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Effect of DC-CIK cell on the proliferation, apoptosis and differentiation of leukemia cells

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ABSTRACT

Objective: To observe the effect of co-culture cytokine-induced killer cells (CIK) and homologous dendritic cells (DC) on the proliferative activity and phenotype change of the DC-CIK cell and the cell killing activity of leukemia HL-60. **Methods:** 50 mL cord blood sample was obtained from infants delivered by full term healthy woman and the cord blood mononuclear cells were isolated by density gradient centrifugation. Non-adherent cells were collectedfor the induction culture of CIK, adherent cells were differentiated into mature DC; cultured mature DC was mixed with and CIK in the proportion of 1:5 for 12 d. Killing activity of DC-CIK co-cultured cell on leukemia HL-60 was detected by MTT assay. **Results:** Compared with CIKs, the co-cultured DC-CIKs presented a markedly higher proliferation and killing activity. **Conclusions:** Co-culture of DC-CIK cells led to a significant increase of the proliferation and cytotoxicity of CIK.

1. Introduction

Cellular immunotherapy is a new treatment for leukemia following chemotherapy and hematopoietic stem cell transplantation, which is mainly because many tumor cells are resistant to immunologic effector cells. Leukemia patients themselves with immune dysfunction and antigen presentation dysfunction, further increase the tumor cells escape immune response[1]. Cytokine-induced killer (CIK) cells have the characteristics of broad spectrum in killing of tumor cells[2] and equally sensitive to multi-drug resistant tumor cells. Dendritic cells (DC) is an efficient antigen-presenting cells which can be united applicated with CIK and increase the specificity of the response of CIK cells[3]. Therefore, in order to effectively remove residual leukemia cells and reduce the relapse rate in patients with leukemia, this paper observed the impact of DC combined CIK on the killing effect of leukemia HL-60 DC cell.

2. Materials and methods

2.1. Materials

Human leukemia HL-60 was purchased from cell bank of Institute of Shanghai Institutes for Biological Sciences, CAS., lymphocyte separation medium was purchased from Tianjin TBD, 1640 were purchased from Sigma Chemical Co., MTT and formazan were purchased from Gibco. ELISA kits of IFN-γ, IL-8 and TNF-α were purchased from Jingmei Biological Engineering Co., Ltd.. Cord blood was provided by obstetrics and gynecology of Xx Hospital (obtained from infants delivered by full term healthy woman under sterile

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conditions and anticoagulated with Sodium heparin).

2.2 .CIK and DC induction, amplification and culture

2.2.1. Culture of DC

Cord blood was obtained from infants delivered by full term healthy woman and anticoagulated with Sodium heparin. Mononuclear cells (MNC) were isolated with lymphocyte separating medium and washed by serum-free RPMI 1640 culture medium. They were cultured in RPMI 1640 medium for 2 h. Adherent cells were suspended in RPMI 1640 culture medium which contained rhGM-CSF 550 U/mL, rhIL 4 500 U/mL, rhSCF 100 μg/L, rhTNF α 10 μg/L for 10 d. Medium was changed every other day for half dose and added with cytokines.

2.2.2. Culture of CIK

Non-adherent cells of cord blood MNC were collected, washed with serum-free RPMI 1640 culture medium and the MNC cell concentration was adjusted to 1×10^6/m. Interferon-α (IFN-α) 1 000 U/mL was addedand and they were cultured in the CO_2 incubator at constant temperature 37 ℃. After 24 h, 1 g/mL CD3 monoclonal antibody, 1 000 U/mL recombinant human interleukin-2 (rh IL-2) and 300 U/mL recombinant human interleukin-1 (rh IL-1) were added. Medium was changed every three days for half dose and added with cytokines to maintain the cell concentration at $(1-2)\times10^6$/mL.

2.2.3. DC co-cultured with CIK

Mature DC was obtained and CIK and they were cultured in the proportion of 1:5 after the cells were counted. Medium containing 500 U/mL rh IL-2.Cells were collected for biological activities assay after 12 d.

2.3. MTT assay detect the killing activity of DC-CIK cell on target cells

HL-60 cells were used as target cells, the CIK and DC-CIK cells cultured for 12 d were used as effector cell mixed in the proportion 5:1, 10:1 and 20:1. Leukemia HL-60 cells was obtained at logarithmic growth phase and concentration was adjusted to 1×10^5/mL, then the CIK and DC-CIK cells were adjusted according to effector-target ratio, respectively. They were divided into three groups, the experimental group, the effector cell group and the target cells group. There were 3 parallel holes at every group. Experimental group were added with effector cells and target cells 100 μL respectively. Effector cell group were added with effector cells and 1640 culture medium 100 μL respectively. The target cell groups were added target cells and 1640 culture medium 100 μL respectively and all cells were cultured in the CO_2 incubator at constant temperature 37 ℃. MTT reagent 10 μL was added after 48 h, formazan solution 100 μL was added after 4 h. Absorbance was measured at wave length of 570 nm with automated immunoanalyser after 4 h, killing rate was calculated as follows: The killing rate (%) = [1 − (A effect target cell well-A effector cell well)/A target cell well] ×100%.

2.4. Immune phenotype and cytokine expression were detected

Cultured cells were collected and the immune phenotype of DC cells, CIK cells, DC-CIK cells were detected with flow cytometry. IFN-γ, IL-8 and TNF-α content of the cell supernatants were measured with ELISA.

2.5. Statistical analysis

The data were analyzed by SPSS 17.0 statistics software and the measurement data were expressed as mean±SD values. *t*-test was used to evaluate the differences between groups. $P<0.05$ was considered as statistical significant difference.

3. Results

3.1. Cell proliferation

Cord blood CIK cells proliferation began at the first three days after culturing. Cells were colony-like suspended growth under invert microscope. Part of the cell became larger and irregular shape. Cells were quickly proliferated from the 5-6 d, most of the cells became larger (Table 1).

Table 1
Comparison of CIK and DC-CIK proliferation multiple at different times.

Groups	3 Days	6 Days	9 Days	12 Days
CIK group	1.9±0.5	5.3±1.5	9.2±2.6	14.3±2.6
DC-CIK group	1.9±0.4	5.3±1.4	9.3±2.8	20.5±3.2*

Note: Compared with CIK group, *$P<0.05$.

3.2. Analysis of cell phenotype

Flow cytometry showed that with the culture time, $CD3^+$ $CD8^+$ cell percentage gradually increased. After co-cultured with DC, $CD3^+$ $CD8^+$ cell percentage was increased more significantly than the simple CIK ($P<0.05$) (Table 2).

Table 2
Cord blood CIK and DC-CIK cells phenotype at different times.

Groups	3 d	6 d	9 d	12 d
CIK group	1.8±0.5	5.2±1.3	9.2±2.1	15.5±2.6
DC-CIK group	1.8±0.4	5.3±1.2	9.3±2.3	21.3±3.0*

Note: Compared with CIK group, *P<0.05.

3.3. Measured the secretions of cytokines of DC-CIK and CIK cells

At 12 d of culturing cord blood CIK and DC cells, it was found that the IFN-C, TNF-α and IL-8 levels of DC-CIK cells were significantly greater than the simple CIK cells (P<0.05) (Table 3).

Table 3
Comparison of cord blood CIK and DC-CIK cells cytokine secretion.

Groups	IFN-γ	TNF-α	IL-8
CIK group	85.2±28.3	76.3±25.9	55.7±17.8
DC-CIK group	227.6±81.9*	137.5±40.2*	78.5±21.5*

Note: Compared with CIK group, *P<0.05.

3.4. DC-CIK and CIK killing activity

HL-60 leukemia cells were used as target cells. CIK and DC-CIK cells both had a strong killing effect on HL-60, and the killing effect became more significant with the increase of effector-target ratio (P<0.05). Under the same effector-target ratio, the killing effect of DC-CIK cells on HL-60 cells was more significant than the CIK cells (P<0.05) (Table 4).

Table 4
Comparison of CIK and DC-CIK killing activity under different effector-target ratio.

Effector-target ratio	CIK group	DC-CIK group
5:1	20.2±2.1	30.8±2.6*
10:1	36.7±2.9$^{\triangle}$	45.6±3.3$^{*\triangle}$
20:1	52.9±3.8$^{\bigcirc}$	62.8±4.5$^{*\bigcirc}$

Note: Compared with the same effector-target ratio, *P<0.05; compared with the 5:1 effector-target ratio, $^{\triangle}P$<0.05, compared with the 10:1 effector-target ratio, $^{\bigcirc}P$<0.05.

4. Discussion

The minimal residual disease in patients is still the main cause of leukemia relapse. Generally, residual leukaemie cell can only be eliminated by the own body antitumor immuno-protection mechanism when all of the leukemic cells less than 10^6[5]. As the key anti-tumor immune cells, DCs are important professional antigen-presenting cells. But usually the patients with leukemia have low immune function and that leading to tumor cells escape autoimmune. Therefore, this study observed the effect of co-culture CIK and homologous dendritic cells (DC) on the killing activity of leukemia HL-60[7-10].

CIK cells come from T cell subsets. They are a group of heterogeneous cells isolated from peripheral blood, bone marrow or umbilical cord blood by multiple cytokines. It has multiple characteristics such as high tumor-cytotoxic effect and broad spectrum in killing of tumor cells. Current research focus on co-cultured CIK with DC. DC are the strongest antigen-presenting cells *in vivo*, also the only antigen-presenting cells which can activate naive T lymphocyte[11-13]. Co-cultured CIK with DC can significantly stimulate the proliferation of naive T cells. This study suggests that cord blood CIK cells began proliferation at the first three days after culturing and quickly proliferated from the 5-6 days. At 12d of culturing cord blood CIK and DC cells, it is found that the proliferation of DC-CIK cells were significantly greater than the simple CIK cells.

The $CD3^+$ $CD8^+$ cells percentage increased more significantly than a simple CIK and the IFN-C, TNF-α and IL-8 which secreted by the $CD3^+$ $CD8^+$ cells were significantly greater than the simple CIK cells. That not only enhanced the killing effect on leukemia cells, but also kill leukemic cells indirectly by regulating the immune system. The reason is because the number of DC-CIK cells proliferation increased significantly after stimulated by antigen, and because effector cells CIK whose efficient anti-tumor activity is closely related to the double-positive cell levels of the $CD3^+$ $CD8^+$ cells and secrete cytokines such as IL-8, IFN-C and TNF-α. That directly or indirectly lead to enhanced tumoricidal activity. CIK cells can release large amounts of toxic particles and inflammatory cytokines which induce tumor cell apoptosis. Co-cultured with DC amplified the cytotoxic activity of CIK cells and promoted the release of inflammatory cytokines and remain active for a long time. Because of the sub-feedback effect, it can inhibite T cell numbers and reduced secretion of IL-10 which has an immunosuppressive function[14-20].

In vitro experiment demonstrated that cord blood can be used as a source of CIK and DC and can co-cultured DC-CIK. It is found that the DC-CIK proliferated significantly, which can enhance its anti-leukemia effect. Cord blood have wide raw material sources thus easy to solve the problem of shortage of materials, which are also unlikely to cause immune rejection, so it is think that cord blood can be

widely used in the clinical application for the co-culture of DC-CIKs.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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