Antiapoptotic Cytokine IL-3 + SCF + FLT3L Influence on Proliferation of Gamma-Irradiated AC133$^+$/CD34$^+$ Progenitor Cells

(AC133$^+$ cells / gamma irradiation / *ex vivo* expansion / cell cycle / apoptosis)

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Abstract. Recovery from radiation-induced bone marrow aplasia depends on appropriate cytokine support. The aim of our work was to find a cytokine combination allowing *in vitro* gamma-irradiated (2.5 Gy) CD34$^+$/AC133$^+$ haematopoietic stem cells to evade radiation-induced apoptosis and to enhance damage reparation, which should enable proliferation and *ex vivo* expansion of cells. Cells were isolated using separation in a Cobe separator followed by immunomagnetic selection by antibody against the AC133 antigen. Thus isolated cells were 80% AC133$^+$/CD34$^+$ and 10% of them expressed the CD33$^+$ antigen. Ten thousand of AC133$^+$ cells formed 1146 CFU-GM and 304 BFU-E. We proved a high expansion efficiency of cytokine combination SCF + IL-3 + FLT3L in comparison with the combination SCF + IL-3 + IL-11 in both, non-irradiated cells and cells irradiated with a dose of 2.5 Gy. The D_0 value for AC133$^+$ cells was determined by the clonogeneity test. The D_0 value for CFU-GM was estimated to be 1.08 Gy and for BFU-E 0.95 Gy. The results of DNA analysis showed that the majority of isolated AC133$^+$ cells were in G_0/G_1 phase of the cell cycle. We proved that the dose of 2.5 Gy induced massive apoptosis (80%) of these cells without progression through the cell cycle, which indicates interphase cell death. Under the influence of cytokine combination (SCF + IL-3 + FLT3L), the surviving 20% of cells entered the cell cycle and, similarly to non-irradiated control cells, on 7th day 35% of cells were in S phase.

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Abbreviations: BFU-E – burst-forming unit erythroid progenitor, CFU-GM – colony-forming unit-granulocyte-macrophage, D_0 – dose that reduces the surviving fraction to 37%, FLT3L – FLT3 ligand, PBPC – peripheral blood progenitor cells, SCF – human recombinant stem cell factor.

The AC133 antibody provides an alternative to CD34 for selection and characterization of cells necessary for both, short- and long-term engraftment, in transplant situations and for studies of *ex vivo* expansion strategies. AC133 is one of a new panel of murine hybridoma lines producing monoclonal IgG antibodies to a novel stem cell glycoprotein antigen with the molecular weight of 120 kD (Miraglia et al., 1997). The AC133 antigen is selectively expressed on CD34$^+$ haematopoietic stem and progenitor cells derived from human foetal liver and bone marrow, and blood (Yin et al., 1997). All of the noncommitted CD34$^+$ cell population, as well as the majority of CD34$^+$ cells committed to the granulocytic/monocytic pathway, are stained with AC133 antibody. In this report, we used the AC133$^+$ population for an experimental study of *ex vivo* expansion of progenitors.

The choice of cytokine combination and culture system will largely determine the fate of the cells used to initiate the culture. Cytokine-mediated expansion has been proposed as a means of increasing the total number of cells as well as both committed and primitive haematopoietic progenitors. Nevertheless, most cytokine combinations have included SCF and IL-3 as an absolute requirement (Brandt et al., 1992). Used on its own, SCF has, at best, only a modest stimulatory activity. However, it has a profound synergistic effect when used in conjunction with other cytokines. FLT3L is a more recently cloned cytokine, which seems to share numerous properties with SCF. FLT3 gene expression is restricted to haematopoietic cells and tissues in human. The receptors for SCF and FLT3L are both members of the family of receptors with tyrosine kinase activity. These receptors transfer the signals to the nucleus using the ras-MAP kinase pathway, which could directly regulate cell survival and proliferation. FLT3L is able to accelerate cell cycling of IL3-dependent haematopoietic progenitors by shortening the G_1 phase of the cell cycle (Fichelson, 1998).

In our previous study we expanded AC133$^+$ cells isolated from peripheral blood of patients suffering of breast cancer using the combination SCF + IL-3 + IL-

11 and after seven days of long-term expansion the number of nucleated cells increased 20-fold (Vávrová et al., 1999). In this work we compared the expansion capabilities of two combinations of cytokines for $AC133^+$ cell expansion: SCF + IL-3 + IL-11 and SCF + IL-3 + FLT3L, in the control group and group irradiated *in vitro* by a dose of 2.5 Gy.

Information about the response characteristic of clonogenic haematopoietic cells exposed to ionizing irradiation *in vitro* is important for prediction of the tolerance of the haematopoietic system to radiation exposure *in vivo*. The D_0 value of 1.34 Gy obtained for CFU-GM and BFU-E of $CD34^+$ cells isolated from human umbilical cord blood (Kreja et al., 1993) was similar as the D_0 value for CFU-GM and BFU-E from adult bone marrow. In our work we established the D_0 value for $AC133^+$ cells isolated from peripheral blood of healthy donors after mobilization by G-CSF.

The next important question is whether radiation damage can be repaired. Cells isolated after mobilization from peripheral blood are nearly all in G_0/G_1 phase of the cell cycle. From experiments carried with haematopoietic cell lines it is known that most of haematopoietic cells die by apoptosis after irradiation (Radford and Murphy, 1994; Vávrová et al., 2001). Apoptotic cell death was also described in haematopoietic cell cultures FDCP-1 and FDCP-mix after removal of growth factors from culture media (Cowling and Dexter, 1994). Drouet et al. (1999) described apoptotic cell death resulting from *in vitro* irradiation of $CD34^+$ cells isolated from monkey bone marrow after doses 2.5–6.0 Gy. Our aim was to evaluate whether $AC133^+$ cells isolated from peripheral blood of healthy donors die by apoptosis after irradiation with 2.5 Gy and whether in the presence of cytokine combination SCF + IL-3 + FLT3L part of these irradiated cells are able to repair damage and proliferate in cell culture *in vitro*. Apoptosis could also be labelled as a proliferation regulator. The presence of some growth factors could have a key role for reversion of the proliferation/apoptosis ratio of primitive haematopoietic cells in benefit to proliferation, which is necessary for haematopoietic cell regeneration of irradiated victims.

Material and Methods

Mobilization procedure and PBPC harvest

$AC133^+/CD34^+$ cells were obtained from peripheral blood of six healthy donors after mobilization by subcutaneous application of G-CSF (Neupogen, Roche, Basel, Switzerland) in a dose of 5 μg/kg/day for three days. Cells were collected on the 4^{th} day after application by leukapheresis. The leukaphereses were performed using an automated COBE Spectra (Lakewood, CO) continuous blood flow cell separator with the following parameters: blood volume processed: 10 000–12 000 ml (median 11 000 ml) per procedure, inlet flow rate: 60–70 ml/min. We used the original COBE MNC version 5.1 software programme.

Reagents

Human recombinant IL-3 was purchased from Sigma-Aldrich chemie GmbH (Munich, Germany). Human recombinant IL-11 and human recombinant SCF were purchased from Stem Cell Technologies Inc. (Vancouver, Canada) and FLT3L from Genzyme diagnostics (Cambridge, MA). Iscove's modified Dulbecco's medium and foetal bovine serum were purchased from Sigma-Aldrich, foetal bovine serum (Sigma-Aldrich), complete methylcellulose medium for colony assays with 5637 CM conditioned medium were supplied from Institute of Haematology and Blood Transfusion (Prague, Czech Republic) and with erythropoietin (Eprex) from Janssen-Cilag AG (Baar, Switzerland). The MiniMACS separation system $AC133^+$ cell isolation kits were supplied by Miltenyi Biotec (Bergisch Gladbach, Germany). Phycoerythrin-conjugated antiCD34 /QBEnd (IgG1)/, fluorescein isothiocynate-conjugated antiCD33 /D3HL60.251(IgG1)/, antiCD34/581(IgG1)/ and antiCD15/80H51gM/ were purchased from Immuno-tech (Miami, FL). Phycoerythrin-conjugated antiAC133 was purchased from Miltenyi Biotec.

$AC133^+$ cell purification and liquid culture

$AC133^+$ cells were purified from fresh PBPC using the MiniMACS purification system. PBPC used for MACS separation of $AC133^+$ cells were directly magnetically labelled using AC133 microbeads. $AC133^+$ cell purity was determined by flow cytometric analysis.

The $AC133^+$ cells were seeded at 1 x 10^4 cell/ml in Iscove's modified Dulbecco's medium containing 10% foetal bovine serum, 100 U/ml penicillin, 100 U/ml streptomycin, 2 mM/ml L-glutamine, 50 ng/ml hSCF, 50 ng/ml hIL-11 or 50 ng/ml FLT3L and 20 ng/ml hIL-3. All cultures were incubated in 5% CO_2 and 5% O_2 at 37°C. Aliquots of expanded cells were analysed by multiparameter flow cytometry over seven- and fourteen-day periods.

Gamma irradiation

$AC133^+$ cells were suspended at a concentration of 5 x 10^4 cells/ml in complete medium. Two ml of aliquots were given into the plate and irradiated at room temperature using a ^{60}Co gamma-ray source with a dose rate 0.66 Gy/min. After irradiation, plates were placed in a 37°C incubator in 5% CO_2 and 5% O_2 and aliquots of cells were removed at various times after irradiation for analysis. The cells were counted and cell viability was determined with the Trypan blue exclusion assay.

Clonogeneic assays for myeloid (CFU-GM) and erythroid (BFU-E) progenitors

Unseparated PBPC from the leukapheresis product, AC133$^+$ cells, irradiated AC133$^+$ cells, as well as expanded cells were grown in 0.9% methylcellulose as described by Coutinho et al. (1993). All semi-solid cultures were performed in duplicates and stimulated with 10% conditioned medium from the 5637 human bladder carcinoma cell line (Kaashoek et al., 1991) and 4 units/ml erythropoietin. CFU-GM and BFU-E colonies were counted after 14 days of incubation in 5% CO_2 and 5% O_2 at 37°C. Dose response curves of AC133$^+$ cells (from three persons) irradiated *in vitro* by an increasing dose of 0.5–5.0 Gy were used for D_0 value calculations.

Immunofluorescence staining and dual-colour flow-cytometric analysis

Enriched AC133$^+$ as well as expanded nucleated cells from suspension cultures were incubated with antiCD34 fluorescein (FITC)-conjugated or antiCD34 phycoerythrin (PE)-conjugated monoclonal antibodies and/or antiCD33-FITC, antiCD15-FITC and antiAC133-PE for 30 min. Flow-cytometric analysis was performed in a Coulter Epics XL flow cytometer. A minimum of 10 000 cells were collected for each 2-colour sample in a list mode file format. List mode data were analysed using Epics XL System II software colour eventing (Coulter Electronic, Hialeah, FL).

Flow-cytometric analysis of the cell cycle and DNA fragmentation

Following the incubation the cells were washed with cold PBS and fixed by 70% ethanol (minimally 30 min at 4°C). After centrifugation (200 *g*, 10 min at 4°C) and ethanol removal the cells were washed by ice-cold PBS, suspended in phosphate buffer and incubated 5 min at room temperature for extraction of low-molecular fragments of DNA. Then the cells were stained in 0.5 ml of Vindelov's solution (Vindelov, 1977), 1 h in a thermostat at 37°C and analysed by flow cytometry in a Coulter Electronic (Hialeah, FL) flow cytometer. A minimum of 10 000 cells analysed in each sample served to determine the percentages of cells in each phase of the cell cycle, using Multicycle AV software. Experiments were performed with AC133$^+$ cells from three persons, non-irradiated and irradiated by a dose of 2.5 Gy.

Statistical analysis

The results were statistically evaluated with Student's t-test. The values represent mean ± SD (standard deviation of the mean). Statistical significance of the difference of means in comparable sets is expressed in the text for $P < 0.05$.

Results

Characteristics of the sorted AC133$^+$ cells and changes in immunophenotype

The purity of fractions was controlled by flow-cytometric analysis using antiCD34 and AC133 monoclonal antibodies. CD34-FITC and AC133-PE expression on MiniMACS AC133$^+$ isolated cells is shown in Fig. 1. Table 1 shows that cells isolated by immunomagnetic separation were 78% AC133$^+$/CD34$^+$ and 77.8% CD34$^+$/CD33$^-$. Cell maturation in culture was assessed by monitoring the serial expression of AC133$^+$, CD34$^+$, CD33$^+$ and CD15$^+$ antigen. In Fig. 2, the decrease in number of primitive AC133$^+$/CD34$^+$ cells in culture after 14 days of expansion is apparent. On the contrary, expression of antigens showing differentiation of cells into granulocytes increased. After 14 days of expansion 86% of cells were CD33$^+$ and 39 % CD15$^+$. The absolute numbers of AC133$^+$ cells expressing various antigens immediately after isolation and after 7 and 14 days of cultivation are recorded in Table 2. It can be seen that after 14 days of cultivation with combination SCF + IL-3 + FLT3L

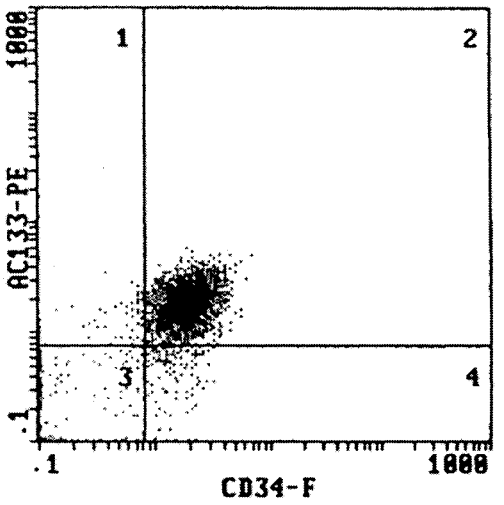

Fig. 1. AC133$^+$ haematopoietic stem and progenitor cells were positively selected from fresh PBPC using AC133 microbeads. The AC133$^+$ cell population was analysed using two-colour fluorescence AC133-PE versus CD34-FITC. A minimum of 10 000 cells were collected.

Table 1. Characteristic of AC133$^+$ cells

Expressing antigen	%
AC133$^+$ PE	81.8 ± 8.0
CD34$^+$ FITC	85.9 ± 9.3
CD33$^+$ FITC	10.4 ± 2.8
CD15$^+$ FITC	2.3 ± 2.3
AC133$^+$ PE/ CD34$^+$ FITC	78.0 ± 11.2
CD34$^+$ PE/ CD33$^-$ FITC	77.8 ± 11.6

The values represent mean of percentage ± SD (6 persons)

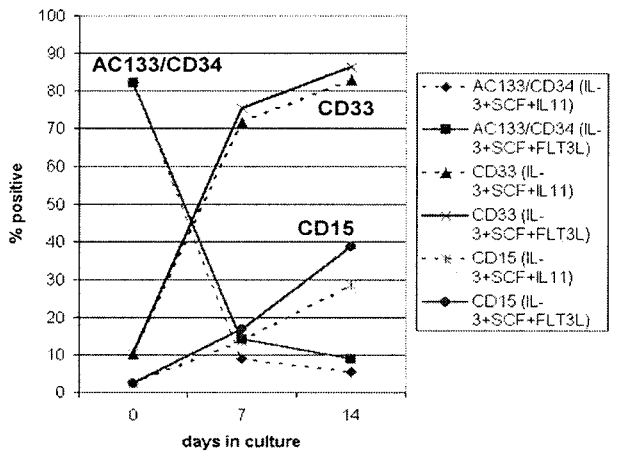

Fig. 2. The proportions of cells expressing CD34, AC133, CD33 or CD15 in the culture. The values represent the mean percentage (6 persons).

decrease of expansion ability was also observed when using the combination SCF + IL-3 + FLT3L; the number of nucleated cells increased after irradiation by 2.5 Gy 4.5-fold, which means a 27-fold decrease in comparison to non-irradiated cells.

Ten thousand of $AC133^+$ cells isolated by immunomagnetic selection formed 1146 (± 416) CFU-GM and 304 (± 70) BFU-E. Figure 5 shows the results of the clonogeneic assay for *in vitro* irradiated $AC133^+$ cells, which form both, CFU-GM and BFU-E colonies. The curve was obtained by irradiation of $AC133^+$ cells, from three healthy donors, by increasing the dose of radiation from 0.5 to 5.0 Gy. Each sample was processed twice, so the presented points represent the median of six measurements. Colonies were evaluated after 14 days of incubation in a thermostat with 5% CO_2 and 5% O_2. The curve shows the number of colonies as a percentage of the number of colonies formed by non-irradiated cells (100%).

Table 2. Absolute number of cells expressing lineage markers on fresh and cultured AC133 miniMACS-isolated cells

antigen	Days in culture 0	7	7	14	14
		SCF+IL–3 +IL-11	SCF+IL–3+FLT3L	SCF+IL–3 +IL-11	SCF+IL–3+FLT3L
$AC133^+$	0.8 ± 0.1	0.8 ± 0.4	2.2 ± 0.8	3.5 ±2.5	11.7 ± 7.0
$CD34^+$	0.9 ± 0.2	1.4 ± 0.7	2.3 ± 0.7	1.4 ± 1.0	2.7 ± 1.5
$AC133^+/CD34^+$	0.8 ± 0.1	0.6 ± 0.2	1.1 ± 0.3	1.0 ± 0.8	1.6 ± 0.6
$CD33^+$	0.1 ± 0.03	9.2 ± 3.5	13.0 ± 5.9	58.8 ± 8.6	142.5 ±35.5
$CD34^+/CD33^-$	0.8 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.2
$CD15^+$	0.02 ± 0.02	1.5 ± 0.7	2.8 ± 1.0	19.0 ±10	50.9 ±25.4

The values represent the cell numbers per ml x 10^4; mean ± SD (standard deviation of the mean) (6 persons).

there was no decrease in the absolute number of $CD34^+$ or $AC133^+$ cells, while a statistically significant increase of neutrophil granulocyte progenitors $CD33^+$ and $CD15^+$ was apparent.

Changes in number of nucleated cells and clonogeneity

Figure 3 shows the number of $AC133^+$ cells expanded *ex vivo* (1 x 10^4 cell/ml) in supplemented Iscove's modified Dulbecco's medium. A statistically significant increase in the number of nucleated cells could be seen in both cytokine combinations (IL-3 + SCF + IL-11 or FLT3L) after 7 and 14 days of cultivation. On the 14th day the number of nucleated cells increased in the group stimulated by the combination SCF + IL-3 + FLT3L 120-fold and in the group stimulated by the combination SCF + IL-3 + IL-11 55-fold; the difference between the groups was statistically significant.

As can be seen in Fig. 4, the expansion ability of 2.5 Gy-irradiated cells was very low. On the 14th day after the beginning of expansion the number of nucleated cells increased after incubation with the combination SCF + IL-3 + IL-11 only 3.2-fold, which means a 17-fold decrease in comparison to non-irradiated cells, where the number of cells after expansion increased 55-fold. A simi-lar

Using Microsoft-Excel software the curve was plotted, the slope of curve counted and the D_0 value established. The D_0 value for $AC133^+$ cells forming CFU-GM was **1.08 Gy** and for cells forming BFU-E **0.95 Gy**.

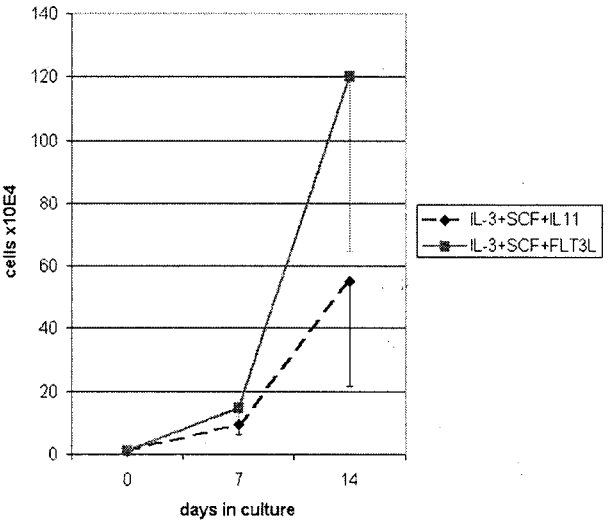

Fig. 3. Increase of the total number of cells in response to SCF, IL-3 and IL-11 or FLT3L after 14-day incubation in liquid culture. The values represent the cell number per ml x 10^4; mean ± SD (standard deviation of the mean), 6 persons.

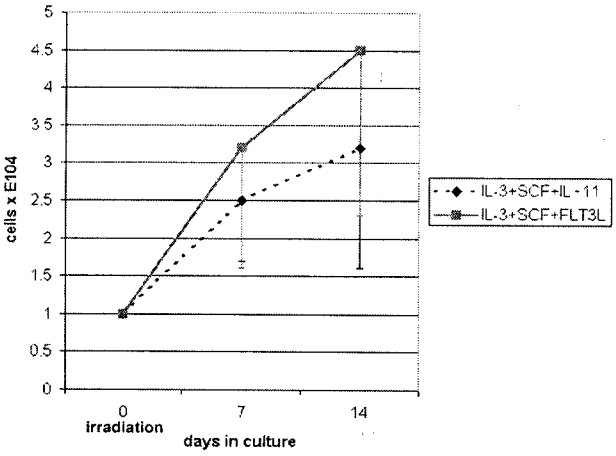

Fig. 4. Increase of the total number of 2.5 Gy-irradiated cells in response to SCF, IL-3 and IL-11 or FLT3L after 14-day incubation in liquid culture. The values represent the cell number per ml x 10^4; mean ± SD (standard deviation of the mean), 6 persons.

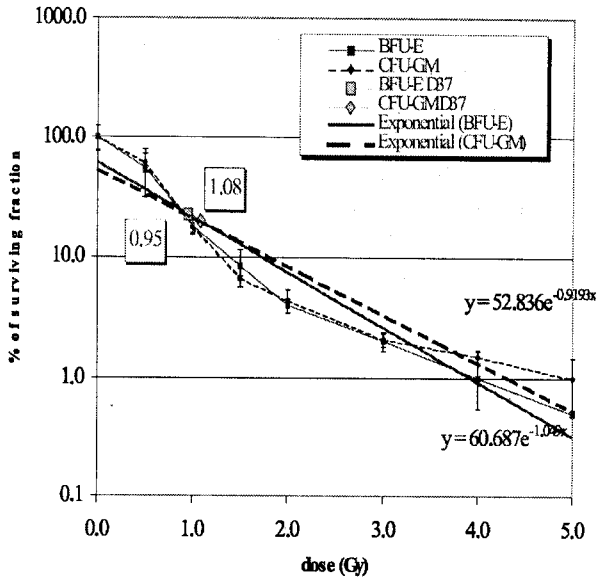

Fig. 5. Radiation response curves of AC133$^+$ haematopoietic progenitors obtained from human peripheral blood. Surviving fractions are presented as a mean value from three experiments (6 dishes). The D_0 value was determined for CFU-GM = 1.08 Gy and for BFU-E = 0.95 Gy.

Effect of cytokine combination SCF + IL–3 + FLT3L on proliferation of AC133$^+$ cells

Figure 6 shows cell cycle progression of AC133$^+$ cells expanded by combination SCF + IL-3 + FLT3L. It can

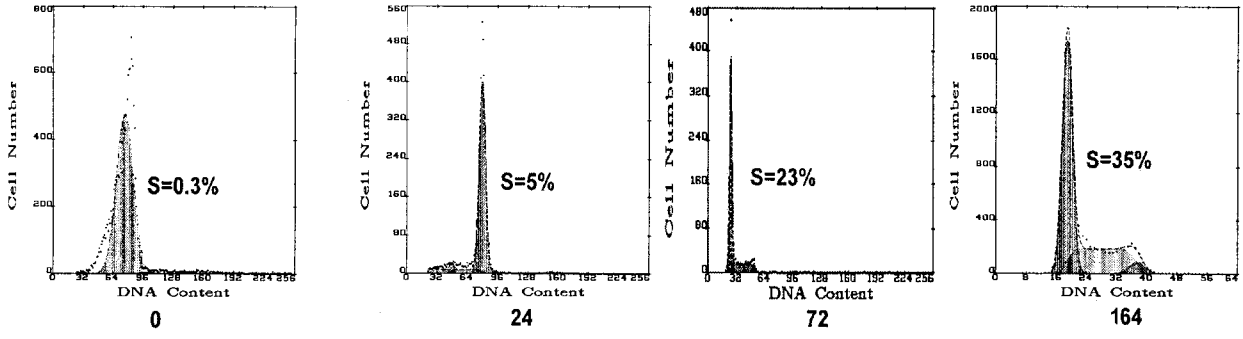

Fig. 6. Flow-cytometric analysis of DNA content and cell cycle of AC133$^+$ cells after immunomagnetic separation and *in vitro* incubation (24–164 h) with combination of cytokines SCF + IL-3 + FLT3L.

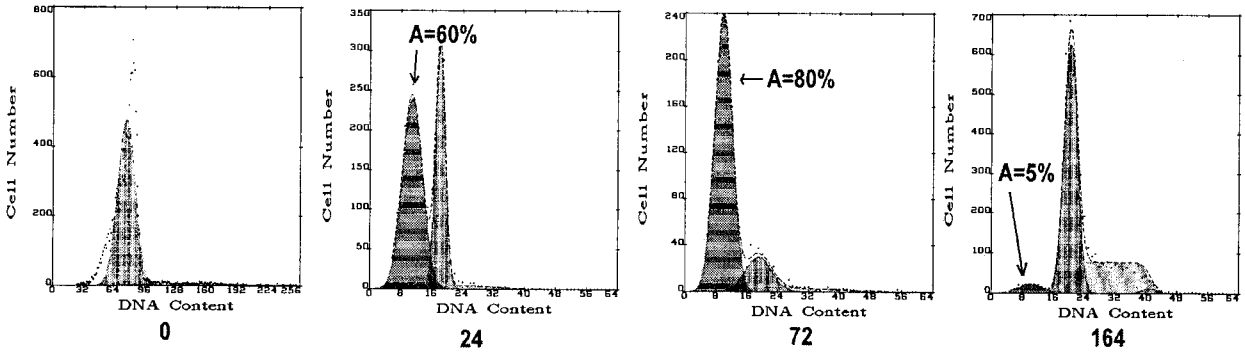

Fig. 7. Flow-cytometric analysis of DNA content and cell cycle of AC133$^+$ cells after immunomagnetic separation and irradiation with a dose of 2.5 Gy. Irradiated cells were cultivated (24–164 h) with combination of cytokines SCF + IL-3 + FLT3L.

be seen that after release of stem cells into peripheral blood by mobilization using G-CSF and their separation in a Cobe separator and miniMACS system nearly all cells were in resting G_0/G_1 phase (0.23% of cells were in S phase). Twenty-four hours after expansion the beginning 5% of cells appeared in S phase and on the 7th day of expansion 35% of cells were in S phase, which means that the cells proliferated.

Figure 7 shows cell cycle progression of cells irradiated with a dose of 2.5 Gy. It was observed that 24 h after irradiation 60% of cells had subdiploid DNA content, which represents apoptotic cells; 40% of cells were in G_0/G_1 phase. After 72 h the number of apoptotic cells increased to 80%, but cells in S phase were also detected (5%). On the seventh day after irradiation there was only a minimum of apoptotic cells (5%) and 36% of cells were in S phase, similarly to the control, non-irradiated group.

Discussion

In our previous study we proved the ability of $AC133^+$ cells to expand in the presence of cytokines SCF + IL-3 + IL-11 in patients with breast carcinoma. After seven days of expansion the number of cells increased 20-fold (Vávrová et al., 1999). In this study of healthy donors we proved 9.3-fold expansion after 7 days and 55-fold expansion after 14 days using cytokine combination SCF + IL-3 + IL-11. It is necessary to realize that different mobilization protocols were used for breast cancer patients (epirubicin and cyclophosphamide on day 1; 24 h after chemotherapy mobilization was started with subcutaneous administration of G-CSF for the next 13 days; Vávrová et al., 1999) and healthy donors (only subcutaneous application of G-CSF for three days). A higher expansion ability was observed with combination SCF + IL-3 + FLT3L – 15-fold after 7 days and 120-fold after 14 days. In our experiments CD33 antigen was expressed only on 10% of $AC133^+$ cells and CD15 antigen on 2% of cells. After 14 days of expansion the percentage of $CD33^+$ cells increased to 85% and the percentage of $CD15^+$ cells to 30% (IL-11) of cells or 40% (FLT3L) of cells, respectively. Our results indicate that during 14 days of cultivation the combination SCF + IL-3 + FLT3L induced a higher increase of proliferative rate of $AC133^+/CD34^+$ in comparison to the combination SCF + IL-3 + IL-11. The results showed no loss of the absolute amount of $AC133^+/CD34^+$ cells, but the amount of $CD34^+/CD33^-$ cells decreased. The increase of progenitors of the granulocyte lineage ($CD33^+$, $CD15^+$ and CFU-GM) was significantly pronounced in comparison to the combination with IL-11. Lyman and Jacobsen (1998) described an increase of $CD34^+$ and CFU-GM cells after addition of FLT3L to the medium with cytokines (SCF + IL-6 + G-CSF + IL-3) after 14 days of expansion of $CD34^+$ cells from umbilical blood.

Our results show that minimum of isolated $AC133^+$ cells was in S phase of the cell cycle (0.3%). However, in the presence of cytokine combination SCF + IL-3 + FLT3L these cells entered the cell cycle and on seventh day of expansion 35% of cells were in S phase. Chute et al. (1999) described that cultivation in the presence of cytokine combination SCF + IL-3 + IL-6 + GM-CSF and endothelial cells induced transition of cells into S phase. Fifty-one percent of $CD34^+/CD38^+$ cells were in S phase after 7 days and 17% of $CD34^+/CD38^-$ cells were also in S phase. This showed that the primitive $CD34^+/CD38^-$ subpopulation could enter the cell cycle under environmental influence and be expanded *ex vivo*. Brant et al. (1999) proved in experiments with baboons that it was possible to use expanded $CD34^+$ cells for autologous transplantation of lethally irradiated baboons and this treatment ensured survival of the animals.

The results showed that cells of a human leukaemic cell line were more radioresistant (D_0 = 2.2 Gy) (Vávrová et al., 2001) than $AC133^+$ cells isolated from peripheral blood of healthy donors after mobilization, where D_0 for CFU-GM was 1.08 Gy and for BFU-E 0.95 Gy. Our results are in good accord with other authors (Baird et al., 1989; Kreja et al., 1993). When using the combination SCF + IL-3 + FLT3L for expansion of 2.5 Gy-irradiated $AC133^+$ the cells expanded 4.5-fold after 14 days, contrary to non-irradiated cells, which expanded 120-fold. The ability of expanded cells to form CFU-GM increased only 2-fold (unpublished). Because the expanded cells were not used for transplantation *in vivo*, based on our results we are not able to determine if there was an increase of primitive stem cells, which are capable of providing long-term haematopoiesis or can guarantee survival of a myeloablated host.

While 23% of non-irradiated $AC133^+$ cells isolated from peripheral blood of healthy donors after mobilization enter S phase of the mitotic cycle during 72 h after *ex vivo* expansion and apoptosis is not observed, *in vitro* irradiation of these cells with a dose of 2.5 Gy induces apoptosis in 80% of cells 72 h after irradiation. However, in the presence of cytokine combination SCF + IL-3 + FLT3L, the surviving 20% of cells are able to divide and on the 7th day after irradiation 35% of cells are in S phase of the cell cycle. Drouet et al. (1999) proved that when the $CD34^+$ cells are irradiated *in vitro* with doses 2.5–6.0 Gy and incubated in the medium without cytokines, 97% of cells die by apoptosis during 24 h, and after 48 h all cells lose their functionality completely. When these authors incubated cells with cytokine combination SCF + IL-3 + FLT3L+ thrombopoietin, 15% of cells were saved from apoptosis after 2.5-Gy irradiation and 12% of cells after 4-Gy irradiation. In accordance with our results after 7 days of *ex vivo* expansion of 2.5 Gy-irradiated cells, the authors observed 5.6-fold increase of the nucleated cell number. The results suggest that early use of antiapoptotic cytokines is important for the expansion of irradiated haematopoietic progenitors, and it is possible to partially avoid induction of apoptosis by ionizing radiation in haematopoietic stem cells. Surviving of nuclear accident victims suffering from radiation-induced bone marrow aplasia could therefore

be significantly influenced by timely application of anti-apoptotic cytokines.

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