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

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 chemiluminescence assay. The cultured cells, incubated with 1 µM luminol (3-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; Berthold, 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.

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The human herpesvirus-7 (HHV-7), which shows many similarities with HHV-6 and human cytomegalovirus (HCMV), is a prevalent virus toward which >90% of the population is seropositive by adulthood. The first clinical manifestation clearly associated with primary HHV-7 infection was exanthema subitum.
which is sometimes associated with thrombocytopenia.3-4

In this study, we investigated the effects of HHV-7 infection on clonogenic megakaryocytic progenitors in semisolid cultures and of cells differentiating along the megakaryocytic lineage in liquid culture. The experiments were carried out on cord blood (CB) CD34+ hematopoietic cells after obtaining maternal informed consent according to the Helsinki declaration of 1975. CB CD34+ cells were isolated using a magnetic cell sorting program (Mini-MACS) and the CD34 isolation kit (both from Miltenyi Biotec, Auburn, CA, USA). Consistently with the previously reported lack of intrauterine transmission of HHV-7,2 analysis of HHV-7 DNA by polymerase chain reaction (PCR) confirmed that CB CD34+ cells were negative for endogenous HHV-7 infection (data not shown).

CD34+ cells were inoculated with cell-free HHV-7, prepared as described elsewhere,1 and seeded in plasmacytoid semisolid cultures, supplemented with stem cell factor (SCF, 50 ng/mL) + thrombopoietin (TPO, 100 ng/mL). The number of megakaryocytic colonies (CFU-meg), scored 14 days following infection, was 34±7 in HHV-7-infected cultures and 27±5 in mock-treated controls (means±standard deviations of four separate experiments), indicating that CFU-meg were not adversely affected by HHV-7.
at least in semisolid assays.

The effect of HHV-7 was next investigated on CD61+ megakaryoblasts, derived from CD34+ cells after 10 days of serum-free liquid cultures, supplemented with SCF+TPO. This approach promotes virus-cell interactions and may more closely resemble in vivo infection, as it allows potential secondary infection. Analysis of cell viability performed between 5 and 8 days post-infection revealed that HHV-7 infection induces marked (p<0.01) cytototoxicity on cultured CD61+ megakaryoblasts, mostly due to a significant (p<0.01) increase of apoptosis (Figure 1A). Cells surviving HHV-7 infection showed a brighter expression of CD42b late megakaryocytic marker than did the mock-treated cultures (Figure 1B), coupled with an increased frequency of mature polyoid megakaryocytes at morphologic analysis (Figure 1C). In fact, there was a significantly higher (p<0.01) number of cells with a diameter greater than 20 μm in HHV-7 cultures than there were in control cultures (53±5% versus 35±8%, respectively, means±SD of four experiments). All these effects were completely abrogated by the neutralizing anti-HHV-7 serum (1:100 dilution, Advanced Biotechnologies, Columbia, MD, USA).

In parallel, we investigated whether HHV-7 infects CD61+ megakaryoblasts and persists in their differentiated progeny. Since the presence of viral DNA could be the consequence of residual virions of the initial inoculum, the occurrence of HHV-7 entry was analyzed by reverse transcriptase-PCR, performed on total RNA extracted from CD61+ infected megakaryoblasts at different days post-infection. HHV-7 RNA was detectable at all the time points examined (Figure 1D).

In immunocompromised hosts, human herpesviruses show prompt ability to reactivate generating disseminated infections. In fact, HHV-6 and HCMV have both been associated with opportunistic pathologic manifestations in AIDS patients (pneumonia, encephalitis, abnormalities of the hematologic picture). In spite of a relative scarcity of definitive evidence to establish the pathogenic potential of HHV-7, it has been demonstrated that reactivation of HHV-7 occurs following bone marrow transplantation and that an increased expression of HHV-7 takes place in lymphoid organs of AIDS patients. For the purpose of this study, it is particularly noteworthy that HHV-7 DNA is present in up to 50% of bone marrow samples of healthy adult donors. Moreover, we have previously shown that in vitro HHV-7 infection of CD34+ hematopoietic progenitors accelerates differentiation along the granulocytic but not the erythroid lineage, without showing cytotoxic effects. On the other hand, we have demonstrated for the first time in this study that HHV-7 severely impairs the survival of CD61+ megakaryocytic cells, and that the megakaryocytes surviving HHV-7 cytopathy show a hastened maturation. Of note, also HCMV selectively inhibits CD42b+ megakaryocytes without affecting CFU-meg progenitors or cells of the erythroid and granulocytic lineages. Taken together, the data of Crapnell et al. 9 and our present data suggest that megakaryocytes are particularly susceptible to the cytopathicity of the two closely related herpesviruses, HCMV and HHV-7.

Several factors, such as direct HHV-7 virion/cell interactions and release of cytokines by infected cells are likely implicated in inducing megakaryocyte apoptosis and in promoting maturation along the granulocytic and granulocytic lineages. Due to the central role of megakaryoblasts in the regulation of megakaryocytosis, our data may contribute to explain the occurrence of thrombocytopenia, frequently occurring in patients with HIV-1 disease. 10

References


Complete remission induced by high-dose erythropoietin and granulocyte colony-stimulating factor in acute erythroleukemia (AML-M6 with maturation)

Alternative therapeutic approaches with low dose chemotherapy and differentiative-maturative treatment by growth factors are under consideration for elderly patients with acute leukemia. Two patients with AML-M6 with maturation, one refractory to standard chemotherapy and the other ineligible for cytotoxic treatment, obtained complete remission from leukemia using high dose recombinant erythropoietin and granulocyte colony-stimulating factor.


In the last years the use of recombinant human erythropoietin (rHuEpo) has increased greatly. Beneficial effects have been reported in myelodysplasia 11 and in avoiding chemotherapy.