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Stem cell factor induces outgrowth of c-*kit*-positive neurites and supports the survival of c-*kit*-positive neurons in dorsal root ganglia of mouse embryos

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SUMMARY

The c-*kit* receptor tyrosine kinase is highly expressed by about 10% of the neurons in the dorsal root ganglia (DRGs) of mouse embryos. We investigated the in vitro effect of stem cell factor (SCF), the ligand for c-*kit* receptor, on DRGs. Recombinant murine SCF (rmSCF) induced the outgrowth of c-*kit*-positive neurites from DRGs of normal (+/+) embryos. The effect of SCF was dose dependent and completely abolished by anti-c-*kit* ACK2 monoclonal antibody (mAb). Some neurites whose outgrowth was induced by nerve growth factor (NGF) were c-*kit*-positive, but anti-NGF mAb did not inhibit the rmSCF-induced neurite outgrowth. rmSCF did not induce neurite outgrowth from DRGs of *W*/*W* embryos that did not express c-*kit* receptors on the cell surface and of W^{42}/W^{42} mutant embryos that expressed c-*kit* receptors without tyrosine kinase activity. rmSCF also had a trophic effect on c-*kit*-positive neurons in the culture of dissociated DRG cells. Most c-*kit*-positive neurons appeared to respond to NGF as well, and the SCF-responsive subpopulation represented about 10% of NGF-responsive neurons. rmSCF did not support the survival of DRG neurons from embryos of *W*/*W* and W^{42}/W^{42} genotypes. These results suggest that the stimulus through the c-*kit* receptor tyrosine kinase has an important role in development of the peripheral nervous system.

Key words: neurite outgrowth, neurotrophic factor, stem cell factor, c-*kit* receptor, dorsal root ganglion, *Sl* locus, *W* locus, nerve growth factor

INTRODUCTION

The c-*kit* receptor tyrosine kinase is encoded by the *W* locus in mice (Chabot et al., 1988; Geissler et al., 1988). The ligand for c-*kit* receptor has been designated as stem cell factor (SCF), steel factor or mast cell growth factor, and is encoded by the *Sl* locus in mice (Williams et al., 1990; Flanagan and Leader, 1990; Zesbo et al., 1990; Huang et al., 1990). Hereafter we refer to the ligand as SCF. It has the transmembrane domain and appears to be a cell surface protein. However, recombinant SCF lacking the transmembrane domain has apparent functions both in vivo and in vitro (Tsai et al., 1991; Godin et al., 1991; Dolci et al., 1991; Matsui et al., 1991). Analyses of *W* and *Sl* mutant mice have shown that the interaction between the c-*kit* receptor and SCF is indispensable for proper development of melanocytes (Mayer and Green, 1968; Silvers, 1979), erythrocytes (Russell and Bernstein, 1968; Russell, 1979), mast cells (Kitamura et al., 1978; Kitamura and Go, 1979) and germ cells (Nakayama et al., 1988; Kuroda et al., 1988). These cells or their precursors express c-*kit* receptor on the surface, whereas cells surrounding them express SCF and appear to support their migration, proliferation or differentiation.

Recent in situ hybridization studies revealed that the c-*kit* receptor and SCF are highly expressed by some neurons in mice and rats (Matsui et al., 1990; Orr-Urtreger et al., 1990; Keshet et al., 1991; Motro et al., 1991; Morii et al., 1992; Hirota et al., 1992). Their expression is highly localized and there are synaptic connections between c-*kit*-positive and SCF-positive neurons (Hirota et al., 1992). These findings suggest that the c-*kit* receptor and SCF are involved in the development of neuronal connections. In this study, we directly demonstrated the function of the c-*kit* receptor and SCF in neural development. Since previous studies have shown that dorsal root ganglia (DRGs) of mice express c-*kit* mRNA (Orr-Urtreger et al., 1990; Motro et al., 1991; Keshet et al., 1991), we cultured DRGs from normal (+/+) and mutant (*W*/*W*, W^{42}/W^{42}, Sl^{d}/Sl^{d}) mouse embryos and examined the effect of recombinant murine SCF (rmSCF) on neurite outgrowth from these DRGs. We also examined the trophic effect of rmSCF on dissociated DRG neurons from +/+ and mutant (*W*/*W*, W^{42}/W^{42}, *Sl*/*Sl*) mouse embryos.

MATERIALS AND METHODS

Mouse embryos

Mice of WB-(+/+, *W*/+, *Sl*/+) and C57BL/6-(+/+, W^{42}/+, Sl^d/+) were maintained in our laboratory. The *W* mutant allele results in a deletion of the transmembrane domain of the c-*kit* receptor. Mast cells of *W*/*W* genotype completely lack c-*kit* receptors on the surface (Nocka et al., 1990; Reith et al., 1990). The W^{42} mutant allele is a point mutation at the tyrosine kinase domain of the c-*kit* receptor, which abolishes the tyrosine kinase activity (Tan et al., 1990). The *Sl* mutant allele is a large deletion of the *Sl* locus. Fibroblasts of the *Sl*/*Sl* genotype do not produce SCF at all (Huang et al., 1990; Zesbo et al., 1990). The Sl^d mutant allele results in a deletion of transmembrane and intracellular domains of SCF (Flanagan et al., 1991).

Embryos of +/+ genotype were obtained by mating either WB-+/+ or C57BL/6-+/+ parents. Embryos of various mutant genotypes were obtained by mating the corresponding heterozygous parents. The time of gestation was calculated by considering the morning after mating as 0.5 day post coitum (p.c.). The mothers were anesthetized by ether and killed by exsanguination 15.5 or 18.5 day p.c. and the embryos were dissected out. Homozygous mutant embryos at 18.5 day p.c. were determined by the apparent decrease of mast cells in the skin (Hayashi et al., 1985). Genotypes of *W*/*W*, *Sl*/*Sl* and Sl^d/Sl^d embryos at 15.5 day p.c. were determined by grafting skin pieces of embryos under the kidney capsule of congenic +/+ recipients according to Niwa et al. (1991). Development of white hair alone was considered to indicate *W*/*W*, *Sl*/*Sl* or Sl^d/Sl^d genotype. Since the coat color of heterozygous W^{42}/+ mice is almost white (Tan et al., 1990), determination of the W^{42}/W^{42} genotype from hair color of grafted skin pieces was difficult. Therefore, the W^{42}/W^{42} genotype of mouse embryos at 15.5 day p.c. was determined by sequencing the c-*kit* gene fragment (nucleotide 1707 through 2678) which was reverse transcribed and amplified by polymerase chain reaction (PCR) as described by Tono et al. (1992).

Chemicals, factors and antibodies

Serum-free medium (Cosmedium) purchased from Cosmo Bio. (Tokyo, Japan) was supplemented with human transferrin (100 μg/ml, Sigma Chemical Co., St Louis, MO), bovine insulin (5 μg/ml, Sigma) and sodium selenite (5 ng/ml, Sigma) and used throughout the present study. The rmSCF was a generous gift of Kirin Brewery Company Ltd (Tokyo, Japan). Nerve growth factor (NGF) 2.5 S purified from mouse submaxillary glands was purchased from Boehringer Mannheim (Mannheim, Germany). Anti-c-*kit* ACK2 monoclonal antibody (mAb) that recognizes the extracellular domain of murine c-*kit* receptor was a generous gift of Dr S. I. Nishikawa of Kumamoto University Medical School (Kumamoto, Japan). Anti-murine NGF mAb was purchased from Boehringer Mannheim.

Histological specimens

Embryos were frozen in liquid nitrogen. Sagittal sections of 6 μm in thickness were prepared and fixed in acetone for 10 minutes. The specimens were incubated with ACK2 mAb (20 ng/ml) for 42 hours at 4°C, then with biotinylated goat anti-rat IgG antibody (1:300, DAKO-PATTS, Copenhagen, Denmark), and finally with streptavidin-biotin-peroxidase complex (1:200, Vector Laboratories, Burlingame, CA). The binding of the streptavidin-biotin-peroxidase complex was visualized with diaminobenzidine. The specimens were counterstained with hematoxylin. Neurons were virtually indistinguishable from non-neuronal cells in frozen sections. The numbers of total cells and those stained with ACK2 mAb were counted using a square micrometer attached to an eyepiece.

Assay of neurite outgrowth

DRGs were dissected from all segments of mouse embryos harvested aseptically at 15.5 day p.c. Thereafter, 15 to 20 DRGs in Hank's solution were placed on 35 mm plastic dishes (Corning, Tokyo, Japan) coated with poly-L-lysine (Sigma). The DRGs were cultured in the above-mentioned serum-free medium at 37°C for 48 hours in a humidified atmosphere of 5% CO2 in air. Neurite outgrowth was expressed as the length of the longest neurite from each DRG. The length of the longest neurite was measured with a square micrometer attached to an eyepiece; 11 to 20 DRGs were used for a single assay. In one experiment, ACK2 mAb (20 μg/ml) or anti-NGF mAb (250 ng/ml) was added to the culture 30 minutes before SCF.

Assay of neuronal survival

DRGs were dissected from mouse embryos at 15.5 day p.c. The DRGs from embryos of the same genotype were pooled and incubated in Ca^{2+}/Mg^{2+}-free Hank's solution containing 0.1% trypsin (Sigma) and 0.002% DNase (Sigma) for 15 minutes at 37°C. The DRGs were then washed three times with the serum-free culture medium supplemented with soybean trypsin inhibitor (100 μg/ml, Sigma). Single cell suspensions were prepared by gentle pipetting and the dissociated cells were plated on 8-chamber culture slides (Nunc Inc., Naperville, IL) coated with poly-L-lysine (Sigma). The plating density was $4.0\text{-}5.7\times10^4$ cells per well. When comparisons were made among DRG neurons obtained from individual embryos, the cell density was adjusted to 4.0×10^4 cells per well. The plated DRG cells were cultured in the serum-free culture medium at 37°C in a humidified atmosphere of 5% CO_2 in air. Surviving neurons were counted under a phase-contrast microscope using a square micrometer attached to an eyepiece. Neurons were identified as phase-bright round cells with a large nucleus.

Detection of cells in S phase

Dissociated DRG cells were incubated with culture medium containing 300 ng/ml BrdU (Wako Pure Chemical Ind, Osaka, Japan) for 48 hours at 37°C. The cultures were fixed with 70% ethanol. Cells that incorporated BrdU were visualized by staining with mouse anti-BrdU mAb (1:300, Becton Dickson, Mountain View, CA), biotinylated horse anti-mouse IgG antibody (1:300, Vector Laboratories) and streptavidin-biotin-peroxidase complex (1:200, Vector Laboratories). The binding of streptavidin-biotin-peroxidase complex was visualized with diaminobenzidine.

RESULTS

Expression of c-*kit* receptors in DRGs

First, we confirmed the expression of c-*kit* receptor in the DRGs of normal (C57BL/6-+/+) mouse embryos at 15.5 day p.c. Frozen DRG sections were stained with the ACK2 mAb that specifically bound the extracellular domain of the c-*kit* receptor (Nishikawa et al., 1991). About 10% of cells in each DRG were c-*kit*-positive, and they were considered to be neurons due to the presence of long processes (Fig. 1). DRGs of different segmental levels showed a similar distribution pattern of c-*kit*-positive neurons. Both central and peripheral axons expressed c-*kit* receptors.

We then examined whether the expression of c-*kit* receptor was abnormal in embryos of various mutant genotypes. In this experiment, embryos at 18.5 day p.c. were used because determination of their genotypes was easier than at 15.5 day p.c. The numbers of c-*kit*-positive cells and the total were not affected by the genotype of the embryos.

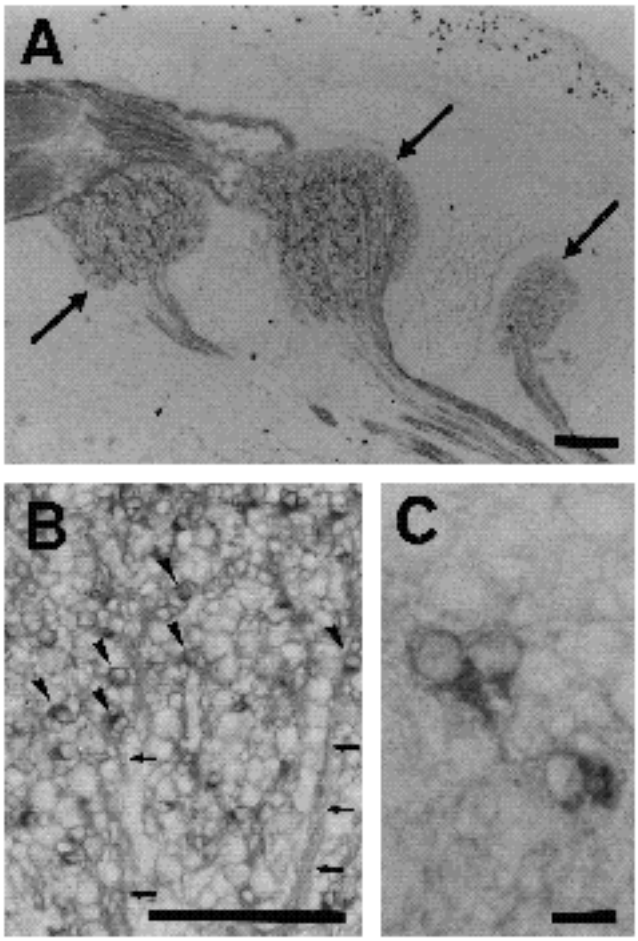

Fig. 1. Expression of c-*kit* receptors in a C57BL/6-+/+ mouse embryo. (A) A sagittal section of the mouse embryo of 15.5 day p.c. was stained with anti-c-*kit* ACK2 mAb; arrows indicate DRGs. (B) A higher magnification of A; arrowheads indicate c-*kit*-positive neurons and small arrows indicate c-*kit*-positive axons. (C) A higher power view of c-*kit*-positive neurons with an apparent process. Bar, 200 μm (A,B), 20 μm (C).

A normal number of c-*kit*-positive cells were counted in DRGs of C57BL/6-W^{42}/W^{42} embryos regardless of the complete abrogation of the c-*kit* kinase activity (Table 1). Moreover, normal numbers of c-*kit*-positive cells were observed in embryos of WB-*Sl*/*Sl* and C57BL/6-Sl^d/Sl^d despite the lack of normal SCF (Table 1). Axons from DRGs of C57BL/6-W^{42}/W^{42}, WB-*Sl*/*Sl* and C57BL/6-Sl^d/Sl^d embryos expressed c-*kit* receptors normally (data not shown).

Induction of neurite outgrowth by SCF

DRGs were dissected from C57BL/6-+/+ mouse embryos at 15.5 day p.c. and cultured in the serum-free medium with or without rmSCF. Non-neuronal fibroblast-like cells migrated from the explant and formed an area surrounding each DRG regardless of the presence or absence of rmSCF. When DRGs were cultured in the presence of rmSCF (100 ng/ml), numerous fine neurites grew beyond the area of fibroblast-like cells and directly attached to the plastic subtrata of culture dishes (Fig. 2A). Most outgrowing neurites were stained with ACK2 mAb (Fig. 2B). In contrast, when DRGs were cultured in the absence of rmSCF, most outgrowing neurites remained within the area of fibroblast-like cells (Fig. 2C). None of outgrowing neurites were stained with ACK2 mAb (Fig. 2D).

Table 1. Proportion of c-*kit*-positive cells in histlogical sections of DRGs from normal and mutant embryos

Embryos	Embryo no.	No. of cells* per 10^{-3} cm^2		Proportion of c-*kit*-positive cells (%)
		c-*kit*-positive	Total	
C57BL/6-+/+	1	28±1	276±12	10.1
	2	30±2	309±15	9.7
	3	29±3	350±30	8.3
	4	35±3	359±16	9.7
	5	32±1	341±8	9.4
C57BL/6-W^{42}/W^{42}	1	28±1	305±17	9.2
	2	32±2	327±25	9.8
WB-*Sl*/*Sl*	1	29±1	329±11	8.8
	2	29±1	319±13	9.1
	3	33±2	310±8	10.6
C57BL/6-Sl^d/Sl^d	1	32±2	347±12	9.2
	2	30±2	305±20	9.8

*Data from embryos of 18.5 day p.c. Means±s.e.m. of 4 microscopic fields (10^{-3} cm^2) from individual embryos.

The magnitude of neurite outgrowth was dependent on the concentration of rmSCF (Fig. 3). Neurites positive for c-*kit* were detectable with 1 ng/ml of rmSCF; neurite outgrowth at a half-maximal level was observed with 10 ng/ml and at a maximal level with 100 ng/ml. The ACK2 anti-c-*kit* mAb completely abolished the rmSCF-dependent outgrowth of neurites (Fig. 3).

NGF induces the outgrowth of neurites from DRGs of mouse embryos (Thoenen and Barde, 1980). We compared the effect of rmSCF on neurite outgrowth to that of NGF. The magnitude of NGF-induced neurite outgrowth as determined by the length of neurites was comparable to that of rmSCF-induced neurite outgrowth. However, the number of NGF-dependent neurites was several times as great as the number of rmSCF-dependent neurites (Fig. 4). NGF-dependent neurites were tightly fasciculated and some of the neurites were stained with ACK2 mAb (data not shown). Although anti-NGF mAb abolished the NGF-dependent outgrowth of neurites, the addition of the same concentration of anti-NGF mAb did not affect the rmSCF-dependent neurite outgrowth (Fig. 3).

We then used mutant embryos of *W*/*W*, W^{42}/W^{42} and Sl^d/Sl^d genotypes to confirm the effect of rmSCF on neurite outgrowth. The genetic background of *W*/*W* is WB, whereas that of W^{42}/W^{42} and Sl^d/Sl^d is C57BL/6. However, when we compared DRGs of C57BL/6/-+/+ and WB-+/+ mouse embryos, neurite outgrowth from both gave comparable results either with or without rmSCF (data not shown).

rmSCF did not induce significant outgrowth of neurites from DRGs dissected from WB-*W*/*W* embryos that completely lack cell surface expression of the c-*kit* receptor (Nocka et al., 1990; Reith et al., 1990) or from DRGs of C57BL/6-W^{42}/W^{42} embryos that lack the c-*kit* kinase activity (Table 2). WB-*W*/*W* and C57BL/6-W^{42}/W^{42} mouse embryos are severely anemic due to hypoproduction of erythrocytes and die in utero or shortly after birth. Failure of

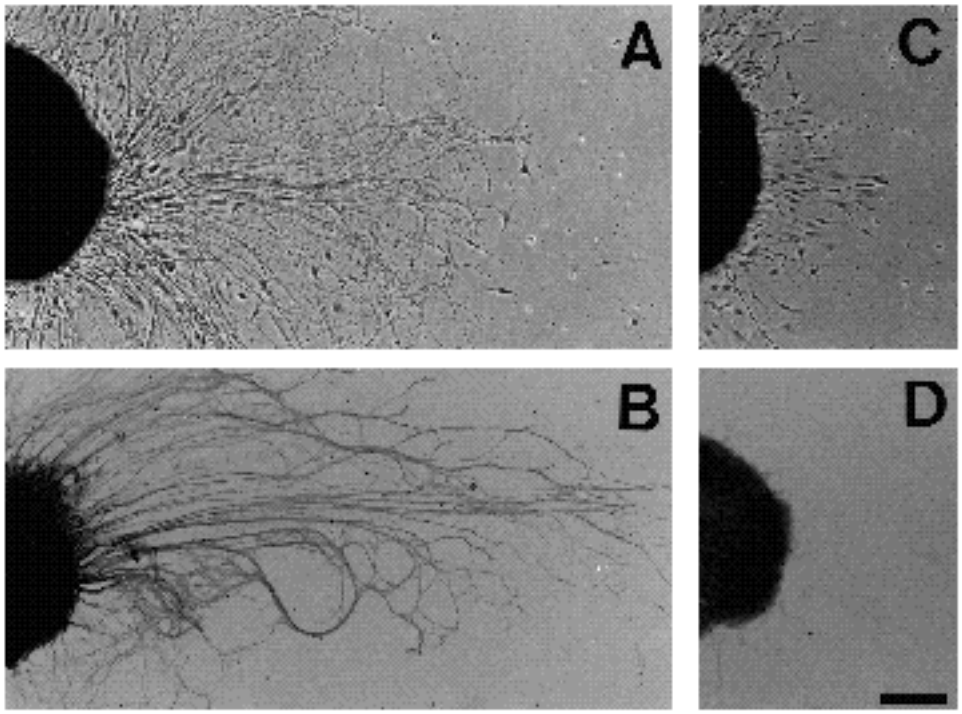

Fig. 2. Outgrowth of neurites induced by rmSCF. DRG was cultured in the presence of rmSCF (A,B) or in the absence of rmSCF (C,D). (A,C) Unstained specimens under the phase-contrast microscope. (B,D) Specimens stained with ACK2 mAb that binds the extracellular domain of the c-*kit* receptor. Bar, 200 μm.

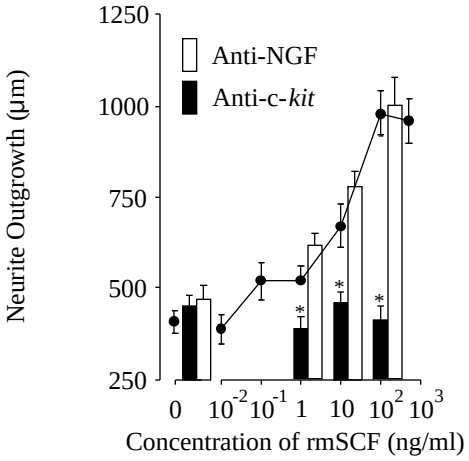

Fig. 3. Neurite outgrowth at various concentrations of rmSCF(closed circles) and the effects of anti-c-*kit* ACK2 mAb and anti-NGF mAb. The effect of rmSCF was abolished by ACK2 mAb (20 μg/ml) (closed columns), but not by anti-mouse NGF mAb (250 ng/ml) (open columns). Each point represents the mean of 12 to 20 samples, and vertical bars indicate the s.e.m. *, $P<0.01$ by the *t*-test when compared with the magnitude of neurite outgrowth from DRGs that were cultured with rmSCF but without antibodies.

neurite outgrowth from DRGs of WB-*W*/*W* and C57BL/6-W^{42}/W^{42} embryos was not attributed to the poor health of these embryos. In fact, rmSCF induced normal outgrowth of neurites from DRGs of C57BL/6-Sl^d/Sl^d embryos that was phenotypically similar to WB-*W*/*W* and C57BL-W^{42}/W^{42} embryos (Table 2).

Trophic effect of SCF on DRG neurons

It is possible that the outgrowth of c-*kit*-positive neurites resulted from the survival of DRG neurons induced by rmSCF. Since quantitation of neuronal survival is difficult in organ cultures of DRGs, those from 15.5-day p.c. embryos were dissociated into single cells by trypsinization. These were cultured and the number of surviving neurons was counted. When DRG cells were cultured without any additional factors, most of neurons died within the first 48 hours. In the presence of rmSCF, however, a significant number of neurons survived. The number of neurons started to decrease 4 days after the initiation of the culture even in the presence of rmSCF (data not shown). Thereafter, non-neuronal fibroblast-like cells vigorously proliferated, replacing the neurons. When the number of surviving neurons counted at 48 hours of the culture was used as an index of the trophic effect of rmSCF, we found that it was dependent on the rmSCF concentration. The effect was significant at 1 ng/ml and maximal at 100 to 300 ng/ml (Fig. 5). These values were comparable to the concentrations of rmSCF that were necessary for the neurite outgrowth from DRGs as shown in Fig. 3.

To rule out the possibility that rmSCF increased DRG neurons by promoting proliferation of neuronal precursor cells, DRG cells were cultured in medium containing BrdU, and cells in S phase of the cell cycle were assessed by BrdU incorporation. None of 143 neurons examined incorporated BrdU, but a large number of non-neuronal cells incorporated BrdU. The addition of rmSCF did not significantly affect the proportion of non-neuronal cells that incorporated BrdU; 75% of non-neuronal cells incorporated it in the presence of rmSCF and 78% did so in its absence.

NGF supports the survival of DRG neurons (Thoenen and Barde, 1980). The neurotropic effect of rmSCF was compared to that of NGF at saturation levels; rmSCF (300 ng/ml) and NGF (50 ng/ml). The number of neurons supported by NGF was 7.2 times greater than that supported

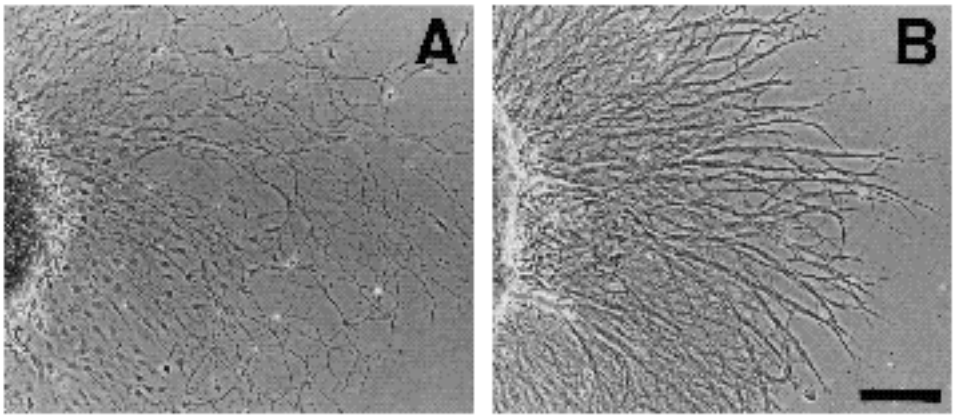

Fig. 4. Outgrowth of neurites induced by either rmSCF or NGF. DRGs of a C57BL/6-+/+ mouse embryo were cultured in the presence of (A) 100 ng/ml of rmSCF or (B) 50 ng/ml of NGF. Bar, 200 μm.

Table 2. Effect of embryo genotypes on neurite outgrowth induced by rmSCF (100 μg/ml)

Genotype of embryos	Embryo no.	Neurite outgrowth (μm)*		Genotype of embryos	Embryo no.	Neurite outgrowth (μm)*	
		rmSCF (−)	rmSCF (+)			rmSCF (−)	rmSCF (+)
+/+†	1	400±20	1110±70‡	*W*/*W*	1	640±60	610±60
	2	500±40	1240±160‡		2	540±40	500±30
	3	370±40	1360±180‡		3	630±50	560±40
	4	600±50	1150±80‡		4	560±40	570±20
	5	490±20	910±60‡		5	560±20	580±30
	6	440±20	1130±150‡		6	590±60	600±50
W^{42}/W^{42}	1	550±20	540±30	Sl^d/Sl^d	1	600±110	1150±110‡
	2	500±30	490±30		2	520±60	1040±100‡
	3	420±30	430±40		3	420±30	1050±60‡
	4	390±30	420±20		4	370±30	1120±110‡
	5	520±30	520±20		5	350±30	1080±90‡
	6	600±50	580±70		6	370±20	960±70‡

*Means±s.e.m. of 11 to 17 DRGs from individual embryos.
†Data from C57BL/6-+/+ embryos.
‡P<0.01 by the t-test when compared with values in the absence of rmSCF.

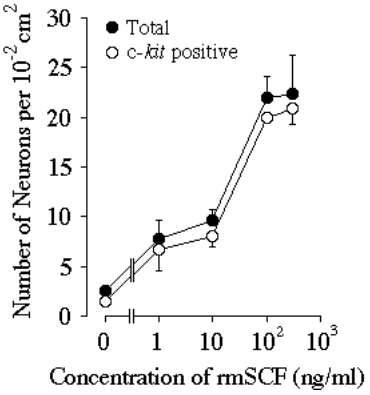

Fig. 5. Neuronal survival at various concentrations of rmSCF. The number of total neurons (closed circles) and that of c-*kit*-positive neurons (open circles) were counted 48 hours after the initiation of the culture. Each point represents the mean of 9 microscopic fields (10^{-2} cm^2 each) and vertical bars indicate the s.e.m.

by rmSCF (Table 3). Moreover, additing of rmSCF to NGF did not increase the number of surviving neurons (Table 3). When neurons whose survival was supported by rmSCF or NGF alone or rmSCF + NGF were stained with ACK2 mAb, 86% of them supported by rmSCF alone were c-*kit*-positive whereas only 12% of them supported by NGF alone and 13% by rmSCF + NGF were c-*kit*-positive. In other words, the total number of surviving neurons was much greater when the culture was supported by NGF than by rmSCF, but no significant difference was observed in the number of c-*kit*-positive neurons between these two conditions. Moreover, rmSCF did not increase the number of c-*kit*-positive neurons when SCF was added with NGF.

We used mutant embryos of WB-*W*/*W*, C57BL/6-W^{42}/W^{42} and WB-*Sl*/*Sl* to confirm the effect of rmSCF and NGF on the survival of DRG neurons. rmSCF did not support the survival of DRG neurons from WB-*W*/*W* and C57BL-W^{42}/W^{42} embryos. In contrast, rmSCF supported the survival of DRG neurons from WB-*Sl*/*Sl* embryos that were phenotypically similar to WB-*W*/*W* and C57BL/6-W^{42}/W^{42} embryos (Table 4). NGF supported the survival of DRG neurons from all mutant embryos examined (Table 4). Moreover, NGF supported the development of c-*kit*-positive neurons from DRGs of C57BL/6-W^{42}/W^{42} and WB-*Sl*/*Sl* embryos as well as DRGs from C57BL/6-+/+ embryos (Table 4).

DISCUSSION

rmSCF induced the outgrowth of c-*kit*-positive neurites from DRGs of mouse embryos. Moreover, rmSCF

Table 3. Neurotrophic effect of rmSCF (300 ng/ml) and NGF (50 ng/ml) on DRG neurons of C57BL/6-+/+ embryos

Factor	No. of neurons* per 10^{-2} cm^2	
	c-*kit*-positive	Total
None	3.6±0.4	6.0±0.7
rmSCF	24.0±2.6†	27.8±2.8†
NGF	24.6±3.5†	200.4±30.1†‡
rmSCF and NGF	26.8±4.4†	205.7±23.3†‡

*Means±s.e.m. of 9 microscopic fields (10^{-2} cm^2). Surviving neurons were counted 48 hours after the initiation of the culture.

†P<0.01 by the t-test when compared with the values from cultures to which no factors were added.

‡P<0.01 by the t-test when compared with the values from cultures to which rmSCF alone was added.

Table 4. Effect of embryo genotypes on neuronal survival promoted by SCF (300 ng/ml) and by NGF (50 ng/ml)

Embryos	Factor	No. of neurons* per 10^{-2} cm^2	
		c-*kit*-positive	Total
C57BL/6-+/+	None	0.4±0.1	1.1±0.2
	rmSCF	19.1±2.1†	20.4±2.3†
	NGF	22.6±2.3†	209.3±15.4†
WB-*W*/*W*	None	N.D.‡	1.1±0.2
	rmSCF	N.D.‡	1.2±0.2†§
	NGF	N.D.‡	180.8±13.0†
C57BL/6-W^{42}/W^{42}	None	0.1±0.1	0.9±0.3
	rmSCF	0.1±0.1†	1.1±0.3†§
	NGF	20.8±1.7†	196.2±14.1†
WB-*Sl*/*Sl*	None	0.1±0.1	0.7±0.1
	rmSCF	19.9±2.2†	21.9±2.6†
	NGF	22.4±2.0†	203.3±12.0†

*Pooled data of 3 embryos of each genotype, and 9 microscopic fields (10^{-2} cm^2) were examined in each culture. Mean ± s.e.m. of 27 (i.e. 3×9) fields.

†P<0.01 by the t-test when compared with the values from cultures to which no factors were added.

‡Not determined because of faint stainings with ACK2 mAb.

§P<0.01 by the t-test when compared with the values from C57BL/6-+/+ embryos.

supported the survival of c-*kit*-positive neurons in DRGs. The action of rmSCF was dose-dependent, and anti-c-*kit* ACK2 mAb abolished the effect of rmSCF on neurite outgrowth. The rmSCF did not induce neurite outgrowth from DRGs of WB-*W*/*W* embryos that lacked the extracellular domain of the c-*kit* receptor and did not support the survival of DRG neurons from WB-*W*/*W* embryos, either. NGF (Thoenen and Barde, 1980), brain-derived neurotrophic factor (Lindsay et al., 1985; Davies et al., 1986), neurotrophin-3 (Maisonpierre et al., 1990), S-100β (Eldik et al., 1991) and transforming growth factor α (Chalazonitis et al., 1992) all promote both neurite outgrowth and neuronal survival. The present result added another example to the association of these two functions.

The extracellular domain of the c-*kit* receptor has immunoglobin-like repeats (Besmer et al., 1986; Yarden et al., 1987). Cultured mast cells of mice express the c-*kit* receptor and some murine fibroblast cell lines express SCF on the surface. The c-*kit* receptor and SCF are used as adhesion molecules between mast cells and fibroblasts (Adachi et al., 1992). Under these conditions, the tyrosine kinase activity of the c-*kit* receptor is not necessary for the adhesion. In fact, cultured mast cells derived from C57BL/6-W^{42}/W^{42} mice may attach to fibroblasts expressing SCF. However, since rmSCF promoted neither the neurite outgrowth nor the neuronal survival from DRGs of C57BL/6-W^{42}/W^{42}, the tyrosine kinase activity of the c-*kit* receptor appears to be necessary for the effect of SCF on neurons.

DRG neurons are heterogeneous in many aspects and several classifications of DRG neurons have been attempted (Lawson, 1992). Our results indicated the presence of a c-*kit*-positive subpopulation in DRG neurons. Since DRG cell cultures supported by NGF alone contained a comparable number of c-*kit*-positive neurons with the cultures supported by rmSCF alone, SCF itself did not appear to be essential for the expression of c-*kit* receptor. However, anti-NGF mAb did not inhibit the rmSCF-induced neurite outgrowth from DRGs and NGF did not appear to be essential for the expression of the c-*kit* receptor. Taken together, the expression of c-*kit* receptor depended neither on SCF nor on NGF. NGF supported development of c-*kit*-positive neurons even from DRGs of WB-*Sl*/*Sl* embryos, indicating that the effect of NGF on c-*kit*-positive neurons is not mediated through endogenous SCF. Since the number of c-*kit*-positive neurons was comparable between cultures supported by NGF alone and by NGF+rmSCF, the c-*kit*-positive neurons appeared to be a subpopulation of NGF-responsive neurons.

The effect of rmSCF was observed at a concentration as low as 1 ng/ml and reached the maximal level at 100 to 300 ng/ml. These values are comparable with the effective concentrations of recombinant SCF for the proliferation of mast cells (Tsai et al., 1991) and for the survival of primordial germ cells (Godin et al., 1991; Dolci et al., 1991; Matsui et al., 1991). This implied that the effect of SCF on DRGs is physiological. There is evidence that axons are guided by diffusible chemoattractants or immobilized cues to reach their targets (Dodd and Jessell, 1988). SCF appears to be expressed within DRGs (Keshet et al., 1991; Motro et al., 1991). Moreover, SCF is expressed by epithelial and mesenchymal cells in the skin (Matsui et al., 1990; Keshet et al., 1991), the peripheral targets of some DRG neurons, and by motor neurons in the spinal cord (Keshet et al., 1991), the central targets of some DRG neurons. SCF expressed by these cells may play a role in guiding axons from DRGs.

There is another possibility. In neural development, neurons are made in excess and a substantial number of them are eliminated during target innervation (Barde, 1989). This naturally occurring neuronal death is believed to have an important role in adjusting the number of neurons to the target size, thus ensuring accurate synaptic connections. From this perspective, the peripheral nervous system is one of the most studied system because of the early identification of NGF. Several lines of evidence have indicated that limiting amounts of neurotrophic factors are produced and support the survival of limited numbers of DRG neurons (Barde, 1989). SCF may have a trophic role for DRG neurons such as that seen in mast cells (Tsai et al., 1991)

and germ cells (Godin et al., 1991; Dolci et al, 1991; Matsi et al., 1991). A recent study of the murine cerebellum showed that c-*kit*-positive neurons internalized the soluble form of SCF and accumulated the SCF in perikarya by retrograde tranportation (Manova et al., 1992). This suggests that SCF to c-*kit* signaling is indeed working in the nervous system. Moreover, the observation supports the neurotrophic role of SCF because uptake by nerve terminals and the subsequent retrograde transportation to neuronal perikarya are the common pathway for neurotrophic factors (Thoenen and Barde, 1980; Hendry et al., 1992).

To our knowledge, abnormalities suggesting defects in neuronal development have not been reported in homozygous or double heterozygous *W* or *Sl* mutant mice. The absence of any obvious neurological defects might be explained by the present results, which show most SCF-dependent neurons are also NGF-dependent. Alternatively, small defects may have escaped detection. Since c-*kit*-positive neurons represent a minor subpopulation of DRG neurons, SCF to c-*kit* signaling might have a role in the selective aspect of neural development. Further careful examination using homozygous or double heterozygous c-*kit* mutants of mice (Silvers, 1979; Russell, 1979) and rats (Niwa et al., 1991) may reveal abnormalities in neuronal development.

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