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SHORT COMMUNICATION

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Granulocyte and macrophage colony-stimulating factors stimulate proliferation of human keratinocytes

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Granulocyte/macrophage colony-stimulating factor (GM-CSF), granulocyte CSF (G-CSF), and macrophage CSF (M-CSF), modulators of hematopoietic cells, are widely used for the treatment of neutropenia after chemotherapy for malignant disease and other neutropenic states [1, 2]. Some keratinocyte-derived cytokines, such as interleukin-1 (IL-1), IL-6, GM-CSF, transforming growth factor (TGF)-α, and tumor necrosis factor (TNF)-α [3–7] are known as autocrine factors. To elucidate possible autocrine mechanisms of these two CSFs, we have investigated stimulatory effects on keratinocyte growth by G- and M-CSFs.

Neonatal human foreskin keratinocytes (more than 5×10^5 cells) in the Keratinocyte Growth Kit (Cascade Biologics, Portland, Ore.) were used. Thawed cells were seeded in modified MCDB 153 [8] (Nissui Seiyaku, Tokyo), that contained 150 μg/ml bovine pituitary extract, 5 μg/ml bovine insulin, 0.4 μg/ml hydrocortisone, 10 μg/ml bovine transferrin and 10 ng/ml human epidermal growth factor. Cells were used at the second or third passages and seeded in six wells at a density of 1.0×10^4 cells/cm^2. The medium was changed to modified MCDB 153 without bovine pituitary extract, and with or without recombinant G-CSF (0.1–10 ng/ml) (Kirin Beer, Tokyo) or recombinant M-CSF (1-100 ng/ml) (Midori Jyuji, Osaka). Control experiments for specificity were performed by the addition of G-CSF- (2×10^{-2} to 10^{-4} dilution) (the kind gift of Kirin Beer) or M-CSF-specific neutralizing antibody (1×10^{-2} to 10^{-4} dilution) (the generous gift of Morinaga Nyugyo, Kanagawa). After 4 and 7 days, cells were detached, collected and counted by trypan blue exclusion on a hemocytometer. [^{3}H]Thymidine (0.5 μCi/well: Dupont/Nen Research Products, Boston, Mass.) was incubated in keratinocytes per well for 24 h. Cultured keratinocytes were detached and harvested with an automatic cell harvester (ACH-96, BioTec, Tokyo). Radioactivity was measured with a liquid scintillation counter (LKB 1216 Packbeta, Wallac Oy, Turk, Finland). The experiments were triplicated and were analyzed by Student's *t*-test for unpaired values.

Two CSFs studied showed dose-dependent stimulation of human keratinocyte growth. Cell number increased at G-CSF concentrations between 0.1 and 10 ng/ml. M-CSF also stimulated proliferation of keratinocytes with similar effects at higher concentrations (10–100 ng/ml) (Fig. 1). G- and M-CSFs induced the increase in [^{3}H]thymidine uptake dose-dependently, indicating parallel results to cell number studies (Fig. 2). Control experiments with the addition of the specific neutralizing antibodies demonstrated no significant increase in cell number or [^{3}H]thymidine uptake.

This study demonstrated that G- and M-CSFs had stimulatory effects on keratinocyte growth in culture. These results indicate that they may be potential autocrine growth factors for keratinocytes, since these two CSFs are produced by keratinocytes as well as by immune cells [9, 10]. G-CSF exerted similar proliferative effects at lower concentrations than M-CSF, suggesting that G-CSF may have relevance to binding to keratinocytes. Receptors for M-CSF are identified as the gene product of the c-*fms*-protooncogene, showing a possible role for M-CSF in non-hematopoietic tissue [11]. Each receptor is expressed in other cells [10], while little information is available for keratinocytes. An investigation of receptors of G- and M-CSFs in human keratinocytes is currently under way.

GM-CSF is synthesized by keratinocytes and has the ability to capacitate isolated murine Langerhans cells for

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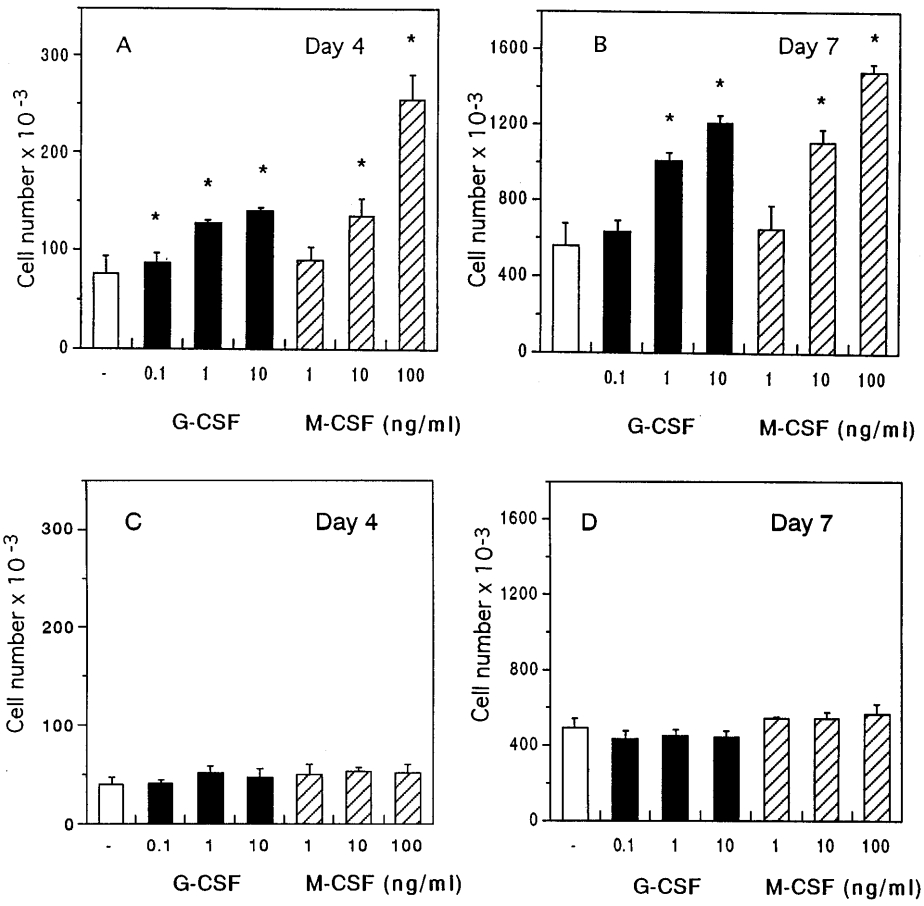

Fig. 1 **A, B** Stimulation of keratinocyte proliferation by G- and M-CSFs after 4 (**A**) and 7 days (**B**). Cells were cultured in modified MCDB 153 without bovine pituitary extract, and with or without G-CSF or M-CSF. * Significant p values of ($0.01 < p < 0.02$) compared with no added CSFs. *Open bars*, no added CSFs; *black bars*, added G-CSF (0.1-10 ng/ml); *hatched bars*, added M-CSF (1–100 ng/ml). **C, D** Control experiments were performed in a separate run by adding specific neutralizing antibodies after 4 (**C**) and 7 days (**D**). Cells were cultured in modified MCDB 153 without bovine pituitary extract, and with a mixture of the cytokine and neutralizing antibody. *Open bars*, no added CSFs; *black bars*, added G-CSF (0.1–10 ng/ml) and G-CSF neutralizing antibody (2×10^{-4} to 10^{-2} dilution); *hatched bars*, added M-CSF (1–100 ng/ml) and M-CSF neutralizing antibody (1×10^{-4} to 10^{-2} dilution)

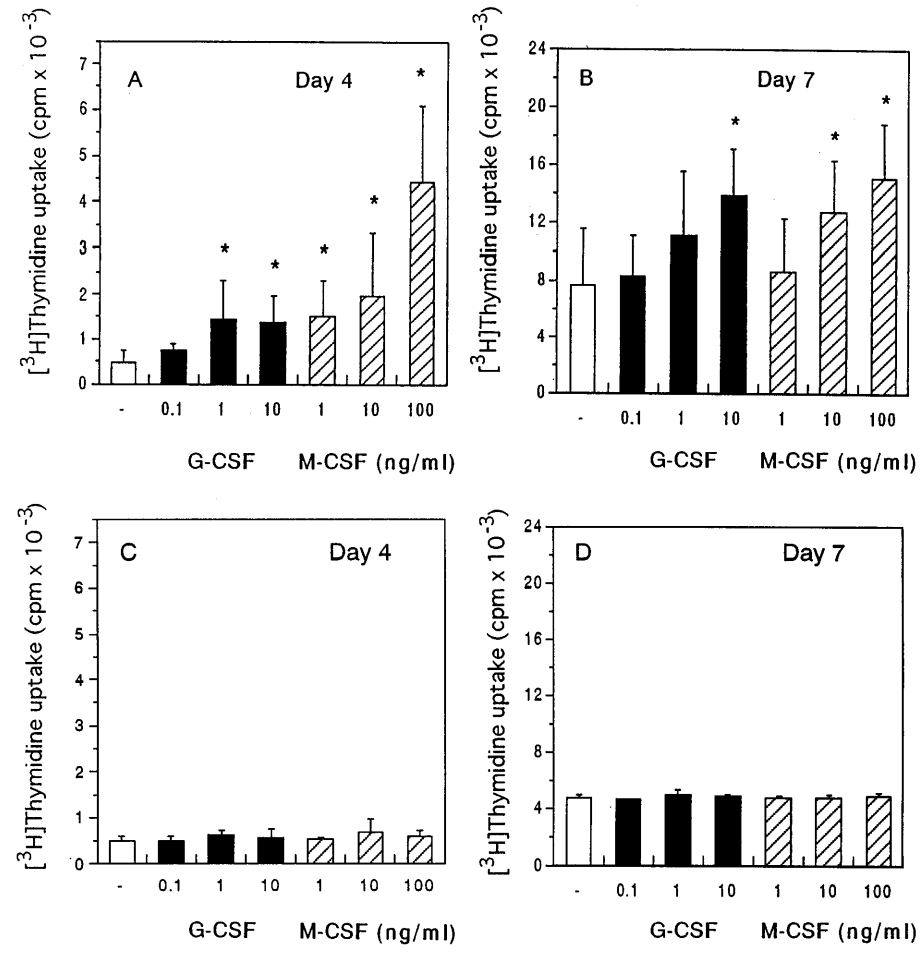

Fig. 2 **A, B** Stimulation of [^{3}H]thymidine uptake in keratinocytes by the CSFs after 4 (**A**) and 7 days (**B**) of culture. [^{3}H]Thymidine (0.5 μCi/well) was incubated in cells per well for 24 h and harvested. * Significant p values ($0.01 < p < 0.05$) compared with no added CSFs. *Open bars*, no added CSFs; *black bars*, added G-CSF (0.1–10 ng/ml), *hatched bars*, added M-CSF (1-100 ng/ml). **C, D** Control experiments were conducted separately with the addition of specific neutralizing antibodies after 4 (**C**) and 7 days (**D**) of culture. *Open bars*, no added CSFs; *black bars*, added G-CSF (0.1–10 ng/ml) and G-CSF neutralizing antibody (2×10^{-4} to 10^{-2} dilution), *hatched bars*, added M-CSF (1–100 ng/ml) and M-CSF neutralizing antibody (1×10^{-4} to 10^{-2} dilution)

their accessory function [4, 12]. Keratinocyte-derived M-CSF presumably controls Langerhans cells in a paracrine manner [10]. M-CSF also inhibits the decrease in rRNA and IA_β mRNA in epidermal Langerhans cells in conjugation with TNF-α and GM-CSF [13]. It is possible that M-CSF, like GM-CSF, may affect T-cell mediated immunity via paracrine effects on Langerhans cells.

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