Antitumor Therapeutic Potential of Activated Human Umbilical Cord Blood Cells against Leukemia and Breast Cancer

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ABSTRACT

In this study, in vitro and in vivo antitumor effects of mononuclear cells from human umbilical cord blood cells (UCBCs) and peripheral blood stem cells (PBCs) harvest obtained by leukapheresis were compared. Interleukin 2 (IL-2)-activated mononuclear cells from UCBCs showed increased cytotoxicity against K562 and Raji hematopoietic malignant cells compared with PBCs (P < 0.05). After IL-2 activation, both UCBCs and PBCs showed significant cytotoxicity against MDA-231 human breast cancer cells. The UCBC population involved in this antitumor activity appeared to be CD56+ natural killer precursors. The cytotoxicity of UCBCs was inhibited in the absence of Ca2+(P < 0.05), supporting a perforin/granzyme-mediated target of cell lysis. In addition, antibodies to Fas ligand blocked cytotoxic activity, suggesting that some of the antitumor cytotoxicity was Fas ligand mediated. In vivo antitumor effects of UCBCs and PBCs were studied using a human leukemic cell-bearing severe combined immunodeficient mouse model. There was a significant increase in the survival of K562 leukemia-bearing mice that also received 5 million in vitro IL-2-activated UCBCs or PBCs i.v. on days 3 and day 5 after tumor transplantation compared with untreated mice (P < 0.01). Similar antitumor cytotoxicity of UCBCs and PBCs was also observed against MDA-231 human breast cancer grown in severe combined immunodeficient mice (P < 0.01). These studies suggest that IL-2-activated UCBCs may be a useful source of cellular therapy for patients with hematological malignancies and breast cancer.

INTRODUCTION

Human umbilical cord blood is a rich source of hematopoietic stem cells for hematopoietic reconstitution in cancer patients after high-dose therapy (1–6). Umbilical cord blood contains significantly more numbers of hematopoietic stem/progenitor cells compared with normal human peripheral blood, as determined by the number of CD34+ cells and colony forming unit-granulocyte/macrophage progenitors (7). Transplantation of UCBCs has several advantages over other sources of stem/progenitor cells for hematopoietic rescue. These include: widespread availability; absence of donor risk; absence of donor attrition; low risk of transmissible infectious diseases; decreased graft-versus-host disease without an increased incidence of relapse, even in mismatched situations; and increased precursors of immune effector cells (8–10). Endogenous hematopoietic reconstitution induced by UCBCs in immunocompromised mice has been reported, indicating another beneficial property of UCBCs for transplantation (11). In vitro antitumor cytotoxicity of UCBCs against several hematological malignancies has been reported previously (12, 13). The natural killer cell-mediated cytotoxicity of UCBCs against standard tumor targets is significantly low compared with mononuclear cells from blood stem cell harvests (14). However, upon in vitro activation with IL-2, even at a low-dose, short-term incubation, the in vivo antitumor cytotoxic activity of UCBCs increases significantly (14). These reports indicate the potential clinical usefulness of UCBCs against hematological cancers and solid tumors such as breast cancer, particularly after in vitro activation with cytokines such as IL-2. Additional preclinical studies comparing mononuclear cell cord blood versus peripheral blood using human tumor models of hematological malignancy and solid tumors to confirm the therapeutic ability of UCBCs are essential.

Therefore, to evaluate the potential clinical use of UCBCs as a cellular immunotherapeutic modality, studies were designed to compare the antitumor effects of IL-2-activated UCBCs and PBCs for in vitro and in vivo antitumor cytotoxicity against human hematological malignant cells and human breast cancer cells. In this report, we have described the results of these studies, including the mechanism of action of cytolysis of tumor cells by activated UCBCs in vitro.

MATERIALS AND METHODS

Cell Collections. All human cell specimens were obtained using protocols approved by the Institutional Review Board at the University of Nebraska Medical Center.

Received 3/27/00; revised 8/22/00; accepted 8/24/00.

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1 This work was supported by a research grant from the Elsa U. Pardee Foundation and a grant from the Susan G. Komen Breast Cancer Foundation.

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3 The abbreviations used are: UCBC, umbilical cord blood cell; PBC, peripheral blood stem cell; IL-, interleukin; MNC, mononuclear cell; NK, natural killer; LAK, lymphocyte activated killer; FasL, Fas ligand; RT-PCR, reverse transcription-PCR; SCID, severe combined immunodeficient; LGL, large granular lymphocyte.
Cord Blood Cells. Human UCBC samples were obtained by the Labor and Delivery nursing staff. Briefly, after the delivery of an infant, the umbilical cord was double clamped and ligated, and venipuncture of the umbilical cord vein was performed using a heparinized syringe prior to expulsion of the placenta. MNCs in the cord blood samples were isolated using lymphocyte separation medium. The cells were further processed in a manner identical to the PBCs described below.

MNC Harvest from Peripheral Blood Apheresis Product. Normal volunteers received no mobilizing cytokine prior to apheresis. PBCs were collected with the Cobe Spectra apheresis machine, as described previously (15). Briefly, an aliquot of the PBC collection was layered and centrifuged over lymphocyte separation medium to obtain MNCs. The MNCs were washed once with RPMI 1640 and resuspended in RF10 medium, which consisted of RPMI 1640 supplemented with 10% fetal bovine serum, 2 mmL-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (15). The MNCs were activated in vitro with IL-2 and then used for cytotoxicity assays and/or for the in vivo therapeutic studies described below.

Cell Line Cultures. The human chronic myeloid leukemia cell line, K562 (NK sensitive), and human B-cell lymphoma cell line, Raji (NK resistant/LAK sensitive), were grown in vitro in 25-cm² culture flasks in RF10 medium. As a representative of solid tumors, MDA-231 human breast cancer cells were grown in DF10 medium (DMEM medium supplemented with 10% fetal bovine serum, L-glutamine, and antibiotics, as above) as monolayer cultures and used in both in vitro and in vivo studies. Raji, K562, and MDA-231 cells were labeled with chromium-51 (Na₂⁵¹CrO₄) and used as target cells for cytotoxicity assays.

Activation of Effector Cells with IL-2. MNCs obtained from UCBCs and PBCs were activated in vitro with IL-2 and assayed for cytotoxicity. Five million cells were cultured in T-25 flasks containing 5 ml of RF10, 50 μM β-mercaptoethanol, and 1000 units/ml IL-2 for 72 h at 37°C in 5% CO₂ and 95% air. Control flasks contained PBCs or UCBCs in 5 ml of the above media without IL-2. After the incubation period, the cells were harvested using a diSPo cell scraper, washed twice with RF10 or DF10 medium, resuspended, counted, and assayed for their cytotoxicity against ⁵¹Cr-labeled K562, Raji, or MDA-231 target cells. Activated cells were also injected into tumor-bearing mice to evaluate their in vivo antitumor activity.

Cytotoxicity Assay. MNCs obtained from UCBC and PBC harvests were tested for cytotoxic activity against ⁵¹Cr-labeled K562, Raji, or MDA-231 tumor target cells before and after in vitro activation, as described previously (15). Briefly, the effector cells and ⁵¹Cr-labeled target cells were mixed to obtain the E:T ratios of 12.5:1, 25:1, 50:1, and 100:1 in 200 μl of RF10 or DF10 medium/well in 96-well plates. The plates were incubated at 37°C for 4 h, and the supernatants were then harvested. The radioactivity in the supernatant was measured using a Beckman 5500 gamma counter. The percentage of target cells lysed was calculated with the following formula:

\[ \% \text{cytotoxicity} = \frac{\text{Experimental cpm} - \text{spontaneous cpm}}{\text{Maximum cpm} - \text{spontaneous cpm}} \times 100 \]

where the experimental cpm is effector + target cells, spontaneous cpm is target cells + medium, and maximum cpm (total release) is target cells + 1% Triton X-100.

Immunophenotyping Analysis. Cell surface immunophenotyping of MNCs from UCBCs and normal PBCs was performed by flow cytometric analysis using a Becton Dickinson FACStar PLUS flow cytometer as described previously (16). Three thousand hundred cells were suspended in 200 μl of PBS containing 0.1% BSA and 0.05% NaN₃ (fluorescence buffer) in 12 × 75-mm culture tubes. Cells were incubated on ice for 30 min with 10 μl of FITC or 5 μl of phycoerythrin-labeled antibody specific for the following cell surface markers: CD3, CD4, CD8, CD49c, CD49d, CD56, CD80, and CD83 (Becton Dickinson Corp., San Jose, CA). After incubation, the cells were washed with 1 ml of fluorescence buffer, centrifuged, and resuspended in 800 μl of PBS for analysis.

Purification of CD3- and CD56-positive Cells. The CD3-positive T cells and CD56-positive NK cell populations were purified from UCBC and PBC MNCs using flow sorting techniques as described by Pirruccello et al. (16). Briefly, the cells were stained with CD3 antibodies conjugated to phycoerythrin and CD56 antibodies conjugated to FITC. CD3-positive and CD56-positive cells were sorted and separated to at least 95% or greater purity using a Becton Dickinson FACStar PLUS flow sorter. The purified cells were then used to determine cytotoxicity activity after in vitro activation with IL-2 as described above.

Role of Perforin and Granzyme in Cytolysis of Tumor Cells by UCBCs. To determine the mode of cytolysis of target cells by activated UCBCs, calcium-free RF10 medium was used in the cytotoxicity assay. Calcium is an essential component for cytolysis mediated by perforin and granzyme present in the effector cells (17). The UCBC effector cells were activated as above and used in an in vitro cytotoxicity assay. The effector and ⁵¹Cr-labeled target cell mixtures were suspended in either calcium-free RF10 medium or RF10 medium with calcium. The rest of the procedure for the cytotoxicity assay was similar to that described above.

Role of Fas Ligand in Cytolysis of Tumor Cells by UCBC. Tumor cell lysis by the effector cells may be mediated by FasL. Some effector cells express FasL on their surface, which recognizes the Fas molecule expressed on target cells. The FasL–Fas binding leads to the activation of apoptosis pathways in the target cells, resulting in death (18). Therefore, to determine the role of FasL in the cytolysis of tumor target cells by the UCBCs, anti-FasL antibodies were used to block FasL–Fas binding. Purified, activated cells from UCBCs were mixed with ⁵¹Cr-labeled target cells, and different concentrations of anti-FasL antibody, NOK1 (PharMingen, San Diego, CA), were added. The control wells contained isotype-matched, nonspecific antibodies. The rest of the cytotoxicity assay was similar to that described above.

RT-PCR Analysis for the Expression of Perforin and FasL Genes in Activated Cells. A RT-PCR technique was used to determine the expression of FasL and perforin genes in activated UCBC cells as we have described previously (19). Briefly, RNA was isolated from activated UCBCs using TRIzol reagent obtained from Life Technologies, Inc. (Gaithersburg, MD). One μg of RNA was reverse transcribed using 150 ng of
random primers following standard procedures, with the exception of the addition of DMSO (1% final concentration), and incubated at 44°C for 1 h. PCR was then performed on 10 μl of cDNA in 50-μl reaction medium including 20 pmol of each primer (for perforin, 5′-GTT GCA TCT CCT CAT GGG ACC AGA CTT-3′ and 5′-TAA GCC CAC CAG CAA TGT GTA TGT GTC TGT-3′; and for FasL, 5′-AGG GGC AGG TTG TTG CCA GA-3′ and 5′-CAC CCC AGT CCA CCC CCT GA-3′), 2.5 mM MgCl₂, 200 μM deoxynucleotide triphosphates and 5% DMSO. The temperature conditions were as follows: 5 min at 95°C, followed by 2 min at 62°C, followed by 25 cycles of 20 s at 72°C, 45 s at 94°C, and 20 s at 62°C. PCR products were visualized by electrophoresis in ethidium bromide-stained, 2% agarose gels. As a control, β-tubulin gene expression was measured using the same procedure and appropriate primers, 5′-AAGAAATCCAAGCTGGAGTTC-3′ and 5′-GTTGGTGGAATTCTGTGAG-3′. All primer sets were designed to span introns to eliminate any potential signal from contaminating DNA in the RNA preparation (19).

In Vivo Experiment with Human Leukemic Target Cells. To evaluate the in vivo effects of activated UCBCs and PBCs against malignant hematological cells, K562 cells were grown in SCID mice, 6–8 weeks of age, with CB17 background (Charles River, Wilmington, MA). Each group consisted of 10 mice. The animals were transplanted with 5 × 10⁶ K562 cells i.v. Three days after tumor transplantation, 1 × 10⁷ MNCs from UCBCs or PBCs activated in vitro with IL-2 were infused i.v. into each mouse. After activation, cells were washed three times with sterile PBS and then used for infusion. The absolute numbers of CD56-positive NK cells infused were 3.4 × 10⁶ cells for UCBCs and 2.2 × 10⁶ cells for PBCs per mouse. The control mice were untreated. The mice were observed for signs of morbidity/death. After necropsy, the spleen, liver, kidney, heart, and lung were fixed in 10% buffered formalin and processed for histological evidence of tumor. Tumors that developed in the control mice were also removed for pathological confirmation of the presence of a tumor.

In Vivo Experiment with Human Breast Cancer Target Cells. A human breast cancer cell line, MDA-231, was used as a solid tumor model. For in vivo therapeutic analysis, SCID mice, 6–8 weeks of age, with CB17 background (Charles River, Wilmington, MA) were used. Animals were transplanted with 3 × 10⁶ MDA-231 cells s.c. in the flank along with Matrigel (Collaborative Biomedical Product, Bedford, MA) to enhance the tumor uptake and growth. The control group of six mice did not receive any treatment, whereas each treatment group consisted of seven mice. Twelve days after tumor transplantation, 5 × 10⁶ in vitro IL-2-activated UCBCs or PBCs were infused i.v. After IL-2 activation, cells were washed three times with sterile PBS and then used for infusion. This cell therapy treatment was repeated three additional times over a period of 2 weeks. In total, the absolute numbers of CD56-positive NK cells infused into these mice were 3.9 × 10⁶ cells/mouse for UCBCs and 3.25 × 10⁶ cells/mouse for PBCs as a result of three infusions. Tumor growth was determined by measuring the tumor volume using a caliper twice a week and plotted on a linear scale. Ten weeks after tumor transplantation, all remaining mice were sacrificed and processed as described above.

Statistical Analysis. Data from control and experimental groups were analyzed using the Tukey-Kramer Multiple Com-
parison Test, and differences were considered significant when $P < 0.05$.

**RESULTS**

**In Vitro Cytotoxicity of UCBCs versus PBCs against Hematological Tumor Targets.** The cytotoxicity levels of normal UCBCs and PBCs against NK-sensitive K562 and NK-resistant but LAK-sensitive Raji tumor target cells were determined after *in vitro* activation with IL-2 (Fig. 1). The cytotoxicity of IL-2-activated UCBCs was significantly ($P < 0.05$) higher than that generated by IL-2-activated PBCs against K562 tumor target cells (Fig. 1A). Similarly, the cytotoxicity of IL-2-activated UCBCs against Raji tumor cells was significantly greater ($P < 0.001$) than the levels in IL-2-activated PBCs at all E:T ratios (Fig. 1B). These results demonstrate the greater antitumor cytotoxicity of UCBCs compared with PBCs against hematological malignant cells.

**In Vitro Cytotoxicity of UCBCs versus PBCs against Solid Tumor Targets.** The cytotoxic levels of IL-2-activated UCBCs or PBCs were also tested against cells derived from solid tumors using MDA-231 human breast cancer cells. The data in Fig. 2 show the cytotoxicity of both UCBCs and PBCs after *in vitro* activation with IL-2. In this case, there was no significant difference between the *in vitro* cytotoxicity of activated UCBCs and PBCs against MDA-231 breast cancer.

**Cytotoxicity Levels of Purified CD3 T Cells and CD56 NK Cells.** Results shown above in Fig. 1 indicated an increased cytotoxicity of activated MNCs from UCBCs against different tumor targets compared with activated MNCs from PBCs. In an attempt to dissect out the effector cell types involved in the cytotoxicity, CD3-positive and CD56-positive cells were purified by flow cytometric cell sorting (>95% purity) from UCBCs and tested for cytotoxicity using the *in vitro* cytotoxicity assay (Fig. 3). The purified CD56 cells showed significant levels of cytotoxicity against both K562 and Raji target cells ($P < .001$), whereas the CD3-positive cells did not, suggesting that the CD56-positive NK cells may be the predominant antigen-nonspecific effector cells in the UCBCs, as is the case of PBCs (data not shown).

**Immunophenotyping of Activated UCBCs versus PBCs.** After IL-2 activation, MNCs from both UCBCs and PBCs were analyzed for the frequency of immune effector cells and the expression of adhesion molecules (Fig. 4). CD56$^+$ NK cells were significantly increased in UCBCs compared with PBCs ($P < 0.05$). The frequencies of other markers, such as CD4$^+$-positive T helper cells, or the expression of adhesion molecules CD49e and CD49d were comparable in both UCBCs and PBCs. Expression of CD54, a marker for intercellular adhesion molecule expression, was significantly lower ($P < 0.05$) in UCBCs compared with PBCs.

**Role of Perforin in Cytolysis of Tumor Cells by UCBC- and PBC-derived MNCs.** To elucidate the mechanism of cytolysis of tumor target cells by activated UCBC MNCs, cytotoxicity assays were performed in the absence of Ca$^{2+}$ to determine whether the calcium-dependent perforin/granzyme-mediated pathway is involved in cytolysis. Fig. 5 depicts the results of these studies, showing significantly decreased ($P < 0.05$) cytotoxicity of IL-2-activated UCBCs against either K562...
or Raji target cells compared with cytotoxicity levels of similar UCBCs in the presence of Ca^{2+}-free medium. These results support the hypothesis that tumor cell lysis by activated UCBCs is mediated through the perforin/granzyme pathway.

**Role of FasL in Cytolysis of Tumor Cells by UCBC-derived MNCs.** FasL-mediated cytolysis of tumor cells by IL-2-activated UCBCs was analyzed with NOK1, an anti-FasL antibody used to block FasL binding to Fas on target cells. Anti-FasL antibodies significantly inhibited (P < 0.001) the cytotoxicity of UCBCs against both K562 and Raji tumor target cells compared with lysis in the absence of anti-FasL antibodies (Table 1). A nonspecific, isotype-matched control antibody did not reduce the toxicity of activated UCBC-derived MNCs, indicating the FasL-specific inhibition of cytotoxicity by the NOK1 antibody.

**Expression of Perforin and FasL Genes in Activated Effector Cells.** Data shown in Fig. 5 and Table 1 indicate that both perforin- and FasL-mediated pathways are involved in cytolysis of tumor cells by UCBC MNCs. The expression of perforin and FasL in activated UCBCs was confirmed by RT-PCR analysis using specific primers for each gene. Expression of the β-tubulin gene was determined as a control. Fig. 6 demonstrates expression of perforin and FasL gene in UCBCs after activation.

**In Vivo Therapeutic Effects of UCBC- and PBC-derived MNCs on Human Tumors.** The in vitro cytotoxicity studies clearly demonstrated antitumor cytotoxic effects of IL-2-activated UCBCs against tumor target cells, and the cytotoxicity of UCBCs was significantly greater than PBCs against hematological malignant cell lines. To translate the in vitro studies to an in vivo situation, the antitumor cytotoxicity of IL-2-activated UCBC MNCs was studied, and the results were compared with that of similarly activated MNC PBCs. Fig. 7 shows the in vivo therapeutic effects of activated UCBCs and PBCs against K562 cells as measured by the survival of tumor-bearing treated versus tumor-bearing control animals. All 10 mice in the untreated control group died by 110 days or less after tumor cell infusion, whereas 50% of the PBC-treated animals and 10% of the UCBC-treated animals were dead in that time frame. One hundred sixty days after tumor inoculation, 90% of the UCBC-treated mice and 50% of the PBC-treated mice survived. These results suggest a significant antitumor activity of both UCBCs and PBCs (P < 0.01). Activated UCBCs appear to have a greater antileukemic effect compared with PBCs.

Because the in vitro cytotoxicity studies demonstrated considerable cytotoxicity against human breast cancer cells, the antitumor effects of activated UCBCs and PBCs against MDA-231 cells grown in SCID mice were investigated. Fig. 8 demonstrates the in vivo antitumor therapeutic effects of IL-2-activated MNCs from UCBCs and PBCs. The tumor size values represent the mean value derived from at least six animals from each group. There was an exponential growth of tumor in control untreated mice and a significantly (P < 0.01) reduced growth in mice treated with IL-2-activated PBCs or UCBCs. UCBCs appeared more effective than PBCs. After 80 days, all mice were sacrificed, and no metastatic tumor was identified. The histological analysis of the primary tumors revealed more

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**Table 1 Effects of anti-FasL antibodies on the cytotoxicity of cord blood-derived mononuclear cells against K562 and Raji target tumor cells**

<table>
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<tr>
<th>E:T Ratio</th>
<th>Control</th>
<th>2.5 µl NOK1</th>
<th>5 µl NOK1</th>
<th>10 µl NOK1</th>
<th>20 µl NOK1</th>
<th>Control</th>
<th>2.5 µl NOK1</th>
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<td>29</td>
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<td>9</td>
<td>14</td>
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* a E:T ratio, 50:1.

* b ND, not done.
necrosis in tumors from the treated group than the untreated control group. However, the tumor nodules appeared to contain some areas with live tumor cells based on cytomorphology.

DISCUSSION

In this study, the in vitro and in vivo antitumor properties of the IL-2-activated MNCs from UCBCs in comparison with IL-2-activated MNCs from PBCs were explored. The NK cytotoxicity levels of UCBCs were very low compared with PBCs prior to in vitro activation (data not shown). After IL-2 activation, however, there was significant antitumor cytotoxicity against NK-sensitive/LAK-resistant and LAK-sensitive tumor target cells (Fig. 1). Similar results have been observed by other investigators (20–26). The activated UCBCs were not only cytotoxic to hematological malignant cells but also considerably cytotoxic to human breast cancer cells (Fig. 2). The level of cytotoxicity of activated cord blood cells against breast cancer was not different from that of similarly activated MNCs from peripheral blood apheresis product. This result is different from what we observed with K562 and Raji tumor targets. The precise reason for this difference is not clear at this time.

The in vitro results reported here suggest that the CD56+ CD3− MNC population provided the antitumor activity of cord blood MNCs. The purified CD3-positive cells did not demonstrate significant cytotoxicity in vitro after IL-2 activation in the studies reported here. LAK precursor cells were identified based on the expression of CD56 cell surface antigens. Although the
majority of the LAK precursors are CD56-positive cells, a very small population of T cells that express both CD3 and CD56 is also capable of generating LAK cells. Only a few LAK precursors expressed the T-cell surface markers CD3 and CD5. Relatively immature NK precursor cell populations have been isolated from umbilical cord blood (23), which was phenotypically different from that observed in adult peripheral blood MNCs. One population was shown not to express NK markers (CD7+, NK−) but developed into phenotypically and functionally active NK cells after incubation with IL-2. Another population of precursor NK cells did express NK markers CD16 and CD56 (CD7+, NK+). Both cell populations were deficient in their ability to generate NK-like activity but readily acquired this activity after activation with IL-2. The CD3+ lymphocytes from UCBCs displayed strong LAK activity after in vitro activation with IL-2 (24). The majority of these CD3+ lymphocytes (>80%) expressed the high-affinity IL-2 receptor consisting of both p55 and p75 polypeptide chains, indicating that UCBC T lymphocytes, in the presence of IL-2, can differentiate into LAK cells. The results presented in this report, as well as reports from other investigators described above, suggest that in the presence of IL-2, human umbilical cord blood T cells are endowed with the ability to function as a first-line defense against aberrant cell growth. Because in the neonatal period both T and B lymphocyte-mediated immune responses are still on their way to full development, the T cells in the cord blood might function as nonspecific functional effector cells. These studies also confirm that the MNCs from human cord blood have the potential to be used in the clinical setting as a source for cellular therapy.

Vujanovic et al. (26) have demonstrated that the LGLs activated in culture with IL-2 became adherent to the plastic surface of the flask. These cells, accounting for >95% LGLs based on morphological criteria, demonstrated potent antitumor activity against tumor cells in vitro. The UCBCs contain a relatively immature immune cell population; these cells may not express appropriate adhesion molecules, such as CD11 and CD18, that would allow them to attach to the flask. Studies have already shown that plastic adherence by LGLs can be markedly reduced with monoclonal antibodies to these cell surface molecules (24). Our results with phenotypic analysis showed that the expression of the adhesion molecule CD54 was significantly less on UCBC lymphocytes compared with PBCs. The precise role of the decreased expression of CD54 on UCBCs is not known. These observations suggest further studies on the role of adhesion molecules in the cytotoxicity of UCBCs in vitro.

More interestingly for this report, UCBCs have demonstrated significantly greater levels of LAK cytotoxicity compared with adult blood or PBCs. Harris et al. (12) found that IL-2-activated UCBCs produced IL-2, IL-4, and granulocyte/macrophage-colony stimulating factor mRNA encoding hematopoietic growth factors, as measured by message and protein levels. IL-2-activated UCBCs may prove useful in the transplantation setting, serving as antitumor effector cells. As in the case of peripheral blood lymphocytes including NK cells (16, 17), the results with UCBCs have shown that both perforin- and FasL-mediated cytolysis of tumor cells are methods used by the in vitro IL-2-activated UCBCs (17, 18). The most important findings of this study were the in vivo antitumor therapeutic effects of UCBCs against both human leukemic and breast cancer cells. Phenotypic analysis of the activated cells used for in vivo studies showed that there were more CD56+ cells in the UCBCs compared with PBCs. It is possible that the increased antitumor activity could be attributable to the increase in CD56-positive NK cells in UCBC populations. These results provide the foundation for further studies to identify the precise phenotype of the antitumor effector cells in UCBCs. We hope that these studies may also open avenues leading to clinical studies to test the antitumor immunotherapeutic effects of UCBCs against cancer.

ACKNOWLEDGMENTS

We thank Natalya Babushkina-Patz for technical assistance. We also thank the Labor and Delivery staff at University of Nebraska Medical Center for assistance; Dr. James Lynch for help in statistical analysis; Michelle Desler, Eileen Yu, and Penni Davis for assistance in preparing the manuscript; and Kristi Berger editorial assistance. We are grateful to Prof. David Crouse for critically evaluating the manuscript for its content and interpretation of the results.

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