Optimized Protocols for Generation of Cord Blood-derived Cytokine-induced Killer/Natural Killer Cells

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Abstract. *The efficacy of various combinations of stem cell factor (SCF), FLT3 ligand, interleukin (IL)-2, IL-7 and IL-15 to induce and expand cord blood-derived cytokine-induced killer (CIK) cells was investigated. There were three treatment groups: group A: SCF combined with IL-2, IL-7 and IL-15; group B: SCF, FLT3 ligand combined with IL-2, IL-7 and IL-15, and group C: IL-2, IL-7 and IL-15, the control group. Proliferation rates of $CD3^+CD56^+$ CIK cells and $CD3^-CD56^+$ natural killer (NK) cells were highest in group B; expansion of CIK cells increased 796.1±278.5-fold, and that of NK cells increased 36.6±3.5-fold. All expanded cord blood-derived CIK/NK cells showed cytotoxic activity against the K562 cell line. Interestingly, the cytotoxicity of group A was highest and significantly higher than that of other groups. These protocols might provide an alternative choice for CIK/NK cell expansion.*

In the past decade, a protocol has been firmly established to rapidly and reproducibly expand *in vitro* T-cells with natural killer (NK) phenotype termed cytokine-induced killer (CIK) cells (1). These cells are characterized by a very high cytolytic potential, starting from human blood from healthy donors or from leukemia/lymphoma patients (2, 3). It is considered that the cytotoxicities of CIK and NK cells are neither major histocompatibility complex (MHC) restricted nor mediated by the T-cell receptor but *via* perforin-mediated mechanisms (1, 4, 5). Infusion of activated CIK cells can promote graft *versus* leukemia (GVL) or antitumor effect without severe transfusion-related graft *versus* host disease (GVHD) (3, 6). Some studies demonstrated that CIK cells have stronger antitumor activity as compared to NK and lymphokine-activated killer (LAK) cells and exhibit almost no cytotoxicity towards normal hematopoiesis progenitor cells (7).

Many clinical outcomes of hemotopoietic stem cell transplantations (HSCT) point to a relationship between GVHD and GVL, and it is now clear that the GVL effect is responsible for impeding leukemia relapse after HSCT or chemotherapy. Cord blood transplantation has been performed for 20 years. Although the incidence and the severity of acute/chronic GVHD in cord blood transplantation are less than that in bone marrow transplantation (BMT) (8, 9, 10), this dose not imply that after cord blood transplantation, the relapse rate, disease-free survival and overall survival of children with acute leukemia are less than those of patients receiving bone marrow transplantation (11). The presence of immature killer progenitor cells in cord blood holds the potential for the GVL effect following its transplantation (12).

CIK and NK cells are both important antitumor effectors in the immunotherapy of malignancies. At the present time, the extensively used cultural system of expanding $CD3^+CD56^+$ CIK cells has almost no effect on expanding $CD3^-CD56^+$NK cells, but provides a high ratio of $CD3^+CD8^+$ T-cells (13, 14). Excessive T-cells within the CIK cell culture system are not beneficial for eliminating tumor cells without prior tumor-specific antigen stimulation but increase the risk of biotherapeutic-related GVHD (15, 16). Although these unwanted $CD8^+$ T-cells can be removed by a magnetic separating device, such as the Clinimax system from Miltenyi, the high cost and cumbersome operation hinders wider clinical application. Hence, setting up a cultural system in which CIK/NK cells expand synchronously in the same culture system would be valuable, economical and practical for clinical application of CIK/NK cells. Our preliminary research showed

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that stimulating effective NK cell growth cytokines, such as the combination of interleukin-2 (IL-2), IL-7 and IL-15, cord blood-derived CIK and NK cells can both be expanded simultaneously (data not shown here). In this study, we speculated cytokines such as stem cell factor (SCF) and FLT3 ligand, which act during the early period of hematopoiesis of stem cells in cord blood, combined with IL-2, IL-7 and IL-15, would be efficient for inducing and expanding cord blood-derived CIK and NK cells. Here,we explored the possibilitly of establishing a highly effective modified expansion system for cord blood CIK/NK cells and compared the cytotoxicity of CIK/NK cells from various cultural protocols against the K562 cell line by (2,3)-*bis*-(2-methoxy-4-nitro-5-sulfophenyl)-(2H)-tetrazolium-5-carboxanilide (XTT) assay.

Materials and Methods

Generation of cord blood-CIK/NK cells. Umbilical cord blood mononuclear cells were isolated by Ficoll density gradient (1.077±0.002 g/ml, Jinmei, Shenzhen, GD, China) centrifugation, washed, and resuspended at 1×10^6 cells/ml in Iscove's modified Dulbecco's medium (IMDM; Gibco, Grand Island, NY, USA) containing 10% fetal calf serum (FCS; Sigma, St. Louis, MO, USA), penicillin 100 U/ml, streptomycin 100 mg/ml, 2 mmol/l L-glutamine, and 50 μmol/l 2-mercaptoethanol. Different combination of cytokines (Becton Dickinson, San Jose, CA, USA) were used for three experimental groups. The protocol for expanding cord blood-CIK/NK cells in group A was SCF (40 ng/ml), IL-2 (80 ng/ml), IL-7 (40 ng/ml) and IL-15 (40 ng/ml). The protocol of group B was mainly the same as that of group A but with the addition of FLT3 ligand (40 ng/ml, Becton Dickinson, San Jose, CA, USA). The protocol of group C was IL-2, IL-7 plus IL-15,at the concentrations given above. All the appropriate concentrations of these cytokines in different groups were up to the results of preliminary experiments. Various cytokines were added into different groups on day 1. Cells were incubated at 37˚C in a humidified atmosphere of 5% CO_2, and every three days were replenished with fresh complete IMDM and cytokines.

Triplicate experiments were carried out for each donor, and there were fifteen different CB donors tested. Representative results shown here are for nine separate experiments from three different donors.

Proliferation and phenotype analysis. On days 0, 14 and 21, viable CIK/NK cell densities were determined by each counting cell numbers with a hemocytometer; the phenotypes of the cells from each group were identified by flow cytometry. respectively. Flow cytometry analysis CIK cells, NK cells, $CD4^+$ T-cells and $CD8^+$ T-cells were labeled with $CD3^-$PerCP/CD56-PE, CD3-PerCP/CD4-FITC and CD3-PerCP/CD8-FITC (Becton Dickinson). A total of 5×10^5 cells were resuspended in 20 μl of 2% newborn calf serum and 1% sodium azide in phosphate-buffered saline (PBS), and incubated with 10 μl of appropriate monoclonal antibody for 30 min at 4˚C. After incubation, the cells were washed twice and resuspended in 1.0 ml of assay buffer. Nonspecific binding was determined using irrelevant mouse immunoglobulin isotypes IgG1-FITC, IgG1-PE, IgG1-PerCP and IgG2b-PE. The fluorescence was analyzed by a Coulter FC500 flow cytometer (Beckman Coulter, Fullerton, CA, USA).

The CB-CIK/NK cells were harvested on day 21, after being stained with 7-amino-actinomycin D(7-AAD) and CD3-PerCP/CD56-PE, flow cytometry showed that the proportion of viable cells exceeded 95% and the percentage of $CD3^+CD56^+$ plus $CD3^-CD56^+$ cells exceeded 60%, thus the cells qualified for the next cytotoxic XTT assay as the effector cells.

Cell lines. The human erythroleukemia cell line K562 was purchased from the Cancer Institute of Sun Yat-Sen University Cancer Center. The cells were maintained in RPMI-1640 supplemented with 10% FCS, 100 U/ml penicillin and 100 g/ml streptomycin,and grown at 37 °C in a humidified atmosphere of 5% CO_2.

Cytotoxic effects of CB-CIK/NK cells against K562 cell line by XTT assay. The cytotoxic potential of the CIK/NK cells expanded by the different protocols against K562 cell line was measured by XTT (Sigma) assay as described in previous studies (17). XTT was prepared in Dulbecco's PBS (PBS; Gibco, Grand Island, NY, USA) at 0.25 mg/ml; color developing reagent, (2,3)-dimethoxy-5-methyl-(1,4)-benzoquinone (coenzyme Q; Sigma) was prepared at concentration of 0.05 mg/ml in PBS. Fresh XTT stock solutions prepared for each experiment contained 1 ml XTT with 8 μl coenzyme Q. A total of 1×10^4 cells/well target cells (K562 cells) were incubated in triplicate sets with effector cells (CIK/NK cells) in U-bottom, 96-well culture plates, with ratios of effector cells to target cells of 20:1 and 10:1. Controls of effector and target cells were set up at the same time. After a 4 h incubation and centrifugation (3000 rpm for 10 min), all supernatants were collected and removed and a 150 μl volume of XTT stock solution containing coenzyme Q was then added and the plates were gently shaken in a shaker incubator for 1 h at 37˚C.The cells were then pelleted by centrifugation (3000 rpm for 10 min) and 100 μl of each supernatant were transferred to fresh 96-well plates , and the absorbance at 490 nm (A490) was measured using a microplate reader (Universal Microplate Reader, ELx 800 UV). The percentage reduction in formazan produced was calculated using the following formula:

$$\text{Cytotoxicity (\%)}=1-\frac{A_{490}(\text{effector+target})-A_{490}(\text{effector control})}{A_{490}(\text{target control})}\times100$$

Statistical analysis. Data are presented as the mean±standard deviation. Statistical analysis was performed using SPSS 16.0 software package. The quantitative data were compared using one-way analysis of variance and least significant difference (LSD) method. *P*-values of less than 0.05 were considered statistically significant.

Results

Generation of cytotoxic CIK/NK cells from cord blood. $CD3^+CD56^+$ CIK cells were rare (0.5%±0.2%) in uncultured cord blood, and $CD3^-CD56^+$ NK cells comprised 12.7%± 6.4% on day 0. After stimulating with SCF, FLT3 ligand, IL-2, IL-7 and IL-15, the proportions and quantities of the expanded CIK/NK cells in groups A, B and C were notably higher than those of uncultured cord blood.

Compared with that of group C, after 21 days' expansion, the percentage of $CD3^+CD56^+$ CIK cells in group B treated with five cytokines peaked to 26.20%±4.05%, also

significantly higher than that of group A (19.84%±2.11%). The proportion of $CD3^+CD56^+$ CIK cells in groups B and C on day 21 were significantly higher on day 14, but in group A, no significant difference was seen between these two time points. The changes in the proportion of $CD3^-CD56^+$ NK cells in the groups of our study were less obviously.In groups A and B, which appeared to plateau after two weeks in culture, there were no significant differences in the production of $CD3^-CD56^+$ NK cells between day 21 and 14. For group C, however on day 21, the proportion of $CD3^-CD56^+$ NK cells was significantly lower than that on day 14 and than that of groups A and B (Table I, Figure 1).

The absolute number of cord blood derived $CD3^+CD56^+$ CIK cells increased significantly after 14 to 21 days of culture, and on day 21, had expanded nearly 550- to 800-fold in the different groups, the number of $CD3^-CD56^+$ NK cells had expanded nearly 15- to 48-fold using different protocols. For groups A and B, the number of CIK and NK cells increased gradually with culture time, but for group C, expansion of NK cells did not follow this tendency, with a drop on day 21 compared to day 14 (Table II, Figure 1).

Proportion of $CD4^+$ T-cells and $CD8^+$ T-cells in CIK/NK cell cultivation. There were only minor changes in the percentage of $CD3^+CD4^+$ T-cells during CIK/NK cell cultivation, and no significant differences were seen between the ratio of $CD3^+CD4^+$ T-cells on day 21 and day 14. The proportion of $CD3^+CD8^+$ T-cells on day 21 was significantly lower ($p<0.01$) than that on day 14. When CIK/NK cells were harvested on day 21, the proportion of $CD3^+CD8^+$ T-cells were merely about 24%, 13% and 10% in group A, B and C, respectively (Table III, Figure 1).

Our result showed that even with only three cytokines, IL-2, IL-7 and Il-15, $CD3^+CD56^+$ CIK cells and $CD3^-CD56^+$ NK cells could be effectively expanded simultaneously, and there were fewer $CD3^+CD8^+$ T-cells in the cultivation.

Cytotoxicity of expanded CB-CIK/NK cells against K562 cell line. The cytotoxic effect of CIK/NK cells expanded in the presence of SCF, FLT3 ligand, IL-2, IL-7 and IL-15 were studied using K562 cells as targets in a XTT cytotoxicity assay. All expanded CIK/NK cells showed cytotoxic activity against the K562 cell line, and the cytotoxicity at an effector:target ratio of 20:1 was significantly higher than that at 10:1. The cytotoxic activity of group A was highest and significantly higher than that of other groups. There was no obvious difference in cytotoxicity against K562 cell line between groups B and C (Figure 2).

Table I. *Phenotype of cord blood-derived CIK/NK cells in groups A, B and C on days 14 and 21 of culture (χ±S, n=9)*. Groups *A, B and C were generated as outlined in the Materials and Methods section*.

Group	$CD3^+CD56^+$ CIK cells (%)		$CD3^-CD56^+$ NK cells (%)	
	d14	d21	d14	d21
A	19.1±2.1	19.8±2.1★	26.2±2.9	28.6±1.5
B	21.3±2.5	26.2±4.1☆	26.5±1.4	29.2±2.5
C	18.4±3.3	24.0±5.0☆	28.7±3.9	21.3±2.0★☆

☆ $p<0.01$ Compared with that of d14 in the same group;
★ $p<0.01$ compared with that of other groups on d21.

Table II. *Expanding fold-change of cord blood-drived CIK/NK cells in groups A, B and C on days 14 and 21 of culture (χ±S, n=9). Groups A, B and C were generated as outlined in the Materials and Methods section*.

Group	CD3+CD56+ CIK cells		CD3-CD56+ NK cells	
	d14	d21	d14	d21
A	447.9±162.6	559.1±174.0	33.3±3.1	35.0±7.9
B	563.8±203.2†	796.1±278.5☆★	36.5±5.9	36.6±3.5
C	319.7±116.2	575.8±221.7☆	37.2±3.5	21.3±4.8★

☆ $p<0.01$ Compared with that of d14 in the same group;
† $p<0.05$ Compared with that of group C on d14;
★ $p<0.05$ Compared with that of other groups on d21.

Table III. *Phenotype of $CD4^+$ and $CD8^+$ T cells in groups A, B and C on days 14 and 21 of culture (χ ±S, n=9). Groups A, B and C were generated as outlined in the Materials and Methods section*.

Group	$CD3^+CD4^+$ T-cells (%)		$CD3^+CD8^+$ T-cells (%)	
	d14	d21	d14	d21
A	20.5±4.8	17.4±4.4	40.8±5.2†	24.4±5.2☆★
B	23.2±2.4	23.1±2.6	48.3±5.9	13.9±2.8☆
C	22.8±5.3	22.4±0.7	46.0±9.2	10.4±1.0☆

☆ $p<0.01$ compared with that of d14 in the same group;
† $p<0.05$ compared with that of group C on d14;
★ $p<0.01$ compared with that of other groups on d21.

Discussion

CIK cells have been shown to be a heterogeneous population with different cellular phenotypes that are generated by incubation of peripheral blood (1, 18) or cord blood (19, 20) mononuclear cells with various cytokines, such as anti-CD3 monoclonal antibody, IL-1, IL-2, IL-12 and interferon gamma. $CD3^+$ T-cells co-expressing the CD56 antigen were first described by Lanier *et al*. (21) in 1986, and a remarkable expansion of this cellular subset was obtained and developed by Schmidt-Wolf *et al*. (22). The higher lytic activity against tumor cells of CIK cells was mainly due to the higher proliferation of $CD3^+CD56^+$ double positive cells;from the studies of Schmidt-Wolf *et al*. (23), in the

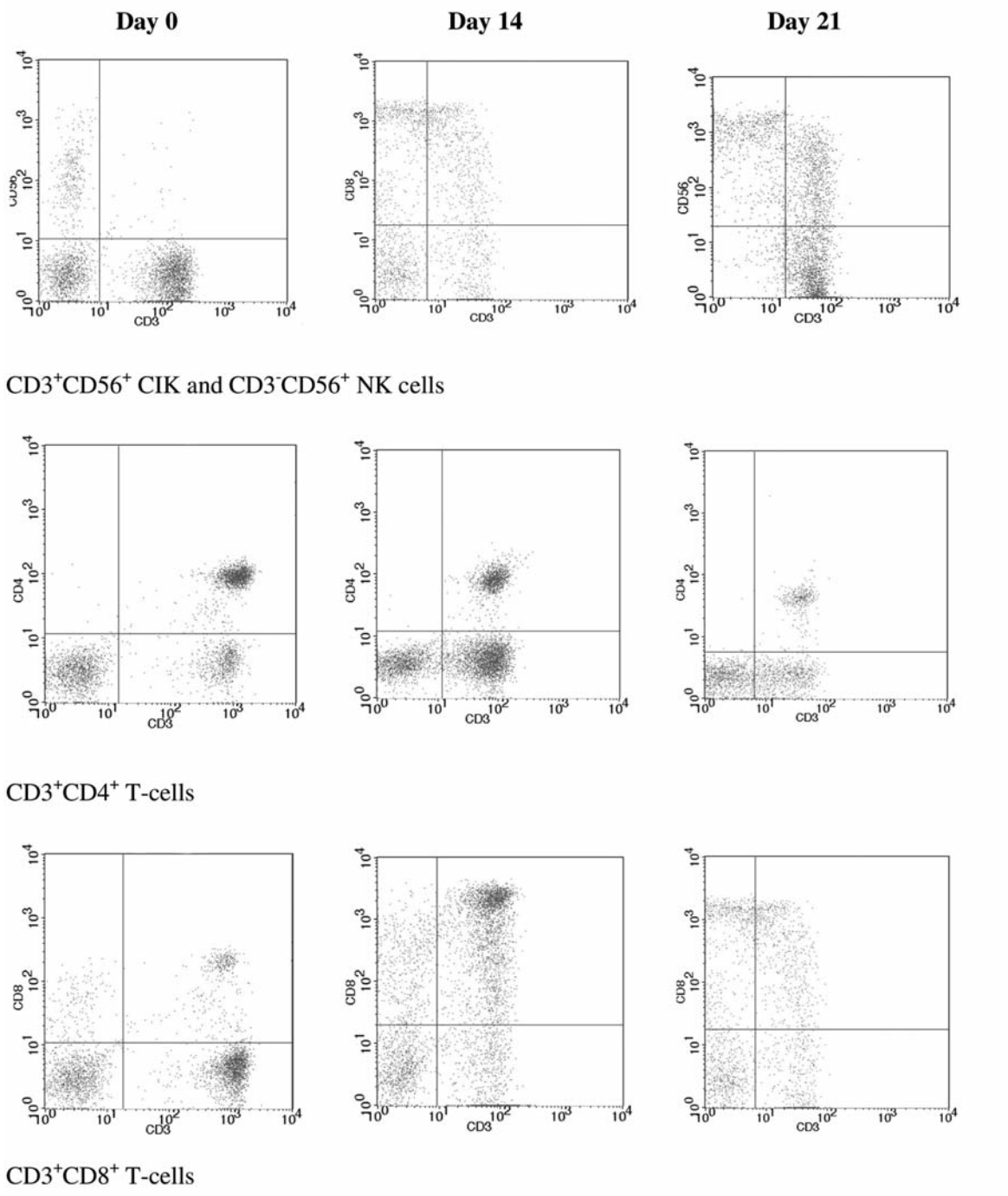

Figure 1. *Phenotype of CIK, NK and T-cells in groups A, B and C on days 0, 14 and 21 of culture. Representative results from three experiments are shown.*

presence of anti-CD3 monoclonal antibody, IL-1, IL-2 and interferon gamma after 14 days culture, the number of peripheral blood-derived $CD3^+CD56^+$ double positive cells can increase by 754-fold. The application of anti-CD3 monoclonal antibody and IL-1 were critical and optimal for the proliferation and cytotoxic activity of CIK cells (22), and this protocol has now been widely adopted as the 'classical' protocol for expanding CIK cells.

The results from Lu and Negrin (13) showed that using the 'classical' protocol, during the expansion of peripheral blood-derived $CD3^+CD56^+$ CIK cells and $CD3^-CD56^+$ NK cells on day 0, 10, 20 and 30, the proportion of CIK cells

was 2.3%, 5.5%, 23% and 28%, respectively, and that of NK cells was only 12%, 5%, 3.9% and 2%, respectively. After one month, $CD3^+CD56^+$ cells expanded nearly 1000-fold; nevertheless, $CD3^-CD56^+$ NK cells expanded less than 10-fold under these culture conditions. Findings of Ren *et al*. (24) demonstrated that with this protocol, $CD56^+$ cells could increase from 8.8±0.3% to 43.1±4.2%, whereas the proportion of $CD16^+$ cells did not change and remained at about 8% during 15 days of culture. Another study of peripheral blood-derived $CD3^+CD56^+$ CIK cells expanded by these 'classical' cytokines also demonstrated that the proportion of $CD16^+CD56^+$ cells decreased from 9.2±8.3% to 4.8±4.0% after culturing (14). The same protocol can also be used for cord blood-derived $CD3^+CD56^+$ CIK cell expansion and leads to same low producity of NK cells (25). All these studies indicate that the combination of anti-CD3 monoclonal antibody, IL-1, IL-2 and interferon gamma used for $CD3^+CD56^+$ CIK cell expansion had a weakly expanding effect on $CD3^-CD56^+$ NK cells. NK cells are also the important antitumor effectors in biotherapy (26, 27), and hence it is important for promoting the GVL effect after HSCT if CIK and NK cells could be induced and expanded in one culture system simultaneously.

At present, $CD3^+CD56^+$ CIK cells are considered to derive from $CD3^+CD56^-$ T-cells but not $CD3^-CD56^+$ NK cells (1, 13). We have previously demonstrated that by using the combination of IL-2, IL-7 and IL-15, successful expansion of both $CD3^+CD56^+$ CIK cells and $CD3^-CD56^+$ NK cells from cord blood is possible. Here we explored the influence of two important early-stage hematopoietic growth factors, SCF and FLT3 ligand, together with the combination of IL-2, IL-7 and IL-15 on induction and expansion of cord blood-derived CIK and NK cells. It was verified that SCF in cooperation with IL-2 can stimulate cell proliferation and increase the sensitive of IL-2 receptors (28). FLT3 ligand is mainly produced by bone marrow mesenchymal cells;the quantity of NK cells in mice lacking Flt3 ligand ($Flt3L^{-/-}$) were clearly reduced (29). FLT3 ligand in conjunction with IL-15 increased the ratio of NK cells derived from $CD34^+$ hematopoietic stem cells were notably than by using IL-15 alone, and increased the expression of both IL-2 and IL-15 receptors (30). After auto-HSCT treatment of cancer patients with subcutaneous injection of FLT3 ligand significantly increased the number of dendritic cells and NK cells *in vivo* (31). For these reasons, we added SCF (protocol of group A), and SCF plus FLT3 ligand (protocol of group B) combined with IL-2, IL-7 and IL-15 (protocol of group C) for 21 days CIK/NK cell expansion. Our study showed that compared to group C, $CD3^+CD56^+$ CIK cells in group A significantly decreased from an average of 24.03% to 19.84%, whereas that in group B was increased from an average of 24.03% to 26.02%. The proportion of $CD3^-CD56^+$ NK cells in groups A and B were both significantly increased from an average of 21.30% to 28.60% and 29.16%, respectively. The protocol used in group B (five cytokines combined) seemed to have an optimal effect on CIK/NK cell proliferation compared to other groups, the expansion of CIK cells was about 800-fold (up to 1313-fold), and that of NK cells was about 36-fold in absolute numbers. These results show that combination of IL-2, IL-7 and IL-15 with SCF alone might reduce $CD3^+CD56^+$ CIK cell yield, but had some synergistic action on $CD3^-CD56^+$ NK expansion,$CD3^+CD56^+$ CIK cells and $CD3^-CD56^+$ NK cells both effectively expanded in the presence of IL-2, IL-7 and IL-15 combined with SCF/FLT3 ligand. After 21 days of cultivation, the proportion of CIK and NK cells in group A was more than 48%, while that in group B was more than 55%, indicating that combination of SCF and FLT3 ligand based on usage of IL-2, IL-7 and IL-15 were helpful for simultaneous expansion of $CD3^+CD56^+$ CIK cells and $CD3^-CD56^+$ NK cells derived from cord blood. Our results might be an important experimental evidence for clinical adoptive immunotherapy.

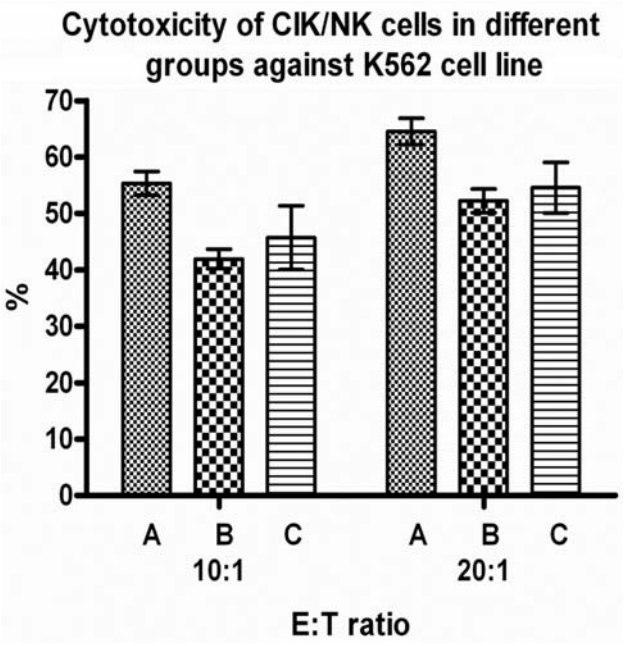

Figure 2. *Cytotoxicity of CIK/NK cells in groups A, B and C against the K562 cell line. Cord blood-derived CIK/NK cells from protocols A, B and C were used as effector cells in a cytotoxicity XTT assay against the K562 cell line at effector:target(E:T) ratio of 10:1 and 20:1. Results are expressed as the mean percentage killing activities±SD. Representative results from nine experiments are shown. Cytotoxicity of CB-CIK/NK cells of groups A, B and C against the K562 cell line at E:T ratios of 10:1 were 55.33%±5.20%, 41.94%±4.18% and 45.68%±5.66%, respectively; and that at E:T ratios of 10:1 were 64.55%±5.74%, 52.25%±5.10% and 54.57%±4.51%, respectively. Killing activities of group A against K562 cell line at E:T ratios of 10:1 and 20:1 were both significantly ($p<0.05$) higher than that of groups B and C, and there was no difference between the cytotoxicities of groups B and C.*

A previous report from Lu *et al*. (13) by FACS sorting the cells then culturing them under the 'classical' CIK cell expansion protocol showed that $CD4^+CD8^-$ T-cells did not develop expression of CD56, whereas the majority of cells that did originated from $CD4^-CD8^+$ cells in peripheral blood. Several studies using the 'classical' CIK cell expansion protocol illustrated that a considerable number of $CD3^+CD4^+$ and $CD3^+CD8^+$ T-cells were present in the culture system, comprising about 45% and 47%, respectively (1), and could even reach up to 67% $CD3^+CD8^+$ T-cells (32). The percentage of $CD3^+CD8^+$ T-cells in graft could be related to GVHD (15, 16). In our study, under the modified CIK/NK cell protocols, the proportions of $CD3^+CD8^+$ T-cells on day 21 and day 14 appeared to have distinct differences; at harvest on day 21, $CD3^+CD8^+$ T-cells only comprised about 10% to 20% of the harvested effector cells, especially in groups B and C. Our flow cytometric data also show that on day 14, the total percentage of CIK cells, NK cells, $CD4^+$ and $CD8^+$ T-cells in some groups were above 100%, this may be due to some CIK cells that also co-expressed CD8 markers overlapping with $CD8^+$ T-cells, so the actual proportion of $CD8^+$ T-cells in the culture system was less than the above data. Sometimes the lower numbers of $CD3^+CD8^+$ T-cells mixed with the immune effectors would be more desirable in clinical adoptive immunotherapy, thus our modified protocol might be an alternative choice for CIK/NK cell expansion.

Finally, we compared the cytotoxic effect of CIK/NK cells expanded using the different protocols. The results indicated that the CIK/NK cells produced by the new protocols also possessed effective killing activities against K562 cell line, but the cytotoxicity was not parallel to the expansion capacity under the different protocols. Previous studies showed that IL-2, IL-7 and IL-15 played important roles in maintaining the cytotoxic activity of killer cells (33, 34), and by Braun *et al*. (35), it has been confirmed that culture system containing SCF can enhance killing activity of lymphokine-activated killer cells against acute myeloid leukemia cells. In this study, we found that the cytotoxicity of CIK/NK cells in group A, with SCF in the culture medium, was higher than that of group C, but if FLT3 ligand was added, such as under the protocol of group B, although the proliferate activity of CIK/NK cells was improved, the cytotoxicity was weakened, this might be due to FLT3 ligand impairing the killing activity of NK cells (36).

In our study, we noticed that combinations using a greater number of cytokines may help to produce more CIK and NK cells, but also may be accompanied by increasing numbers of $CD8^+$ T-cells in the cultivation system. Cultivation protocol of group B with SCF and FLT3 ligand added to IL-2, IL-7 and IL-15 might be optimal because it had higher a capacity for expanding both CIK and NK cells, with relatively lower $CD8^+$ T-cell production. Apart from increasing the killing activity of the CIK/NK cells against the K562 cell line, the protocol with only SCF plus IL-2, IL-7 and IL-15 (group A) showed no obvious advantage, and gave rather fewer CIK cells and more unwanted $CD8^+$ T-cells compared to that of the basic IL-2, IL-7 and IL-15 protocol (group C). It is concluded that using the different combinations of SCF, FLT3 ligand, IL-2, IL-7 and IL-15, we may be able to develop modified protocols for synchronous and effective expansion of cord blood-derived $CD3^+CD56^+$ CIK and $CD3^-CD56^+$ NK cells, containing fewer $CD3^+CD8^+$ T-cells.This might provide an alternative choice for CIK/NK cell expansion in clinical applications.

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