Ex vivo expansion of CD34+ cells and immunocytes from umbilical cord blood*

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Abstract  Objective: Umbilical cord blood stem cell transplantation (CBSCT) has approached significant success in leukemia treatment, but it is associated with higher rates of delayed or failed engraftment and relapse. This may be caused by immature immune cells of umbilical cord blood. We try to expand stem/progenitor cells and T, NK, DC immunocytes from umbilical cord blood for transplantation and immunotherapy. Methods: CB MNCs were cultured and analyzed for progenitor/stem cells, immunocytes at day 0, 3, 7 and 14 by using flowcytometry. Results: The combinations of SCF, IL-3, IL-6, plus IL-2 or/and IL-4 showed significantly expanded results both for UCB MNCs and CD34+ cells. CD34+ percentage went up from fresh CB 1.6% to the highest group E (SCF+IL-3, 5, 2, 4) 11.1%. The average expansion multiples of CD34+ cells at 7th culture days were from 10 to 50 (SCF+IL-3, 6, 2, 4). The CD3+ T cells was (18.7±4.3)% in fresh cord blood, and decreased sharply in the medium without cytokine, while markedly increased in groups with cytokines combination, in group B, E, G and F, their level were about 2 times of fresh control. The fresh UCB contained (3.6±1.9)% CD56+ cells, NK cells only were expanded in groups with IL-2. DCs markers CD1a, CD80, CD83 and CD86 expressed a lower level at day 3 in all test groups, and then increased sharply in groups E, F and G with IL-4 cytokin at 7th culture days. Conclusion: T cells, NK cells and DCs as well as stem/progenitor cells could be expanded in the same medium from CB MNCs with the combinations of cytokines. The combination of SCF, IL-2, IL-3, IL-6 and IL-4 showed a balanced expansion result of both CD34+ cells and immunocytes at 7th culture days.

Key words  cord blood; stem cell; ex vivo expansion; T cell; NK cell; DCs

Umbilical cord blood stem cell transplantation (CBSCT) has approached significant success in treatment of lethal congenital or malignant disorders, but CBSCT with low incidence of GVHD is associated with higher rates of delayed or failed engraftment and relapse than bone marrow transplants. This may be caused by immature immune cells, compared with the corresponding cells from adults. More studies have been reported that cord blood T lymphocytes are immature in phenotype and function so that little cytotoxic is generated after allogeneic transplantation1, 2. Some clues have shown that donor derived T lymphocytes with antileukemic activity, but without normal host tissue reactivity (GVL without GVHD), could be isolated in vitro, and this may implicate that they are two separately phenomena9. These suggest it is possible to avoid GVHD while keeping or even enhancing GVL effect if the contents of graft cells were regulated in ex vivo culture systems.

Encouraging results have been obtained with cord blood, where extensive amplification of primitive progenitor cells was observed4, 5. Ballen K et al6 incubated the cord blood with a four-factor cytokine mixture of interleukin (IL)-3, IL-6, IL-11 and stem cell factor (SCF), and resulted in increased survival of irradiated NOD-SCID recipients' post-transplantation of the expanded cord blood. Robinson KL7 reported that ex vivo combination of IL-2, IL-12, anti-CD3 and IL-7 significantly enhanced the proliferation, activation, maturation and cytotoxic potential of UCB T cells of UCB MNC. NK T cells in human cord blood are very small populations, when lymphocytes in cord blood were cultured with rIL-2 for 14 days in vitro, CD56+ T cells were able to expand up to 25% of T cells8. Dendritic (DC) cells are the most potent antigen presenting cells (APCs) in the initiation of immune response and immunological tolerance. Human DCs can differentiate from CD34+ hematopoietic progenitor cells in cord blood, adult bone marrow and also from other blood precursor cells. Rosenzwajg M9 demonstrated that IL-4 could promote cultured cord blood CD34+ cells differentiation and maturation of DCs.

In this study, we expanded cord blood ex vivo with combinations of cytokines and observed stem/progenitor cells, T, NK and DCs proliferation and differentiation.
Materials and methods

CB cells collection and separation

UCB samples were obtained at the end of full-time deliveries from healthy donors at Nanfang Hospital. Samples were collected into sterile bags containing 25 mL of citrate-phosphate dextrose anticoagulant and diluted 1:1 in phosphate-buffer saline (PBS). Mononuclear cells (MNC) were separated in Ficoll-Isopaque (density 1.077 g/mL) for density centrifugation at 1800 rpm for 20 min. The isolated MNCs were incubated for 30 min at 4 °C with 20 μL FITC CD86-PE. All Abs and isotype-matched FITC-, PE- and allophycocyanin (Cy)-conjugated Abs, washed in PBS, stored at 4 °C in 0.5 mL of 1% paraformaldehyde until analysis. More than 10 000 cells were acquired per assay on a FACscalibur with CellQuest software 1.2.2 version (Becton Dickinson, San Jose, CA, USA). HSCs were labeled with CD34-PE, CD38-FITC, CD45-Cy from Immunotech; T subset cells were labeled with CD3-FITC, CD56-Cy, NK cells were labeled with CD3-FITC, CD56-PE; DCs were labeled with CD1a-FITC, CD80-PE, CD83-PE; CD34+ cells proliferation

When UCB MNCs were cultured in 15% FCS IMDM medium with adding different cytokines for 2 weeks, the results of percentage of CD34+ cells grown in cultures showed in Fig. 1. The fresh UCB contained (1.63±0.66)% CD34+ cells, the other two groups B and C appeared that at 14th culture day. The CD34+ expansion at detection day 3, 7 and 14. Group A, D, E, F and G exhibited CD34+ cells proliferation every 3 days. The suspended cells were incubated at 37 °C, in a 5% CO2 in a fully humidified atmosphere. The fresh UCB contained (34.7±10.4)% CD34+, the other two groups B and C appeared that at 14th culture day. The CD34+ expansion at detection day 3, 7 and 14.

Statistical analysis

Data were presented as mean±S.E.M. (standard error of mean values). The difference between two groups was analyzed by paired Student's t-test.

Results

CD34+ cells proliferation

When UCB MNCs were cultured in 15% FCS IMDM medium with adding different cytokines for 2 weeks, the results of percentage of CD34+ cells grown in cultures showed in Fig. 1. The fresh UCB contained (1.63±0.66)% CD34+ cell in average. All test groups showed increasing effect on CD34+ cells expansion at detection day 3, 7 and 14. Group A, D, E, F and G exhibited CD34+ cells proliferated at 7th culture day, while the other two groups B and C appeared that at 14th culture day.

Cytokines and antibodies

All cytokines were recombinant human material: SCF, IL-2, IL-3, IL-4, IL-6 and IL-7 were purchased from Pro-Tech Inc, Rocky Hill, NJ. All rat anti-human antibodies CD34, CD3, CD38, CD45, CD56, CD1a, CD80, CD83 and CD86 were productions of Immunotech (France) and labeled with FITC, PE or Cy, respectively.

Cells expansion and culture

The obtained cells were suspended at 2×10⁶ cells/mL in 15% FCS IMDM medium supplemented without (control) or with (test groups) following concentration of human recombinant cytokines: 50 ng/mL SCF, 10 ng/mL IL-2, 2 ng/mL IL-3, 10 ng/mL IL-4, 20 ng/mL IL-6 and 5 ng/mL IL-7. There were 7 test groups: CTR: IMDM+FCS; A: IMDM+FCS+SCF+IL-3, 6; B: IMDM+FCS+SCF+IL-3, 6, 7; C: IMDM+FCS+SCF+IL-3, 6, 2; D: IMDM+FCS+SCF+IL-3, 6, 2, 4; E: IMDM+FCS+SCF+IL-3, 6, 2, 4, 5 (x); G: IMDM+FCS+SCF+IL-3, 6, 2, 4, 5; H: IMDM+FCS+SCF+IL-3, 2, 4, 5; I: IMDM+FCS+SCF+IL-3, 2, 4, 5. Cultures were maintained for 14 days with half change and cytokine addition every 3 days. The suspended cells were incubated at 37 °C, in a 5% CO2 in a fully humidified atmosphere, collected and analyzed for progenitor/stem cells, immunophenotype at day 0, 3, 7 and 14.

Flow cytometry analysis

1×10⁶ cells were incubated for 30 min at 4 °C with 20 μL Abs, washed in PBS, stored at 4 °C in 0.5 mL of 1% paraformaldehyde until analysis. More than 10 000 cells were acquired per assay on a FACScalibur with CellQuest software 1.2.2 version (Becton Dickinson, San Jose, CA, USA). HSCs were labeled with CD34-PE, CD38-FITC, CD45-Cy from Immunotech; T subset cells were labeled with CD3-FITC, CD45-Cy, NK cells were labeled with CD1a-FITC, CD80-PE, CD83-FITC CD86-PE. All Abs and isotype-matched FITC-, PE- and allophycocyanin (Cy-) conjugated were purchased from Immunotech.

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