Functional maturation of myeloid cells during *in vitro* differentiation from human cord blood CD34+ cells

CD34+ cells purified from human cord blood were expanded in the presence of several cytokines. The cultured cells were assayed for myeloid effector functions, including phagocytic activity, respiratory burst and microbicidal activity. The results showed that phagocytic activity was observed as early as day 6, irrespective of the type of cytokines used. By contrast, respiratory burst and microbicidal activity peaked on day 15 and were dependent on the type of cytokines used. In particular, granulocyte-colony stimulating factor and interleukin-6 markedly enhanced respiratory burst and microbicidal activity.

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One of the most promizing candidates of cytotherapy is the CD34+ progenitor cell that has been expanded *ex vivo* and induced to granulocytic differentiation.[1] CD34+ cells may be used in the treatment of neutropenia following high-dose chemotherapy, accidental radiation exposure, or hematopoietic stem cell transplantation. The clinical usefulness of *ex vivo* expanded hematopoietic progenitor cells (HPCs) was proposed a decade ago.[2]

We have studied the phenotypic changes of myeloid differentiation during *ex vivo* expansion of human umbilical cord blood (CB) CD34+ cells.[3] The *ex vivo* expanded cells can be considered for clinical use on the premise that effector functions of the expanded cells have been proved to be acquired normally. However, no previously published study has examined the effector functions of maturing myeloid cells expanded from human CB. In the present study, we have investigated the effector functions of maturing myeloid cells during *ex vivo* expansion of human CB CD34+ progenitor cells.

CD34+ cells, purified from human CB as previously described,[3] were cultured in Iscove's modified Dulbecco's medium (IMDM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (BioWhittaker, Walkersville, MD, USA) and combinations of several cytokines including thrombopoietin (TPO; 50 ng/mL; Kirin Brewery, Maebashi, Japan), flt3-ligand (FL; 50 ng/mL; Chemicon, Temecula, CA, USA), stem cell factor (SCF; 50 ng/mL; Kirin Brewery), granulocyte colony-stimulating factor (G-CSF; 100 ng/mL; Kirin Brewery), granulocyte-macrophage colony-stimulating factor (GM-CSF; 100 ng/mL, Kirin Brewery), interleukin (IL)-3 (5 ng/mL; Endogen, Woburn, MA, USA), and/or IL-6 (10 ng/mL; Endogen).

Phagocytic activities were measured by flow cytometry after the cells had been incubated with fluorescent latex beads. Respiratory burst activities of the cultured cells during production of superoxide anion and hydrogen peroxide were measured by the nitroblue tetrazolium (NBT) reduction test and chemiluminescence assay. The microbicidal activity of the cultured cells was measured by opsonophagocytic assay for *Candida albicans* opsonized with human gamma globulin. The results showed that the phagocytic activity of the cultured cells was observed as early as day 6 and continued unabated until day 18 (Figure 1). The proportions of the cultured cells showing phagocytic activity did not differ significantly irrespectively of the type of cytokine used. The NBT reduction test revealed different patterns of formazan deposits in the cultured cells depending on the type of cytokine used (*data not shown*). In the cultures with only TPO and FL, formazan crystals were deposited in 22±6% (n=6) of the cells that had been expanded for 14 days. Addition of IL-6 or G-CSF increased the amount of crystals formed in each cell as well as the proportion of the cells with formazan crystals.

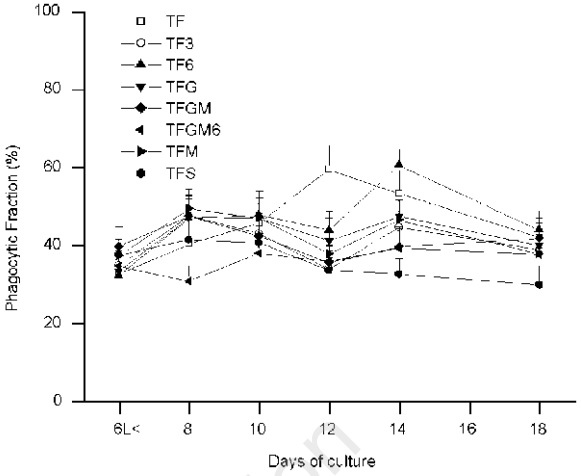

Figure 1. Phagocytic fractions of the cultured cells during *in vitro* differentiation from human cord blood CD34+ cells. The cultured cells (1.0×10^5 cells in 1 mL) were mixed with 5 µL of 2.67% Fluoresbrite™ PC Red latex beads (average diameter 1 µm, Polysciences, Warrington, PA, USA). After 1 hr of incubation at 37°C under agitation, the cells were fixed by mixing with equal volume of 1% paraformaldehyde. The unphagocytosed beads were eliminated by centrifugation on the density of FBS at 300×g for 5 min at room temperature. After the cell pellet had been resuspended with 0.5 mL of PBS, the fluorescence was measured by a FACSCalibur flow cytometer (Becton Dickinson). Data represent mean + SD of four to six separate experiments. T, thrombopoietin; F, flt3-ligand; G, granulocyte colony-stimulating factor; M, granulocyte-macrophage colony-stimulating factor; S, stem cell factor; 3, interleukin-3; 6, interleukin-6.

Quantitative measurement of the produced superoxide anion and hydrogen peroxide by chemiluminescence assay showed that respiratory burst activity began to increase from day 6 and peaked on day 15 (Figure 2A). In parallel with the results of the NBT test, G-CSF and/or IL-6 significantly increased the respiratory burst. Microbicidal activity, as measured by opsonophagocytic assay for *C. albicans*, began to increase from day 8 and peaked on day 15 (Figure 2B). G-CSF, GM-CSF, and/or IL-6 significantly increased the microbicidal activity (*data not shown*).

The results showed that the expanded cells had phagocytic, respiratory burst and microbicidal activities, while the extent of each effector function differed depending on the type of cytokine used and the duration of culture. Meanwhile, phagocytic activity appeared as early as day 6 and did not differ significantly irrespectively of the type of cytokine used, suggesting that it is one of the most primitive functions acquired during the early stage of the differentiation of myeloid cells. Respiratory burst and microbicidal activities, on the other hand, began to increase from day 6 or 8 and peaked on day 15, and were dependent on the type of cytokine used. These results suggest that respiratory burst and microbicidal activities are somewhat refined functions, require special growth factors and appear late during myeloid maturation. Taken together, these results are comparable to those of the classical study by Glasser *et al*.[4] on the granulocytes from BM and peripheral blood (PB) that were physically separated into different stages of maturation.[4] In the present study, we have proved that functionally competent myeloid cells can be generated from human CB CD34+ cells by *ex vivo* expan-

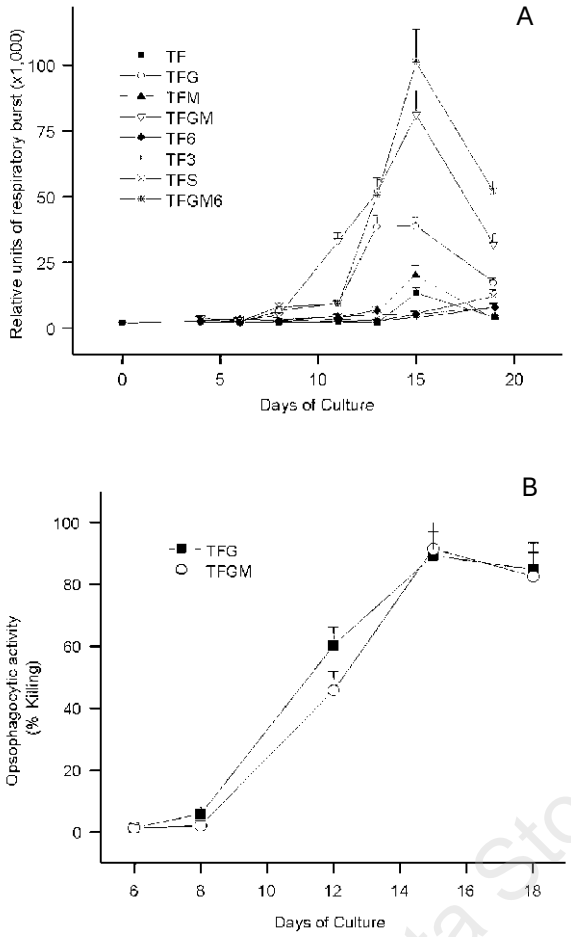

Figure 2. Respiratory burst (A) and microbicidal activities (B) of the cultured cells during *in vitro* differentiation from human cord blood CD34⁺ cells. Respiratory burst was measured by the chemiluminesce assay. The cultured cells, incubated with 1 µM luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma) were stimulated with 2 µg/mL of phorbol myristate acetate (Sigma) and the amounts of produced oxygen metabolites were measured by a luminometer (Multi-biolumat LB9505C; Berthhold, Germany). Microbicidal activity was measured by the opsonophagocytic assay for *Candida albicans*. Yeast cells of *C. albicans* were opsonized by incubation with human gamma globulin (Green Cross Co., Seoul, Korea). The opsonized yeast cells were incubated with the cultured cells in a shaking incubator at 37°C for 1 hr. The mixtures were washed and diluted in DW to lyse the cells to release the intracellular yeast, and the numbers of colony-forming units (CFUs) were determined by spread-plating on Brain Heart Infusion agar plates. Microbicidal activity was expressed as % killing, which is defined as (CFUs of control – CFUs experiment)/CFUs of control × 100, where the control is the yeast cells not incubated with the cultured cells. In this experiment, the opsonophagocytic activities were measured at the cell/yeast ratio of 500/1. Data represent mean + SD of four to six separate experiments. T, thrombopoietin; F, flt3-ligand; G, granulocyte colony-stimulating factor; M, granulocyte-macrophage colony-stimulating factor; S, stem cell factor; 3, interleukin-3; 6, interleukin-6.

sion. However, acquisition of each effector function was different depending on the type of cytokine used for *ex vivo* expansion.

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Human herpesvirus-7 infection impairs the survival/differentiation of megakaryocytic cells

In vitro infection of CD61⁺ megakaryocytic cells with human herpesvirus-7 (HHV-7) induced a drastic increase of apoptosis. Moreover, cells surviving HHV-7 cytotoxicity showed enhanced megakaryocytic maturation with respect to control cultures. These data suggest that HHV-7 reactivation in the bone marrow of HIV-1 infected individuals may contribute to impair megakaryocytopoiesis.

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The human herpesvirus-7 (HHV-7), which shows many similarities with HHV-6 and human cytomegalovirus (HCMV),[1] is a prevalent virus toward which >90% of the population is seropositive by adulthood.[2] The first clinical manifestation clearly associated with primary HHV-7 infection was exanthema subitum,