or murine intestinal cell lines with SCF resulted in a cellular state that was resistant to Salmonella invasion. Finally, mice having mutations in the white spotting (W) locus, which encodes the SCF-receptor (c-kit), were significantly more susceptible to oral Salmonella challenge than their control littermates. Taken together, the above results suggest that an important intestinal tract response to Salmonella infection is an enhanced production of SCF and its subsequent interactions with c-kit.

Stem cell factor (SCF), sometimes referred to as mast cell growth factor or c-kit ligand (KL) and its receptor, c-kit, have been extensively studied and are the subject of many recent reviews (1, 2). SCF exists in both membrane-bound and soluble forms, with the soluble form representing a cleavage product of a surface bound protein (3, 4). The c-kit protein (CD117), the receptor for SCF, is a tyrosine kinase-containing receptor of the PDGF/CSF-1R receptor family (5, 6). SCF:c-kit interactions have been shown to play different roles in a wide range of physiological functions. SCF:c-kit interactions are important for mast development and function (1, 2), melanocyte development (7, 8), germ cell development (9, 10), and may play a role in the development and function of the nervous system (11, 12). Additionally, SCF:c-kit interactions have been shown to be important for the enteric nervous system (13-15) and for development of TCRγδ+ intraepithelial lymphocytes (IEL) (16). Thus, SCF:c-kit interactions appear to be important for certain aspects of intestinal tract physiology. However, the importance of SCF:c-kit interactions in the intestinal tract response to infection has not been fully explored. Using a mouse ligated intestinal loop model, we have recently shown that cholera toxin (CT) induces the production of elevated levels of SCF in the intestinal tract, and that SCF:c-kit interactions are necessary for CT-induced intestinal fluid secretion (17). In the present study, we have investigated whether SCF-production and/or SCF:c-kit interactions are important intestinal tract responses to Salmonella infection. In this paper, we show the following: (a) Salmonella typhimurium induces enhanced production of SCF in human and mouse intestinal epithelial cell lines and in mouse ligated intestinal loops, (b) pretreating human or mouse intestinal cell lines with SCF results in a cellular state that is resistant to Salmonella invasion, and (c) mice with mutations in the white spotting (W) locus, which encodes the SCF-receptor (c-kit), are more susceptible to oral Salmonella challenge than their control littermates. Taken together, the above results suggest that an important intestinal tract response to Salmonella infection is an enhanced production of SCF and its subsequent interactions with c-kit.
Materials and Methods

Mice. All mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were housed under pathogen-free conditions. Female mice at 6-8 wk of age were used from the following mouse strains: WBB6F1/J/Wv and WBB6F1/J +. In some experiments, mice were challenged orally with varying numbers of Salmonella. LD50 values were determined by the method of Reed and Muench (18).

Reagents. Salmonella typhimurium TML-R66 were used in all experiments. Cholera toxin (CT) was obtained from List Biological Laboratories, Inc. (Campbell, CA). Recombinant rat and human SCFs were purified as previously described (19).

Cell Lines. The human colon epithelial cell lines HT-29 and T-84 were obtained from the American Type Culture Collection (Rockville, MD). The human intestinal myofibroblast cell line 18CO has been previously described (20) and was a generous gift from Dr. Don Powell (UTMB). The MODE-K cell line is a mouse intestinal epithelial cell line which has been previously characterized (21) and was obtained from Dr. Dominique Kaeserian (Unité’ d’ Immunologie et de Strategie Vaccinale Institute Pasteur de Lyon, Cedex, France). All cell lines were grown in Dulbecco’s modified Eagle’s medium containing 10% heat-inactivated fetal calf serum, 2 mmol/L L-glutamine and antibiotics. In some experiments, RNA was obtained from different cell lines at varying times post-exposure to Salmonella. In these experiments, cells were grown to confluency in 75-cm² flasks (5 × 10⁷ cells). Cells were grown in antibiotic-free medium and exposed to Salmonella (MOI 1:10) for 90 min. Cells were then washed twice and cultured with medium containing 2X antibiotics and RNA obtained at varying times post-exposure.

Northern Blot Analysis. Human and murine SCF probes have been previously described (22, 23). A cDNA probe for the housekeeping gene glyceraldehyde-3 phosphate dehydrogenase (G3PDH) was obtained from Clontech Laboratories (Palo Alto, CA). The SCF-cDNA was nick-translated in preparation for RNA hybridization. Northern blot analysis was performed as we have previously described (21, 25). Total mucosal RNA or RNA from different cell lines was extracted using guanidine isothiocyanate and purified by cesium chloride gradient centrifugation. RNA samples were electrophoresed through a 1.4% agarose gel in the presence of formaldehyde and transferred to nylon membranes. Filters were UV crosslinked and baked at 80°C for 2 h and prehybridized for 18-24 h at 42°C in 6X SSPE, 5X Denhardt’s solution, 0.5% SDS, 50% formamide, and 300 μg/ml denatured salmon sperm DNA. Hybridization was performed at 42°C for 18-24 h in 6X SSPE, 5X Denhardt’s solution, 0.1% SDS, 50% formamide, 100 μg/ml denatured salmon sperm DNA and 1 × 10⁶ CPM/ml [³²P]-DNA. Initial washes were performed at room temperature with 5X SSPE/0.5% SDS for 15 min, followed by 15 min under the same conditions. Filters were then washed twice in SSPE/0.5% SDS at 42°C for a total of 15 min. Autoradiography was performed with intensifying screens at -70°C with an exposure of 1-3 d to XAR-5 film.

Ligated intestinal loop assay: The ligated loop surgery was performed on mice as previously described (24, 25). Mice were fasted for 24 h before surgery. A midline abdominal incision was made to expose the small intestine, and a single segment (~5 cm) was ligated with 00 silk suture. Loops were injected with one of the following: 100 μl of phosphate-buffered saline (PBS) only, PBS containing S. typhimurium (10⁷ CFU), or PBS containing cholera toxin (1 μg). Incisions were closed with staples and mice were sacrificed at varying times post-surgery. At that time, loops were removed, measured, fluid volume determined, fluid saved for SCF-ELISA, and total RNA isolated as described below.

Quantitation of Bacteria-infected Cells. The agarose-gel overlay assay was used to quantitate the number of intestinal epithelial cells containing Salmonella. This assay has been previously described (26). Briefly, triplicate wells of MODE-K cells or 18CO cells were treated overnight with varying concentrations of rSCF or medium alone. Before exposure to Salmonella, cell monolayers were maintained in antibiotic-free medium and then exposed to Salmonella (MOI for MODE-K 1:4; MOI for 18CO 10:1) for 1 h. Noninternalized bacteria were then counterselected by exposing monolayers to medium containing antibiotics for 2 h at 37°C. Monolayers were then extensively washed with antibiotic-free medium and the number of bacteria-infected cells (CFU/well) determined.

Mouse SCF ELISA. An ELISA for quantitating mouse SCF was developed using the following reagents: (a) capture antibody was a rat monoclonal antibody specific for mouse SCF (JES9-28F4) and (b) detecting antibody was a purified and biotinylated rabbit IgG specific for mouse SCF (17). Immunol-2 plates (Dynatech Laboratories, Chantilly, VA) were first coated with 2 μg/ml of capture antibody for 24 h at 4°C. Wells were then washed twice with PBS/Tween-20 and blocked for 2 h with PBS containing 10% fetal calf serum. Wells were then washed twice with PBS/Tween-20 and ligated loop fluid and varying concentrations (10 ng/ml to 100 μg/ml) of rSCF added to wells for overnight incubation at 4°C. The ELISA was developed by sequential addition of detecting IgG (1 μg/ml) followed by avidin-peroxidase and substrate. Optical densities (405 nm) were next read after 30-45 min and SCF concentrations determined from a SCF standard curve. The SCF-ELISA was sensitive to a level of 400 pg/ml.

Results and Discussion

Salmonella infection of the gastrointestinal tract results in fluid secretion and inflammation. Using a mouse ligated intestinal loop model, we have previously shown that ligated intestinal loops challenged with S. typhimurium have elevated levels of mRNA for IL1, IL6, TNFα and IFNγ and that these cytokines can be detected in the fluid obtained from such infected intestinal loops (24, 25). The cellular source for these cytokines is still not completely understood. Mast cells represent potentially important sources for several of these cytokines and could play an important role in the intestinal tract inflammatory response (27). Mast cells have been shown capable of producing a number of different cytokines including IL1, IL6, TNFα, and IFNγ (28). Additionally, mast cells are abundant in mucosal tissues and have been shown to phagocytize and kill bacteria (29, 30). SCF has been shown to be an important mediator of mast cell development, mast cell survival, mast cell chemotaxis, and mast cell production of different cytokines (1, 2). Besides these functions, SCF has also been shown to be important for homeostasis of the intestinal immune system. SCF: c-kit interactions were shown to be required for the maintenance and generation of γδ⁺ IELs (16). Recently, we have shown that CT induces enhanced production of SCF by the intestinal tract and that SCF: c-kit interaction(s)
Figure 1. Ligated intestinal loops challenged with S. typhimurium have elevated levels of SCF mRNA. Ligated intestinal loops from C57BL/6 mice were challenged with one of the following: (a) PBS (lanes 1 and 2), (b) Cholera toxin (1 μg, lane 3), or (c) S. typhimurium (10^7 cfu, lanes 4 and 5). RNA was obtained at 2 h post-challenged. Each lane represents RNA obtained from an individual ligated intestinal loop. Northern blot analysis was carried out with a cDNA probe for SCF (A) or for G3PDH (B).

Figure 2. High levels of SCF are present in the fluid obtained from ligated intestinal loops challenged with S. typhimurium. Fluid was obtained at 10 h from ligated loops from C57BL/6 mice challenged with one of the following: (a) PBS (lanes 1 and 2), (b) Cholera toxin (1 μg, lane 3), or (c) S. typhimurium (10^7 CFU, lanes 4 and 5). The data presented was from fluid obtained from four mice per condition. Control loops (injected with PBS) were gavaged with a volume of PBS that was equivalent to the volumes of fluid contained in Salmonella-challenged loops. Ligated intestinal loops injected with CT or Salmonella yielded roughly equivalent levels of fluid: Salmonella = 615 ± 45 μl per loop; CT = 585 ± 70 μl per loop. SCF levels are expressed as either ng per ml of fluid or ng per loop.

Figure 3. Human and murine intestinal epithelial cell lines exposed to Salmonella have increased levels of SCF mRNA. Levels of SCF mRNA were assessed by Northern blot analysis from intestinal cell lines from mouse (MODE-K) and human (HT-29 and 18CO) origin as described in Materials and Methods section. RNA was obtained from MODE-K cells exposed to medium only for 4 h (lane 1, control), or from MODE-K cells at the following times post-exposure to S. typhimurium: 30 min (lane 2), 1 h (lane 3), 2 h (lane 4), and 4 h (lane 5). RNA was obtained from HT-29 after the following treatments: medium only for 1 h (lane 1, control), S. typhimurium for 30 min (lane 2), 1 h (lane 3), 2 h (lane 4), and 4 h (lane 5). RNA was obtained from T84 cells after 4 h with medium only (lane 1, control) and after 4 h exposure to S. typhimurium. Northern blot analysis was carried out with a cDNA probe for either mouse or human SCF (A) or for G3PDH (B).
most dramatically enhanced. The above results suggest that exposure of the intestinal tract to *Salmonella* results in an elevated production of SCF and that one potential source for SCF production is the intestinal fibroblast and/or epithelial cell.

We (31) and others (32, 33) have previously shown that pretreatment of human or mouse epithelial cells or fibroblasts with different cytokines results in a cellular state that is resistant to subsequent invasion by *Salmonella* or other invasive bacteria. Since *Salmonella* invasion induced an enhanced production of SCF, we investigated whether SCF pretreatment of fibroblasts or epithelial cells could induce a cellular state that is resistant to *Salmonella* invasion. Cultures containing MODE-K or 18CO (a human intestinal fibroblast cell line) were pretreated with varying concentrations of rSCF and then assessed for their susceptibility to *Salmonella* invasion using a previously described quantitative invasion assay (26). As seen in Fig. 4, SCF pretreatment of MODE-K cells or 18CO cells resulted in a cellular state that was resistant to *Salmonella* invasion. This effect was dependent upon the concentration of SCF, and when high concentrations of SCF were used, a 80–95% reduction in *Salmonella* infected cells was achieved. These results suggest that SCF:c-kit interactions may play a role in host defense by inducing a cellular state that does not allow for efficient bacterial invasion of epithelial cells and/or fibroblasts.

To further explore the possible role of SCF:c-kit interactions in host defense, we investigated whether WW^v^ mice have alterations in their resistance to oral *Salmonella* challenge. To investigate this possibility, we determined the LD50 for WW^v^ versus their littermate controls (++) for oral challenge with *S. typhimurium*. Groups of mice (6 per group) were given varying doses of *Salmonella* (10^2^ to 10^8^ CFU/mouse) orally and then assessed daily for 10 d. The LD50 for WW^v^ mice was 10^5^, while the LD50 for their littermate controls was 10^7^. Fig. 5 presents results from a representative experiment where WW^v^ and their littermate controls (++) (10 mice per group) were given (orally) 5 X 10^6^ CFU of *Salmonella* and followed daily for survival. Results from these experiments showed clearly that WW^v^ mice were more susceptible to oral challenge with *Salmonella*.

Puddington et al. (17) have shown that WW^v^ and Sl/Sld mice have a significantly decreased number of γδ^+^ IEL and an increased number of αβ^+^ IEL. This shift in γδ^+^ versus αβ^+^ IEL occurred in WW^v^ mice from 6 to 14 wk of age. The mice used in our studies were 8–14 wk of age, and therefore probably lacked or had significantly reduced numbers of γδ^+^ IEL. Thus, a major question concerning our in vivo results is whether the enhanced susceptibility of WW^v^ mice to oral *Salmonella* challenge is due to altered numbers of γδ^+^ versus αβ^+^ IEL and/or to some other SCF:c-kit-dependent function.

Epithelial cells are now recognized as an important cell for regulating both natural and acquired immune functions at mucosal surfaces (34). An important component of this epithelial cell regulation could be the enhancement of SCF and/or c-kit expression after infection. Enhanced expression of SCF/c-kit by epithelial cells could result in mast cell chemotaxis, enhancement of mast cell cytokine production and survival, regulation of γδ^+^IEL, inhibition of bacterial invasion of adjacent epithelial cells, and/or to some as yet characterized function(s) that is dependent upon SCF:c-kit interactions. In summary, results presented in this study show that an early response of the intestinal tract to *Salmonella* infection is the enhanced production of SCF and that a potential source of this SCF is the intestinal epithelial cell.

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**Figure 4.** SCF inhibits *Salmonella* invasion of intestinal cell lines. Triplicate wells of MODE-K cells (A) or 18CO cells (human myofibroblast cell line, B) were treated overnight with varying concentrations of SCF or medium alone. Before exposure to *Salmonella*, cell monolayers were washed in antibiotic-free medium and then exposed to *S. typhimurium* (MOI for MODE-K 1:4, MOI for 18CO 10:1) for 1 h. Noninternalized bacteria were then counted by exposing monolayers to medium containing antibodies for 2 h at 37°C. Monolayers were then extensively washed with antibiotic-free medium and the number of bacteria-infected cells (CFU/well) determined using an agarose-agar overlay assay.

**Figure 5.** WW^v^ mice have enhanced susceptibility to oral *Salmonella* challenge. WW^v^ mice and their control littermates were given an oral dose of 5 X 10^6^ CFU of *S. typhimurium*. 10 mice per group were followed for 12 d and assessed daily for survival. The difference in cumulative mortality between WW^v^ versus ++ control littermate mice was statistically significant (P <0.001).
ReFerences

1. Galli, S.J., K.M. Zsebo, and E.N. Geissler. 1994. The kit ligand, stem cell factor. *Adv. in Immunol.* 55:1–96.
2. Galli, S.J. 1990. Biology of Disease: new insights in "The riddle of the mast cells": microenvironmental regulations of mast cell development and phenotypic heterogeneity. *Lab. Invest.* 62:5–33.
3. Huang, E.J., K.H. Nocka, J. Buck, and P. Besmer. 1992. Differential expression and processing of two-cell associated forms of the kit-ligand:KL-1 and KL-2. *Mol. Biol. Cell.* 3:349–362.
4. Flanagan, J.A., D.C. Chan, and P. Leder. 1991. Transmembrane form of the kit ligand growth factor is determined by alternative splicing and is missing in the SIa mutant. *Cell.* 64:1025–1032.
5. Manova, K., and R.F. Bachvarova. 1991. Expression of c-kit encoded at the W locus of mice in developing embryonic germ cells and presumptive melanoblasts. *Dev. Biol.* 146:312–324.
6. Nishikawa, S., M. Kusakabe, K. Yashinaga, M. Ogawa, S. Hayashi, T. Kuniyada, T. Era, T. Sakakura, and S.I. Nishikawa. 1991. In utero manipulation of coat color formation by a monoclonal antibody: two distinct waves of c-kit dependency during melanocyte development. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:2111–2118.
7. Yoshinaga, K., S. Nishikawa, M. Ogawa, S.-I. Hayashi, T. Kuniyada, T. Fujimoto, and S.I. Nishikawa. 1991. Role of c-kit in mouse spermatogenesis as a specific site of c-kit dependency during melanocyte development. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:2111–2118.
8. Nishikawa, K., S. Nishikawa, M. Ogawa, S.-I. Hayashi, T. Kuniyada, T. Fujimoto, and S.I. Nishikawa. 1991. Role of c-kit in mouse spermatogenesis as a specific site of c-kit expression and function. *Development.* 113:689–699.
9. Tajima, Y., H. Onoue, Y. Kitamura, and Y. Nishimune. 1991. Biologically active kit ligand growth factor is produced by mouse Sertoli cells and is defective in SID mutant mice. *Development.* 113:1031–1035.
10. Hirota, S., A. Ito, E. Morii, A. Wanaka, M. Tohyama, Y. Kitamura, and S. Nomura. 1992. Localization of mRNA for c-kit receptor and its ligand in the brain of adult rats: an analysis using *in situ* hybridization histochemistry. *Mol. Brain Res.* 15:477–554.
11. Orr-Urtreger, A., A. Avivi, Y. Zimmer, D. Givol, Y. Yarden, and P. Lonai. 1990. Developmental expression of c-kit, a proto-oncogene encoded by the W locus. *Development.* 109:911–923.
12. Hulzinga, J.D., L. Thuneberg, M. Klippel, J. Malyzy, H.B. Mikkelsen, and A. Bernstein. 1995. W/kit gene required for intestinal cells of Cajal and for intestinal pacemaker activity. *Nature (Lond.).* 373:347–349.
13. Ward, S.M., A.J. Burns, S. Torishashi, and K.M. Sanders. 1994. Mutation of the proto-oncogene c-kit blocks development of interstitial cells and electrical rhythmicity in murine intestine. *J. Physiol.* 480(Suppl.1):91–97.
14. Maeda, H., A. Yamagata, S. Nishikawa, K. Yoshinaga, S. Kobayashi, K. Nishi, and S.I. Nishikawa. 1992. Requirement of c-kit for development of intestinal pacemaker system. *Dev. Biol.* 116:369–375.
15. Puddington, L., S. Olson, and L. Lefrancois. 1994. Interaction between stem cell factor and c-kit are required for intestinal immune system homeostasis. *Immunity.* 1:733–739.
16. Reed, L.J., and H. Muench. 1988. A simple method of estimating fifty percent endpoints. *Am. J. Hyg.* 27:493.
17. Valpentich, J.D., and D.W. Powell. Intestinal subepithelial myofibroblasts and mucosal immunophysiolog. *Curr. Opin. Gastroenterol.* 10:645–651.
18. Vidal, K., I. Grosjean, J.P. Revillard, C. Gespach, and D. Kaiserlian. 1993. Immortalization of mouse intestinal epithelial cells by the SV40-large T gene. *J. Immunol. Meth.* 166:63–73.
19. Zsebo, K.M., D.A. Williams, E.N. Geissler, V.C. Broudy, F.H. Martin, H.L. Atkins, R.Y. Hsu, N.C. Burkett, K.H. Okino, D.C. Murnock et al. 1990. Stem cell factor is encoded at the SI locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. *Cell.* 63:213–224.
20. Martyn, F.H., S.V. Suggs, K.E. Langley, H.S., L. Ting, K.H. Okino, C.F. Morris, I.K. McNiece, F.W. Jacobson, E.A. Mendiaz et al. 1990. Primary structure and functional expression of rat and human SCF DNA. *Cell.* 63:203–211.
21. Klimpel, G.R., M. Asuncion, J. Haithcoat, and D.W. Niesel. 1995. Cholera toxin and *Salmonella typhimurium* induce different cytokine profiles in the gastrointestinal tract. *Microbial. Path.* 14:217–227.
22. Niesel, D.W., C.E. Chambers, and S.L. Stockman. 1985. Quantitation of HeLa cell monolayer invasion by *Shigella* and *Salmonella* species. *J. Clin. Microbiol.* 22:897–902.
23. Marshall, J.S., and J. Bienenstock. 1994. The role of mast cells in inflammatory reactions of the airways, skin, and intestine. *Curr. Opin. Immunol.* 6:853–859.
24. Gordon, J.R., P.R. Bard, and S.J. Galli. 1990. Mast cells as
a source of multifunctional cytokines. *Immunol. Today.* 11: 458–463.

29. Malaviya, R., E.A. Ross, J.I. MacGregor, T. Ikeda, J.R. Little, B.A. Jakschik, and S.N. Abraham. 1995. Mast cell phagocytosis of FimH-expressing enterobacteria. *J. Immunol.* 152: 1907–1914.

30. Malaviya, R., B. Jakschik, and S.N. Abraham. 1994. Mast cell degranulation induced by Type I fimbriated *Escherichia coli* in mice. *J. Clin. Invest.* 93:1645–1654.

31. Niesel, D.W., C.B. Hess, Y.J. Cho, K.D. Klimpel, and G.R. Klimpel. 1986. Natural and recombinant interferons inhibit epithelial cell invasion by *shigella spp.* *Infect. Immunol.* 52: 828–835.

32. Bukholm, G., and M. Degré. 1983. Effect of human leukocyte interferon on invasiveness of *Salmonella species* in HEp-2 cell cultures. *Infect. Immunol.* 42:1198–1202.

33. Bukholm, G., and M. Degré. 1985. Effect of human gamma interferon on invasiveness of *Salmonella typhimurium* in HEp-2 cell cultures. *J. Interferon. Res.* 5:45–53.

34. Kagnoff, M.F. 1996. Mucosal immunology: new frontiers. *Immunol. Today.* 17:57–59.