

Nerve growth factor prevents apoptosis of cord blood-derived human cultured mast cells synergistically with stem cell factor

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Summary

Background Stem cell factor (SCF) has been identified as a critical survival factor of human mast cells. Other cytokines which possess survival promotion activity on human mast cells are less known.

Objective We examined the survival promotion activity of nerve growth factor (NGF) on cord blood-derived human cultured mast cells.

Methods Expression and function of NGF receptors on the mast cells were examined by RT-PCR, flowcytometric analysis, immunoprecipitation and western blotting. The survival promotion activity of NGF to the mast cells was examined. To evaluate the proliferating activity of NGF on the human cultured mast cells, flow cytometric analysis with propidium iodide staining was applied. To confirm whether the human mast cell growth activity of NGF was caused by a suppression of apoptosis, the proportion of the cells containing *in situ* DNA fragmentation was counted.

Results The human cultured mast cells expressed the high affinity receptor p140^{trk} but not the low affinity receptor p75^{LNGFR}. NGF induced the phosphorylation of p140^{trk}. NGF alone could not support the survival of the mast cells, however, the addition of NGF to the culture medium containing recombinant SCF led to a significant increase of the number of survival mast cells. No significant changes of the cell cycle from G₀/G₁ phase to the S/G₂ + M phases were observed by NGF. In contrast, the addition of NGF to the medium with SCF showed a significant inhibitory effect on the apoptosis of the mast cells.

Conclusion NGF may act as a key factor to promote the survival of human mast cells synergistically with SCF through the prevention of apoptosis.

Keywords: mast cells, apoptosis, nerve growth factor, stem cell factor

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Introduction

Nerve growth factor (NGF) is a well-characterized neurotrophic factor essential for survival, differentiation, and function of peripheral sympathetic and sensory neurones and of

basal forebrain cholinergic neurones in the central nervous system [1,2]. In addition to the neurotrophic activities, its biological action has been extended to immune systems. For example, NGF enhances both T and B cell-mediated immune responses [3,4], promotes growth survival, phagocytosis, and superoxide production of mature murine neutrophils [5–7], and induces shape changes of rabbit platelets [8]. NGF causes mediator release from basophils [9] and promotes human hematopoietic colony growth, favouring basophil/mast cell differentiation [10–12]. *In vivo* administration of

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NGF to neonatal rats increases the size and the number of mast cells [13], and NGF induces mast cell degranulation *in vitro* [14]. Aloe [15] reported that the injection of anti-NGF antibody to adult rats led to a significant decrease in the number of peritoneal mast cells (PMC) with severe morphological alternations comparable to apoptotic features. Moreover, NGF induces the phenotypic changes from murine IL-3 dependent bone marrow-derived cultured mast cells to connective tissue-type mast cells [16]. There are also some clinical reports which showed the evidence of increased NGF levels in allergic human diseases [17,18].

Biological actions of NGF are mediated through two types of specific cell surface receptors with different affinity [19], and the expression of the receptors confirms the direct effect of NGF on the cells. The low-affinity NGF receptor is composed of a 75-kDa glycoprotein (p75^{LNGFR}), and does not have an enzymatic domain. On the other hand, the high-affinity NGF receptor is a 140-kDa molecule containing a tyrosine kinase domain that is encoded by the *trk* proto-oncogene isolated from human colon carcinoma (p140^{trk}).

Although stem cell factor (SCF) has been identified as a critical human mast cell survival factor, other cytokines which possess survival promotion activity on human mast cells are less known. Recently we have found that NGF is able to suppress apoptosis of rat PMC through p140^{trk} [20], whereas there has been no report to show the effect of NGF on mast cell survival of other species. Cord blood-derived human cultured mast cells was recently showed to be expressed p140^{trk} but not p75^{LNGFR} [21,22]. Therefore, we examined the effect of NGF on the survival of human mast cells. In the present study, we demonstrated that NGF alone could not inhibit apoptosis of the cells, however, NGF showed a promotional effect on the human cultured mast cells synergistically with SCF.

Materials and methods

Growth factors and antibodies

NGF isolated in the 2.5S form from murine submaxillary glands was kindly provided by Drs A. M. Stanis and J. Bienenstock (McMaster University, Hamilton, Ontario, Canada). Neurotrophic biological activity of the preparation was determined as described previously [11]. Recombinant human (rh) SCF and rhIL-6 were kindly provided by Kirin Brewery Co. (Maebashi, Japan). Alkaline phosphatase (AP)-conjugated mouse monoclonal antibody (mAb) specific to tryptase were purchased from Chemicon International, Inc. (Temecula, CA, USA). Mouse antihuman p75^{LNGFR} mAb was purchased from Cederlane Laboratories (Ontario, Canada), rabbit antihuman p140^{trk} antiserum and mouse anti-Trk-A mAb from Oncogene Research Products (Cambridge, MA, USA). Rabbit antimouse NGF fractionated

antiserum was obtained from Sigma Chemical Co. (St. Louis, MO, USA). We purchased goat antimouse Ab from Cappel (Durham, NC, USA), fluorescein isothiocyanate (FITC)-conjugated goat antirabbit IgG, phycoerythrin (PE)-conjugated donkey antimouse IgG and goat antihuman IgG from Jackson ImmunoResearch Laboratories (West Grove, PA, USA), and PE-conjugated mouse antihuman *c-kit* Ab from Nichirei (Tokyo, Japan).

Cell line

A human rhabdomyosarcoma cell line (RD cells) obtained from Health Science Research Resources Bank (Osaka, Japan) was maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS: Cytosystems, Castle Hill, Australia). Cells were cultured at 37 °C in a 5% CO₂ humidified incubator.

Cord blood-derived human mast cells

The methods to obtain the cord blood-derived human mast cells have recently been established [23–25]. Briefly, mononuclear cells were obtained from the heparinized cord blood after a centrifugation with Ficoll-paque (Pharmacia Biotech, Uppsala, Sweden) and suspended in Media I, which was a modified DMEM component plus 10 µg/mL insulin, 10 µg/mL transferin, 5 × 10⁻⁵ M 2-mercaptoethanol (2-ME), 25 mM HEPES, and 2.6 ng/mL NaSeO₃ (IBL Men-eki Seibutsu Kenkyujo, Gunma, Japan), containing 10% heat-inactivated FCS, 100 ng/mL rhSCF and 50 ng/mL rhIL-6, and they were cultured at 37 °C in a 5% CO₂ humidified incubator. Non-adherent cells were passaged in the culture medium weekly. The purity of mast cells was determined by staining with May-Grünwalds and Giemsa's solution (Muto Pure Chemicals Ltd, Tokyo, Japan) and by immunocytochemical staining for tryptase according to the method described by Irani *et al.* [26].

RNA purification, RT PCR analysis and cloning the products

Total cellular RNA was extracted by the method of acid guanidinium thiocyanate (GT)-phenol-chloroform extraction [27,28]. Oligonucleotide primers were synthesized by Kurabou Co. (Kurashiki, Japan) and their sequences are summarized as follows; p140^{trk} primers were 5'-TGGAG AAGAA GGACG AAACA-3' (sense, located at nucleotides 1277–1296) and 5'-GCCTT GACAG CCACC AGCAT-3' (antisense, at 1669–1688); p75^{LNGFR} primers, 5'-GACAG TGGCA TCTCC GTGGA CA-3' (sense, at 1023–1044) and 5'-ACACC GGGGA TGTGG CAGTG GA-3' (antisense, at 1374–1395). Primers used for β-actin were commercially available primers from Stratagene (La Jolla, CA, USA).

For PCR, the reaction mixtures or non-RT samples were added to a PCR buffer with primers (5 µg/mL of each primer) and dNTP mix (Pharmacia Biotech) to a total volume of 100 µL, and amplified using 35 cycles with 2 U of AmpliTaq Gold (Perkin-Elmer Corporation, Norwalk, CT, USA) under the following conditions; denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 1 min. Final extension was at 72 °C for 7 min. To visualize the PCR products, they were analysed by an electrophoresis in 1% agarose gel containing 0.5% ethidium bromide and visualized by UV excitation. PCR products were gel-purified with GeneClean II (Bio 101, Vista CA, USA), subcloned into the T/A cloning vector with a pGEM-T Easy vector ligation system (Promega Corporation, Madison, WI, USA), and subjected to DNA sequencing. DNA sequence was performed with a Dye Terminator Cycle Sequencing FS Ready Reaction kit (Perkin-Elmer Corporation) using ABI PRISM 377 DNA sequencer (Perkin-Elmer Corporation).

Flow cytometry

To detect the specific receptors for NGF on the human cultured mast cells, indirect immunofluorescence staining was provided as described previously [20] with slight modification. For p75^{LNGFR} detection, after washing once, 1×10^6 mast cells were resuspended in 100 µL of ice-cold phosphate-buffered saline (PBS) containing 1% FCS and 0.1% sodium azide with anti-p75^{LNGFR} mAb (5 µg/mL) and incubated for 30 min at 4 °C. After washing, the cells were incubated with FITC-conjugated second Ab for 30 min at 4 °C. After blocking with control mouse IgG (Sigma) for reduce extra-reaction against second Ab, cells were finally incubated with PE-conjugated anti-*kit* Ab (5 µg/mL) for 30 min at 4 °C. For p140^{trk} detection, the cells were fixed with 2% paraformaldehyde in Hanks' balanced salt solution for 15 min at 4 °C and washed once with 0.05% Triton X-100 (Wako Pure Chemical Industries, Osaka, Japan) to permeabilize cell membranes, because the anti-p140^{trk} antiserum recognizes the C-terminus of the *trk* proto-oncogene products. The cells were then incubated with anti-p140^{trk} antiserum (1:100) and PE-conjugated anti-*kit* Ab for 30 min at 4 °C. After washing, cells were stained with FITC-conjugated second Ab against rabbit for 30 min at 4 °C. After washing three times, each specimen was resuspended in FACS sheath solution, and analysed by a FACS flow cytometer. RD cells, which express both p75^{LNGFR} and p140^{trk} on their surface, served as positive controls. Negative controls were the cells incubated with isotype matched immunoglobulin instead of a specific primary Ab.

Immunoprecipitation and western blot analysis for p140^{trk}

For detection of the phosphorylation of p140^{trk}, 1×10^7

cultured mast cells were lysed in 500 µL of ice-cold lysis buffer (20 mM Tris buffer with 1% Nonidet P-40, 137 mM NaCl, 10% glycerol, 10 µg/mL aprotinin, 1 mM sodium orthovanadate, and 100 mM 4-[2-aminoethyl]-benzenesulphonyl fluoride hydrochloride) and treated by three times of freezing and thawing. The lysate was cleared by a centrifugation, incubated with Protein-A Sepharose CL-4B (Pharmacia Biotech), which was pre-incubated with goat antimouse Ab and sequentially with 1 µg of anti-Trk-A mAb, with a gentle mixing overnight at 4 °C, and then washed three times with the lysis buffer. Immunoprecipitates were subjected to SDS-PAGE using 6% polyacrylamide gel, and electroblotted onto a Hybond-ECL nitrocellulose membrane (Amersham, Arlington Heights, IL, USA). Membranes were incubated in a blocking buffer (0.01 M Tris, pH 7.4, 0.1 M NaCl, 0.1% Tween 20, 1% BSA) with a gentle shaking at 37 °C for 1 h, and then immunoblotted with a horseradish peroxidase (HRP)-conjugated antiphosphotyrosine Ab (Upstate Biotechnology, Lake Placid, NY, USA) at 1:1000 dilution at 37 °C for 1 h. The phosphorylated proteins were detected using an enhanced chemiluminescent (ECL) detection system (Amersham). Then the membrane was washed with stripping buffer (6.25 mM Tris buffer with 2% SDS and 100 mM 2-ME) for 30 min at 50 °C, incubated again in a blocking buffer and reblotted with anti-Trk-A mAb.

Survival assay

Ten-week cultured cord blood-derived human mast cells, which were 100% positive for tryptase, were washed three times with Media I in the absence of any cytokines, then replated in 24-well culture dishes (Corning Glass Works, Corning, NY, USA) at a density of 3×10^5 cells in 1 mL of each medium supplemented with NGF and/or rhSCF, and maintained at 37 °C in a 5% CO₂ humidified incubator. Cell number and viability were examined according to the trypan blue exclusion test.

Analysis of DNA synthesis

DNA synthesis of the cultured mast cells was investigated with a CycleTEST plus DNA reagent kit (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). Briefly, 5×10^5 cells were washed twice with a buffer solution containing sodium citrate, sucrose, and DMSO. After a centrifugation, trypsin in a spermine tetrahydrochloride detergent buffer, trypsin inhibitor and ribonuclease A in a citrate buffer were added sequentially and incubated for 10 min each at room temperature. Then specimens were incubated with propidium iodide (PI) for 10 min at 4 °C, and analysis was performed with CellFIT software on a FACS flow cytometer (Becton Dickinson Immunocytometry Systems).

In situ detection of apoptotic cells

In situ detection of DNA fragmentations was performed by TdT-mediated dUTP-fluorescein nick end labelling method using an *in situ* cell death detection kit, AP (Boehringer Mannheim GmbH, Mannheim, Germany). The cultured mast cells incubated with/without 50 ng/mL NGF, anti-NGF antiserum, or normal rabbit serum were cytocentrifuged on glass slides, fixed with 4% paraformaldehyde in PBS for 30 min at room temperature, and then incubated in a permeabilization solution containing 0.1% Triton X-100 and 0.1% sodium citrate for 2 min on ice. The slides were reacted with a mixture of terminal deoxynucleotidyl transferase (TdT) and nucleotide mixture to label the 3'-OH ends of DNA. Incorporated fluorescein into the tails of DNA molecules was identified by antifluorescein Ab conjugated with AP. The nuclei of multiple DNA fragment-containing cells were stained red by a Fast Red kit (Dako Corporation, Carpinteria, CA, USA). Cell nuclei were counterstained with Gill's hematoxylin (Sigma Chemical Co.).

Statistics

The analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was performed for statistical analysis of the data, and $P < 0.05$ was taken as the level of significance.

Results

Expression of NGF receptors on cord blood-derived human cultured mast cells

The effect of NGF on target cells is dependent on direct binding of NGF to its specific cell surface receptors. We examined the expression of two types of NGF receptors, p140^{trk} and p75^{LNGFR} on cord blood-derived human cultured mast cells. As shown in Fig. 1a, p140^{trk} specific primers yielded the expected product of 412 bp in the human cultured mast cells. In the absence of RT, PCR yielded no product, indicating the absence of genomic DNA contamination (data not shown). On the other hand, p75^{LNGFR} mRNA was not detected in the mast cells. The expected 373-bp product was observed in RD cells, indicating that the lack of RT PCR products was not due to defective primers. We further analysed PCR products by subcloning, being subjected to their sequences, and confirmed that the PCR product for p140^{trk} expressed in both the human cultured mast cells and RD cells, and the product for p75^{LNGFR} in RD cells were identical with their expected sequences of cDNA from GeneBank.

Then, flow cytometric analysis was performed in parallel using RD cells as positive controls. The cultured mast cells

showed positive reaction for p140^{trk}, whereas the mast cells did not show positive reaction for p75^{LNGFR} (Fig. 1b). The expression of p140^{trk} was also recognized in the 4-week cultured mast cells detected by surface Kit expression (data not shown).

Because p140^{trk} has a tyrosine kinase domain, its autophosphorylation is immediately induced by a binding of NGF to the receptor [29,30]. To investigate whether p140^{trk} on the cord blood-derived human cultured mast cells is functional, the cells were treated for 5 min with 50 ng/mL NGF, and subjected to immunoprecipitation and western blot analysis. The mast cells were starved in DMEM with 1% BSA for 2 h before NGF stimulation. An immunoblot analysis of p140^{trk} with antiphosphotyrosine mAb showed that NGF induced tyrosine phosphorylation of p140^{trk} in the cultured mast cells (Fig. 1c). K252a has been shown to be able to specifically inhibit tyrosine kinase activity of p140^{trk} [31,32]. When the mast cells were pretreated with 50 ng/mL K252a (Calbiochem-Novabiochem Corporation, La Jolla, CA, USA) 30 min before the incubation with 50 ng/mL NGF, the autophosphorylation of p140^{trk} was decreased (Fig. 1c).

Survival-promoting activity of NGF on cord blood-derived human cultured mast cells

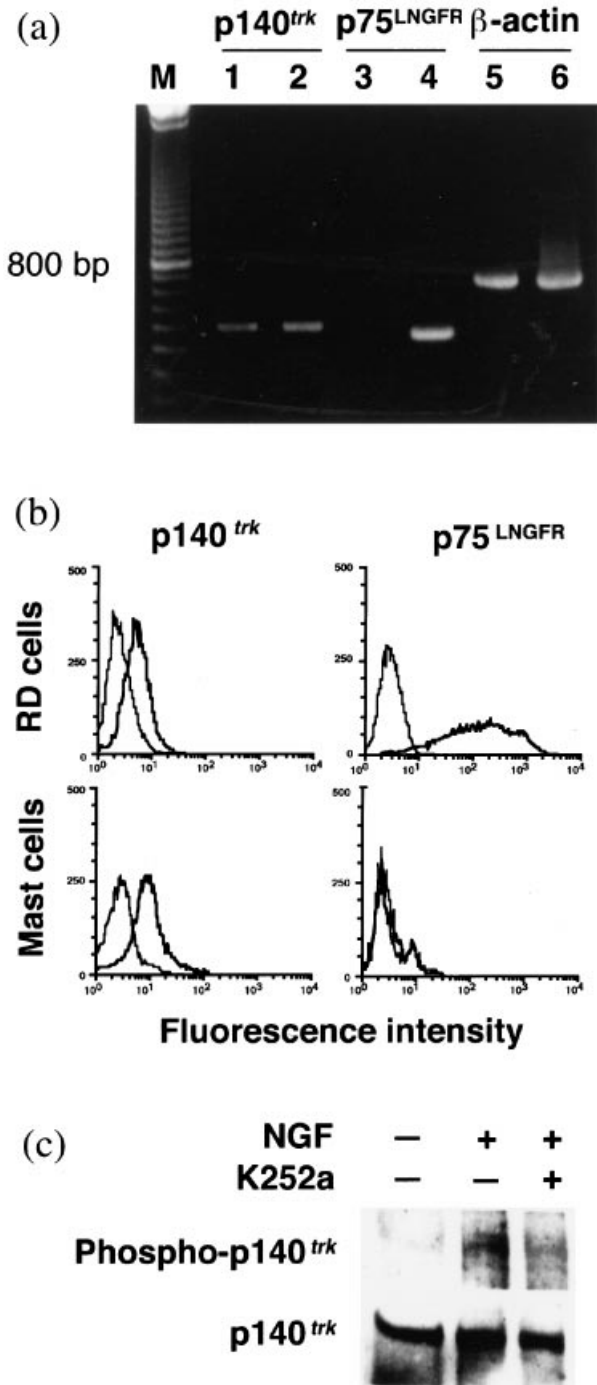
The effect of NGF on the survival of the cultured mast cells in the serum-containing medium was examined. We used the 10-week cultured mast cells in this experiments and further incubated the cells for another week. As shown in Fig. 2, the addition of 50 ng/mL NGF to the culture medium containing 100 ng/mL rhSCF resulted in an increase of the number of the human mast cells after further 1 week in comparison with the medium with rhSCF alone. To confirm the specificity of the proliferative effect of NGF on the human mast cells, we examined the effect of antiserum to 2.5S form of NGF, which was shown to completely block the biological nerve-stimulating activity of NGF. When the anti-NGF antiserum was added to the medium containing NGF and rhSCF or rhSCF alone, the number of the mast cells were not different from the control, showing that polyclonal antibodies against NGF completely and specifically abolished the proliferating activity of NGF to the cultured mast cells.

On the other hand, the number of viable mast cells significantly decreased after further 1-week culture without rhSCF (Fig. 2), suggesting that NGF alone could not support the survival of the human mast cells. Normal rabbit serum showed no inhibitory effect against NGF.

Proliferating activity of NGF on cord blood-derived human cultured mast cells

Possible two mechanisms can be considered in the growth-promoting effect of NGF on the mast cells. The first is a

proliferation of the mast cells, and the second an inhibition of the cell death. To evaluate the proliferating activity of NGF on the human cultured mast cells, flow cytometric analysis with PI staining was applied. In a DNA histogram of 10-week cultured mast cells, more than 95% of the cells were distributed in the G₀/G₁ phase, and very low percentages of proliferation were observed (data not shown).



Then, we separated the mast cells with surface Kit expression by magnetic cell sorting system (MACS; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) from the 4-week cultured cells, which represented magnificent growth in the medium containing rhSCF and rhIL-6, and performed flow cytometric analysis with PI staining. The proportion of S/G₂ + M phase in the beads-separated *c-kit* positive cells incubated with rhSCF alone for 24 h was 10.7% and that in the cells maintained in absence of any cytokines was 10.5%. No significant change in the proportion was observed by the addition of 50 ng/mL NGF (Table 1). In addition, the proportion of S/G₂ + M phase in the cultured cells incubated with 50 ng/mL NGF alone for 24 h was 9.7%, indicating that NGF itself did not induce a proliferation of the human mast cells. On the other hand, the proportion of S/G₂ + M phase in the separated mast cells in the cultured medium was 21.4%, suggesting that the proliferating effect on the human mast cells in the medium containing with 100 ng/mL rhSCF and 50 ng/mL rhIL-6 may not be derived from SCF but IL-6.

NGF prevents apoptosis of cord blood-derived cultured human mast cells

The mast cells cultured in the medium without rhSCF for 1 week showed a wrinkled appearance under phase-contrast microscopy, and the number of the cells became smaller than the cells cultured with rhSCF. To determine whether such cell death was caused by apoptosis, *in situ* DNA

Fig. 1. (a) Expression of p140^{trk} and p75^{LNGFR} mRNA in cord blood-derived human cultured mast cells. Total RNA was reverse-transcribed and amplified for 30 cycles using specific primers for p140^{trk} and p75^{LNGFR}. PCR products were electrophoresed in 1% agarose gel. As a sized marker, a 100-bp ladder with the 800 bp as a bright band was used. Lane M is the sized marker, lane 1, 3, and 5 are the cultured mast cells, and lane 2, 4, and 6 are RD cells as a positive control. (b) Flow cytometric analysis of the expression of NGF receptors on cord blood-derived human cultured mast cells. RD cells as a positive control, and the 12-week cultured mast cells were stained with FITC-conjugated second Ab after the treatment with anti-p140^{trk} antiserum or anti-p75^{LNGFR} mAb. Negative controls were the cells incubated with isotype matched immunoglobulin instead of a specific primary Ab. (c) Phosphorylation of 140^{trk} in the cultured mast cells by NGF. The cultured mast cells were pre-incubated in the medium without FCS and any cytokine for 2 h, and then treated by the medium alone, medium containing 50 ng/mL NGF, or NGF and 50 ng/mL K252a for 5 min. After immunoprecipitation with an anti-Trk-A mAb, the lysates were boiled, resolved on SDS-PAGE using 6% polyacrylamide gel, and probed for phosphotyrosine with the mAb (Phospho-140^{trk}). The membrane was washed in stripping buffer, and reblotted with anti-Trk-A mAb (140^{trk}).

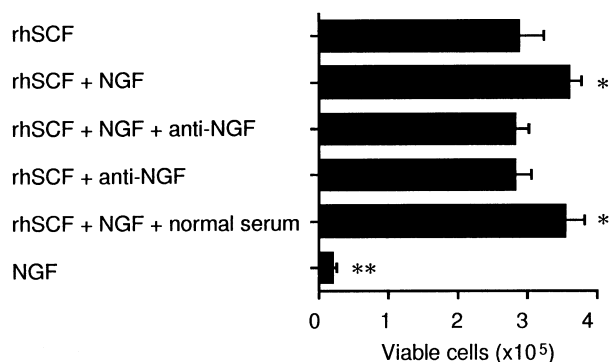


Fig. 2. Viable human mast cell numbers after further 1 week culture with NGF. The 10-week cultured human mast cells were cultured in the medium containing 10% heat-inactivated FCS at a density of 3×10^5 cells/mL with factor(s), and the cell number was counted by the trypan blue exclusion test further 1 week after the incubation. Added factor(s) were 100 ng/mL rhSCF, 50 ng/mL NGF, anti-NGF antiserum derived from rabbit, or normal rabbit serum. Each value represents the mean $\pm$ SEM of three separate experiments performed in triplicate. * $P < 0.05$ and ** $P < 0.01$, when compared with rhSCF alone.

fragmentation of the cells was examined. The nuclei of dying mast cells were stained red with the evidence of a multitude of DNA fragmentation, because the intensity of the staining was dependent on the amount of incorporated nucleotides into the 3'-OH ends of DNA fragmentation. The proportion of the apoptotic cells without rhSCF was significantly higher than that with rhSCF (data not shown). To confirm whether the human mast cell growth activity of

Table 1. Proliferation activity of NGF on the 4-week cultured beads-separated *c-kit* positive cells

Cytokine(s)	Cell cycle distribution		
	G ₀ /G ₁	S	G ₂ + M
None	89.5	6.8	3.7
NGF	90.3	6.5	3.2
RhSCF	89.3	8.2	2.5
RhSCF + NGF	89.5	8.4	2.1
RhSCF + rhIL-6	78.6	17.3	4.1
RhSCF + rhIL-6 + NGF	78.8	17.0	4.2

The human mast cells were separated from the 4-week cultured cells with anti-*kit* Ab by MACS and incubated in Media I at a density of 1×10^6 cells/mL with/without cytokine(s) for additional 24 h. Added cytokine(s) were 50 ng/mL NGF, 100 ng/mL rhSCF, or 50 ng/mL rhIL-6. Then, flow cytometric analysis with PI staining was applied.

NGF was caused by a suppression of apoptosis, the proportion of the cells containing *in situ* DNA fragmentation was counted. As shown in Figs 3 and 4, the addition of 50 ng/mL NGF to the medium with rhSCF showed a significant effect on the suppression of apoptotic cell number.

Discussion

Although NGF has been reported to be a survival factor for rat PMC, and to prevent apoptosis of the cells [20,33], human mast cells have been shown not to respond to NGF [21,24]. In this study, to check the survival promoting effect of NGF on human mast cells, we investigated the expression of specific receptors for NGF on the surface of cord blood-derived human cultured mast cells and a biological activity of NGF for the survival of the cells. The analysis using RT-PCR and flow cytometry showed that p140^{trk} but not p75^{LNGFR} was expressed on cord blood-derived human cultured mast cells, and immunoprecipitation and western blot analysis revealed that NGF elicited a rapid phosphorylation of p140^{trk} on tyrosine residue of the cells, in accordance with the findings on rodent mast cells [20] and recent reports on human mast cells [21,22]. Then we demonstrated that the pretreatment with K252a, a tyrosine kinase inhibitor, inhibited p140^{trk} kinase activity, suggesting that the effect of NGF was mediated through the p140^{trk}, as previously described in rat PMC [20].

Nilsson *et al.* [21] and Yanagida *et al.* [24] reported that, in the absence of SCF, NGF showed no effect on the survival of human mast cells. However, in this present study, we showed that addition of NGF to the rhSCF-containing medium made the number of viable mast cells increased significantly. Polyclonal antibodies against NGF completely abolished this activity of NGF but not rhSCF. These results indicated that NGF promoted the survival of

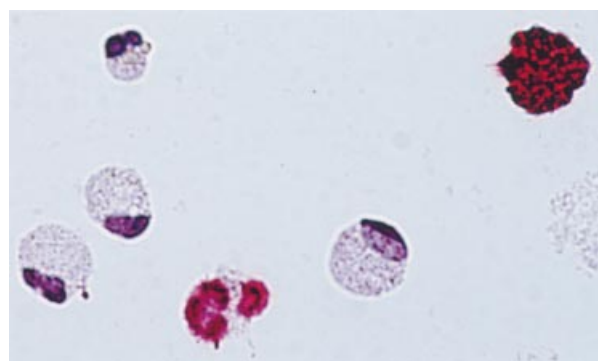


Fig. 3. *In situ* detection of DNA fragmentations by TdT-mediated dUTP-fluorescein nick end labelling method. The nuclei of dying mast cells were stained red with the evidence of a multitude of DNA fragmentation.

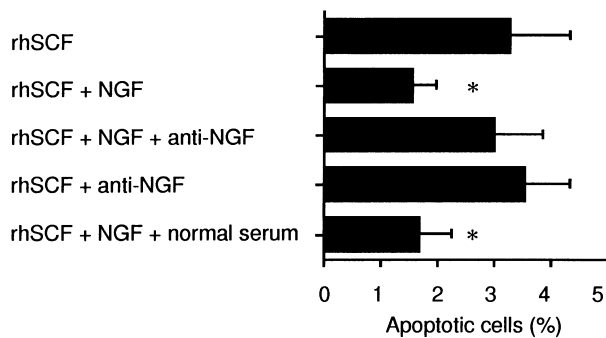


Fig. 4. Proportion of apoptotic cells on human mast cells after further 1 week culture with NGF. *In situ* detection of DNA fragmentations was performed by TdT-mediated dUTP-fluorescein nick end labelling method. Added factor(s) were 100 ng/mL rhSCF, 50 ng/mL NGF, anti-NGF antiserum derived from rabbit, or normal rabbit serum. Each value represents the mean $\pm$ SEM of three separate experiments performed in triplicate. * $P < 0.05$, when compared with rhSCF alone.

the human mast cells in the presence of SCF. *In vivo* human mast cells exist in the environment always supported with cell-bound SCF, which is produced by fibroblasts, stroma cells, and epithelial cells of the airway [34]. Therefore, the effect of NGF to the mast cells *in vitro* should be estimated in the presence of SCF.

Two possible mechanisms could be considered as the action of NGF to increase mast cell numbers; the proliferation of mast cells and/or the inhibition of the cell death. Although combination of rhSCF and rIL-6 induced active DNA synthesis, no significant changes on DNA synthesis were observed by the addition of NGF, suggesting that NGF does not have a proliferating activity to human mast cells. We also ascertained that NGF alone could not prevent apoptosis of the cultured human mast cells, unlike rat PMC, of which NGF alone has been reported to support the viability [20]. In contrast, the addition of NGF to the medium containing rhSCF showed a significant inhibitory effect on the apoptosis of the human cultured mast cells. These results indicated that NGF showed a survival promotion effect on the human mast cells synergistically with SCF by the prevention of their apoptosis.

The expression of p140^{trk} but not p75^{NGFR} is also recognized in human basophils [9], indicating an interaction between the nervous system and the effector phase of allergic reaction. Actually, it has been shown that NGF is accumulated in the site of inflammatory response and that NGF plasma levels are increased in allergic diseases [17,18]. In the sites of allergic inflammation, NGF may act as a key factor to promote a survival of human mast cells by the prevention of apoptosis synergistically with SCF, and play a possible modulatory role for allergic inflammation.

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