

Epidermal Stem Cell Factor Augments the Inflammatory Response in Irritant and Allergic Contact Dermatitis

Journal of Investigative Dermatology advance online publication, 17 January 2008; doi:10.1038/sj.jid.5701247

TO THE EDITOR

Stem cell factor (SCF) is the ligand for the KIT tyrosine kinase and is expressed by keratinocytes in human interfollicular epidermis where it exists predominantly as a transmembrane protein with an extracellular receptor-binding domain (Longley *et al.*, 1993). SCF can also be produced as a soluble bioactive molecule by alternate mRNA splicing or by solubilization of the membrane-bound form by protease cleavage at one of several extracellular juxtamembrane sites (Anderson *et al.*, 1990; Longley *et al.*, 1997). In human skin, KIT is expressed by epidermal melanocytes, and activation of KIT by keratinocyte-derived SCF is necessary and sufficient for epidermal melanocyte survival (Kunisada *et al.*, 1998). By contrast, SCF is not normally expressed postnatally by epidermal keratinocytes in mice, a fact that explains why murine interfollicular epidermis lacks significant numbers of melanocytes at most anatomic sites and why murine epidermis is clear (pink or white), even in "black" mice (Yoshida *et al.*, 1996). Expression of transgenic SCF in murine basilar keratinocytes causes postnatal persistence of interfollicular epidermal melanocytes and deposition of melanin that mimics human epidermis; and mice with skin that is "humanized" in this manner may be useful for studying photobiology and photocarcinogenesis and for studying melanocyte biology, including normal and aberrant pigmentation and the development of melanoma (B.J. Longley, unpublished studies; D'Orazio *et al.*, 2006). However, to our knowledge, there is no published work addressing the potential contribution of epidermal keratinocyte SCF to cutaneous inflammatory or immune responses that could further affect these processes.

neous inflammatory or immune responses that could further affect these processes.

We have investigated the role of keratinocyte SCF in irritant and allergic contact dermatitis using three strains of mice bearing different copy numbers of a transgene that expresses predominantly membrane-bound epidermal SCF that can be solubilized by cleavage by enzymes such as chymase (K14-SCF transgenics, TG1; Longley *et al.*, 1997; Kunisada *et al.*, 1998). The mice were designated as 1 $\times$, 4 $\times$, and 10 $\times$, corresponding to the relative copy number. (All procedures were approved by the Institutional Animal Care and Use Committees of Columbia University and the University of Wisconsin-Madison. Please see Supplementary Materials online for a more detailed description of the mice, reagents, and techniques.)

Compared to non-transgenic littermates, SCF-transgenic mice showed significantly greater ear swelling after the application of croton oil, a contact irritant (Figure 1). The intensity of the ear swelling response increased in proportion to the level of expression of the transgene and could be partially blocked by SU9529, a specific inhibitor of KIT (Ma *et al.*, 2002) (KIT suppression data not shown). It should be noted that the reaction of the 10 $\times$ mice, expressing the highest levels of SCF, was so vigorous that some of the animals developed ulceration, which may have caused underestimation of their ear swelling response.

The SCF-transgenic mice also showed an increased antigen-specific allergic response to dinitrofluorobenzene (Figure 2). The response was

associated with increased antigen-specific proliferation of lymph node cells (described in Supplementary Materials) and was transferable; draining lymph node cells from sensitized SCF-transgenic mice caused more than fourfold greater swelling 24 hours after transfer to *nu/nu* mice than did draining lymph node cells from sensitized wild-type mice. Therefore, antigen-specific draining lymph node cells appear to contribute to the greater allergic response observed in SCF-transgenic mice *in vivo*.

The fact that increased ear swelling occurred in several different transgenic lines and was positively associated with the transgenic "gene dosage" is evidence that this phenomenon is due to the targeted effect of epidermal keratinocyte SCF expression rather than to a random insertional event. In addition, we found that the heightened inflammatory response of the transgenic mice was not associated with an increase in dermal mast cell density (Table S1), indicating that SCF acts in the early phases of contact dermatitis through a mechanism independent of its role as a mast cell mitogen.

How might keratinocyte SCF affect cutaneous inflammation, especially allergic responses mediated by lymphocytes that do not express KIT? It is quite likely that mast cells, which express KIT and are known to contribute to both irritant and allergic contact reactions (Galli and Kitamura, 1987; Kerdel *et al.*, 1987), are key intermediaries. An heuristic hypothesis is that the initial activation of resident dermal mast cells triggered by exposure to an irritant or allergic contactant causes the release of chymase or other enzymes, which in turn cause solubilization of membrane-bound keratinocyte SCF by cleavage at one of several extracellular juxtamembrane sites (Longley *et al.*, 1997). The

Abbreviation: SCF, stem cell factor

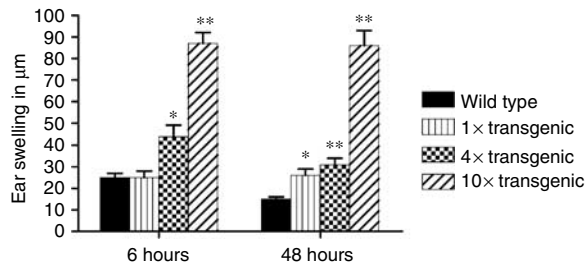


Figure 1. Irritant ear swelling response to croton oil. A dose-response relationship was seen between the level of epidermal SCF expressed by transgenic mice and the magnitude of ear swelling (mean $\pm$ SE) that developed in response to croton oil at both 6 and 48 hours after exposure ($P < 1 \times 10^{-13}$ by fixed-effect ANOVA (analysis of variance)). Swelling was significantly greater in 4 $\times$ and 10 $\times$ transgenics than in WT mice at 6 hours, and greater in each of the transgenic substrains than in WT mice at 48 hours.

*Significantly different from WT at $P < 0.001$; **Significantly different from WT at $P < 0.000001$; two-sided Mann-Whitney U -test, $n = 66$ (WT), $n = 17$ (1 $\times$), $n = 14$ (4 $\times$), $n = 33$ (10 $\times$).

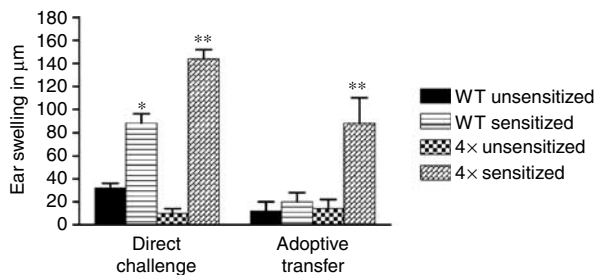


Figure 2. Allergic ear swelling response to DNFB. Sensitized SCF-transgenic mice exhibit a greater allergic ear swelling response to challenge with DNFB (dinitrofluorobenzene) than do sensitized control mice, and the increased response can be transferred by draining lymph node cells. Bars show mean ear swelling response ($\pm$ SE) by sensitized WT or 4 $\times$ SCF-transgenics (direct challenge group), or ear swelling response in *nu/nu* mice given lymph node cells from sensitized WT or 4 $\times$ SCF-transgenic mice (adoptive transfer group). Measurements in all groups were obtained 24 hours after challenge. Each of these experiments was performed at least twice with similar results. *Direct challenge*: *Significantly different from unsensitized WT or unsensitized transgenic, $P < 0.01$; **Significantly different from all other direct challenge groups (unsensitized and sensitized WT, and unsensitized transgenic), $P < 0.01$; two-sided Mann-Whitney U -test, $n = 6$ for each group. *Adoptive transfer*: **Significantly different from all other adoptive transfer groups (unsensitized and sensitized WT, and unsensitized transgenic), $P < 0.001$; two-sided Mann-Whitney U -test, $n = 6$ for each group.

acute stimulation of mast cells by SCF is known to lower the threshold for mast cell degranulation and cause release of proinflammatory mediators in response to both immunologic and non-immunologic stimuli (Columbo *et al.*, 1992; Nakajima *et al.*, 1992; Wershil *et al.*, 1992), so we further propose that release of soluble SCF from epidermal keratinocytes could result in a positive feedback loop with still more mast cell degranulation and keratinocyte SCF solubilization, as well as recruitment of other inflammatory cells (Longley *et al.*, 1997). We also recognize that the effect could involve $\gamma\delta$ -T cells or NK cells, which may also express KIT (Colucci and Di Santo, 2000; Wang *et al.*, 2000).

Although the precise mechanisms involved in this phenomenon remain to be further elucidated, our findings demonstrate unequivocally that the production of SCF by epidermal keratinocytes may contribute to the cutaneous inflammatory response, highlighting another critical difference between murine and human skin. Recognition of the contribution of the SCF-KIT pathway to contact dermatitis suggests a previously unreported target for the development of specific anti-inflammatory agents. Our findings also suggest that the relevance of traditional murine experimental models for human contact dermatitis should be re-evaluated in light of the fact that SCF is expressed

in abundance by epidermal keratinocytes in humans but not in mice.

CONFLICT OF INTEREST

Columbia University holds a patent on the use of these mice for drug discovery. Dr Longley is listed as the Inventor and could receive royalties. No royalties have ever been collected and the mice are being deposited in the Jackson Laboratories and will be made available at cost for academic use. The other authors declare no conflicts of interest.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Dr Robert E. Tigelaar for his thoughtful discussions, and Dr Shan Zeng for her assistance counting murine dermal mast cells. This work was supported in part by NIH R01AR/CA43356-01A5, 2R01AR43356, and AR4453502.

Eric L. Carter¹, Sean O'Herrin², Carla Woolery² and B. Jack Longley²

¹Department of Dermatology, Columbia University College of Physicians and Surgeons, New York, New York, USA and ²Department of Dermatology, University of Wisconsin-Madison, Madison, Wisconsin, USA.

E-mail: ELC4@columbia.edu or bjlongley@wisc.edu

SUPPLEMENTARY MATERIAL

Supplementary Materials and Methods.

REFERENCES

- Anderson DM, Lyman SD, Baird A, Wignall JM, Eisenman J, Rauch C *et al.* (1990) Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. *Cell* 63:235-43
- Colucci F, Di Santo JP (2000) The receptor tyrosine kinase *c-kit* provides a critical signal for survival, expansion, and maturation of mouse natural killer cells. *Blood* 95: 984-91
- Columbo M, Horowitz EM, Botana LM, MacGlashan DW Jr, Bochner BS, Gillis S *et al.* (1992) The human recombinant *c-kit* receptor ligand, rhSCF, induces mediator release from human cutaneous mast cells and enhances IgE-dependent mediator release from both skin mast cells and peripheral blood basophils. *J Immunol* 149:599-608
- D'Orazio JA, Nobuhisa T, Cui R, Arya M, Spry M, Wakamatsu K *et al.* (2006) Topical drug rescue strategy and skin protection based on the role of Mc1r in UV-induced tanning. *Nature* 443:340-4
- Galli SJ, Kitamura Y (1987) Genetically mast-cell-deficient *W/W^v* and *Sl/Sl* mice. Their value for the analysis of the roles of mast cells in biologic responses *in vivo*. *Am J Pathol* 127: 191-8
- Kerdel FA, Belsito DV, Scotto-Chinnici R, Soter NA (1987) Mast cell participation during the elicitation of murine allergic contact hypersensitivity. *J Invest Dermatol* 88:686-90

- Kunisada T, Lu SZ, Yoshida H, Nishikawa S, Nishikawa S, Mizoguchi M *et al.* (1998) Murine cutaneous mastocytosis and epidermal melanocytosis induced by keratinocyte expression of transgenic stem cell factor. *J Exp Med* 187:1565–73
- Longley BJ, Morganroth GS, Tyrrell L, Ding TG, Anderson DM, Williams DE *et al.* (1993) Altered metabolism of mast-cell growth factor (*c-kit* ligand) in cutaneous mastocytosis. *N Engl J Med* 328:1302–7
- Longley BJ, Tyrrell L, Ma Y, Williams DA, Halaban R, Langley K *et al.* (1997) Chymase cleavage of stem cell factor yields a bioactive, soluble product. *Proc Natl Acad Sci USA* 94:9017–21
- Ma Y, Zeng S, Metcalfe DD, Akin C, Dimitrijevic S, Butterfield JH *et al.* (2002) The c-KIT mutation causing human mastocytosis is resistant to STI571 and other KIT kinase inhibitors; kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. *Blood* 99:1741–4
- Nakajima K, Hirai K, Yamaguchi M, Takaishi T, Ohta K, Morita Y *et al.* (1992) Stem cell factor has histamine releasing activity in rat connective tissue-type mast cells. *Biochem Biophys Res Commun* 183:1076–83
- Wang T, Alam R, Langley KE, Klimpel GR (2000) Stem cell factor and IL-2 act synergistically in inducing intraepithelial lymphocyte proliferation and cytokine production: upregulation of the IL-2 receptor gamma-chain and signaling via JAK-3. *Cell Immunol* 205:62–71
- Wershil BK, Tsai M, Geissler EN, Zsebo KM, Galli SJ (1992) The rat *c-kit* ligand, stem cell factor, induces *c-kit* receptor-dependent mouse mast cell activation *in vivo*. Evidence that signaling through the *c-kit* receptor can induce expression of cellular function. *J Exp Med* 175:245–55
- Yoshida H, Hayashi S-I, Shultz LD, Yamamura K, Nishikawa S, Nishikawa S *et al.* (1996) Neural and skin cell-specific expression pattern conferred by Steel factor regulatory sequence in transgenic mice. *Dev Dyn* 207:222–232

Supplementary Materials

Detailed Descriptions of Materials and Experiments

Mice: The generation and characterization of transgenic mice expressing modified epidermal SCF have been previously described (Kunisada *et al.*, 1998). Five different transgenic lines, expressing four different levels of the transgene (as measured by semiquantitative polymerase chain reaction), were maintained by mating transgenic males born in our colony with females of the parent C57Bl/6 strain purchased from The Jackson Laboratory (Bar Harbor, ME). The mice used in the present study were the product of at least 10 backcrossings to the parent strain, and were drawn from three of the transgenic lines: one line with the lowest level of expression of the transgene (“1X”), a second with a level of expression four times higher (“4X”), and a third with a level of expression ten times higher than the substrain with the lowest level of expression (“10X”). Male and female SCF-transgenic mice and their pooled normal, congenic littermates were studied at 6 – 14 weeks of age. The animals were anesthetized with an intraperitoneal injection of a mixture of ketamine and xylazine (Henry Schein Veterinary, Melville, NY) prior to each procedure, including measurements of ear thickness. All procedures were approved by the Institutional Animal Care and Use Committees of Columbia University and the University of Wisconsin-Madison.

Reagents: Solutions of croton oil (Sigma Chemical Company, St. Louis, MO) were freshly prepared in acetone:olive oil 4:1. Dinitrofluorobenzene (DNFB), dinitrosulfobenzene (DNSB), trinitrobenzene sulfonate (TNBS), and ingredients of the vehicles were obtained from Sigma Chemical Company.

Elicitation and Ear Swelling Measurements of Irritant and Allergic Contact

Dermatitis: Irritant contact dermatitis was induced by applying 10 μ L croton oil 1% (v/v) to each side of the right ears of the mice. To study allergic responses, mice were sensitized by application of DNFB to shaved dorsal skin and later challenged on ear skin with a dilute solution of DNFB. Ear thickness was measured using an engineer's spring-loaded micrometer prior to the application of contactant, and again at 6 and 48 hours after treatment with croton oil, or 24 hours after treatment with DNFB. For each ear, the increase in thickness from baseline was taken as the irritant ear swelling due to croton oil or allergic response to DNFB. These experiments were performed at least 4 times, and the results are representative.

Croton Oil Irritant Dermatitis: At 6 hours, mean ear swelling in the substrain of transgenic mice with the highest epidermal SCF expression (10X mice, N = 33) was 0.087 $\pm$ 0.005 mm (mean $\pm$ SE) versus 0.025 $\pm$ 0.002 mm in the wild-type (WT) littermate controls (N = 66; $p < 1 \times 10^{-25}$) (Figure 1). At 48 hours, 10X SCF-mice had a mean swelling of 0.086 $\pm$ 0.007 mm versus control swelling of 0.015 $\pm$ 0.001 mm ($p < 1 \times 10^{-23}$). Mean ear swelling in the 4X substrain of SCF-transgenic mice (N = 14) was 0.044 $\pm$ 0.005 mm at 6 hours ($p < 0.001$), and 0.031 $\pm$ 0.003 mm at 48 hours ($p < 0.00001$). Among the 1X SCF-mice (N = 17), with the lowest level of epidermal SCF expression, mean ear swelling did not significantly differ from that of WT mice until 48 hours: 0.025 $\pm$ 0.003 mm at 6 hours ($p = 0.90$), and 0.026 $\pm$ 0.003 mm at 48 hours ($p < 0.001$).

At both 6 and 48 hours after croton oil exposure, a dose-response relationship was seen between the level of epidermal SCF expressed by the transgenic mice and the magnitude of ear swelling that developed in response to treatment with croton oil ($p < 1 \times 10^{-13}$). This was particularly apparent at 6 hours, when the mean ear swelling in the high-expressing 10X mice was nearly 3.5-fold greater than in the 1X mice ($p < 0.001$), and twice as great as in the 4X mice ($p < 0.001$). By 48 hours, the 10X mice still showed a significantly greater degree of ear swelling than either of the two other transgenic substrains ($p < 0.001$ with either). However, the difference between the 4X and 1X mice was no longer statistically significant at this time point, probably owing to a decrease in the mean ear swelling of the 4X group.

DNFB Allergic Dermatitis: To determine whether expression of epidermal SCF affects allergic contact dermatitis, we first compared the ear swelling response of SCF-transgenic and WT mice to DNFB, a contact allergen. Mice were painted on shaved backs with 100 μ l 0.5% solution of DNFB in an acetone:olive oil carrier (4:1). Five days after DNFB application, ear thickness was measured to obtain baseline. Immediately following, both sides of the left ear were painted with a 0.25% solution of DNFB, the right ear painted with only carrier. Ear thickness was measured again 24 hours later. As was the case with irritant dermatitis, SCF-transgenic mice exhibited a greater ear swelling response than WT mice (Figure 2).

Draining lymph node cells from DNFB-treated SCF-transgenic mice show greater *ex vivo* proliferation to DNFB than draining lymph node cells from DNFB-treated WT Mice

To compare antigen-specificity of the effector cell response, 4X SCF-transgenic and WT mice were sensitized with DNFB or carrier alone on shaved dorsal skin. Axial, brachial, and inguinal lymph node cells were collected and pooled five days after sensitization on the back, stained with CFSE, and stimulated with syngeneic irradiated antigen presenting cells conjugated to either DNSB, a cross-reactive analog of DNFB, or to an irrelevant antigen, TNBS. After incubation for 88 hours, cells were harvested, counted, and immunostained for CD4 and CD8. Controls included donor mice sensitized with carrier alone followed by subsequent DNSB stimulation. Proliferation was determined in the CD4⁺ and CD8⁺ cell populations by flow cytometry. Controls of carrier-alone sensitization followed by subsequent DNSB stimulation and of DNFB sensitization followed by TNBS (irrelevant antigen) stimulation were not significantly different between control and 4X mice. However, more robust antigen-specific *ex vivo* proliferation was observed in cells from SCF-transgenic versus WT mice. Of CD8⁺ lymph node cells, 12% more proliferated and 10-fold more entered the third cycle of division when derived from SCF-transgenic mice than when derived from WT mice. The effect was less with CD4⁺ cells, with SCF-transgenic-derived CD4⁺ cells showing a 2-fold increase in proliferation and no detectable CD4⁺ cells entering the third cycle of division from either SCF-transgenic or WT mice. Therefore, the presence of epidermal SCF during the elicitation phase results in a more robust effector phase, with a greater antigen-specific proliferative response detectable *ex vivo*.

The allergic response in SCF-transgenic mice can be adoptively transferred

To compare antigen specificity of the effector cell response, three 4X SCF-transgenic and three WT mice were sensitized with DNFB on shaved dorsal skin. Five days later brachial, axillary, and inguinal lymph nodes were harvested, pooled, and injected into 6 *nu/nu* recipients per group. Eighteen hours later mice were challenged with DNFB on ears, and ear thickness measured after another 24 hours. The draining lymph node cells from the SCF-transgenic mice caused more than 4-fold greater swelling 24 hours after transfer than did draining lymph node cells from similarly treated WT mice. Therefore, the antigen-specific draining lymph node cells appear to contribute to the greater ear swelling observed *in vivo*.

Histology and Quantification of Mast Cell Density

In one experiment, following the induction of irritant contact dermatitis, the mice's right and left pinnae were removed at the base and fixed in 10% formalin solution, prior to embedding in paraffin. Five-micron sections were cut and stained with toluidine blue for enhanced visualization of mast cells. For each coded specimen, the mast cells per linear unit of cartilage in the combined papillary and reticular dermis (and in some cases the epidermis) were counted under 400x magnification by 2 microscopists blinded to the groupings.

The magnitude of the irritant contact sensitivity response is not dependent on dermal mast cell density

It has been established that a lack of mast cells results in diminished ear swelling in response to irritants (Wershil *et al.*, 1986; Wershil *et al.*, 1988), and a trivial explanation for the increased ear swelling response we observed in the SCF-transgenic mice is that there are simply more mast cells in the dermis of SCF-transgenic mice due to a systemic or local effect such as chronic low-level leakage of solubilized epidermal SCF. To determine whether increased numbers of dermal mast cells were required for the heightened response to croton oil, we counted dermal mast cells before and 48 hours after the induction of irritant contact dermatitis (Table S1). We did find a significant increase in the number of dermal mast cells in the 10X transgenics compared to all other groups, but the differences between the WT mice and the two substrains of transgenic mice with lower expression of epidermal SCF (1X and 4X) were small and did not correlate with the magnitude of the ear swelling response. This was best illustrated in the 4X mice, which had the greatest ear swelling response of the latter three groups (wild type, 1X and 4X transgenics) but actually had lower numbers of dermal mast cells than the WT mice at baseline. Thus, these data disprove the hypothesis that the magnitude of the ear swelling response in the SCF-transgenic mice simply reflects an increase in baseline numbers of dermal mast cells. We speculate that the increased numbers of mast cells in the high-expressing 10X mice might be secondary to chronic low-level leakage of epidermal SCF caused by inflammation due to everyday “wear and tear.”

In some tissue sections obtained from the transgenic mice after croton oil treatment, there appeared to be exocytosis of mast cells into the basal layer of the epidermis, the source of SCF (data not shown). Some of the mast cells in the papillary dermis in close

approximation to the epidermis appeared to be undergoing mitosis, previously reported in acute irritant and allergic contact dermatitis (Dvorak and Mihm, 1976; Gordon and Galli, 1990), suggesting that with time there may be some local expansion of the mast cell population. However, there was no increase relative to baseline in overall mast cell density at 48 hours after croton oil treatment in any of the transgenic mice or their wild-type littermates, indicating that the induction of a contact sensitivity reaction to croton oil is not associated with a rapid increase of dermal mast cells.

SUPPLEMENTAL REFERENCES

- Dvorak AM, Mihm MC (1976). Morphology of delayed-type hypersensitivity reactions in man. Ultrastructural alterations affecting the microvasculature and the tissue mast cells. *Lab Invest* 34: 179-191.
- Gordon JR, Galli SJ (1990). Phorbol 12-myristate 13-acetate-induced development of functionally active mast cells in *W/W^v* but not *Sl/Sl^d* genetically mast cell-deficient mice. *Blood* 75: 1637-1645.
- Wershil BK, Mekori YA, Murakami T, Galli SJ (1986). Diminished croton oil-induced acute inflammation in mast cell deficient (*W/W^v* and *Sl/Sl^d*) mice. *Fed Proc* 45: 1104 (abstract).
- Wershil BK, Murakami T, Galli SJ (1988). Mast cell-dependent amplification of an immunologically nonspecific inflammatory response. *J Immunol* 140: 2356-2360.

SUPPLEMENTAL TABLE S1

Mouse Ear Mast Cell Counts

	BASELINE mean (N)	48h AFTER CROTON OIL mean (N)
WT	47.6 (25)	50.3 (67)
1X	28.7 (10)	60.9 (17)
4X	44.7 (20)	52.3 (14)
10X	94.5 (20)	86.4 (32)