One of the most promising candidates of cytotherapy is the CD34+ progenitor cell that has been expanded ex vivo and induced to granulocytic differentiation. CD34+ cells may be used in the treatment of neutropenia following high-dose chemotherapies, 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.4 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,1 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 combination 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 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.

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 activity. Microbicidal activity, as measured by opsonophagocytic assay for C. albicans, began to increase from day 8 and peaked on day 15 (Figure 2A). The proportions of the cultured cells showing 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.6 on the granulocytes from BM and peripheral blood (PB) that were physically separated into different stages of maturation.7 In the present study, we have proved that functionally competent myeloid cells can be generated from human CB CD34+ cells by ex vivo expansion.
sion. However, acquisition of each effector function was different depending on the type of cytokine used for ex vivo expansion.

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References

Human herpesvirus-7 infection impairs the survival/differentiation of megakaryocytes

In vitro infection of CD61+ megakaryocytic cells with human herpesvirus-7 (HHV-7) induced a drastic increase of apoptosis. Moreover, cells surviving HHV-7 cytolysis 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 impairment of megakaryocyte maturation.

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,