Lymphokine-Activated Killer Cells Selectively Kill Tumor Cells in Bone Marrow Without Compromising Bone Marrow Stem Cell Function In Vitro

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Although it has been demonstrated that lymphokine-activated killer (LAK) cells kill tumor cells in a selective way without being toxic to a variety of normal cells, contradictory results exist about the possible toxicity of natural killer (NK) and LAK cells for hematopoietic progenitor cells. Therefore, the cytolytic activity and growth inhibitory effects of LAK cells on normal bone marrow progenitor cells and the ability of LAK cells to eliminate neoplastic hematopoietic cells from populations of bone marrow cells in vitro was studied. The results of these experiments show the following: (1) LAK cells have little cytolytic activity against normal bone marrow cells; (2) normal bone marrow cells fail to cold target compete for the killing of the hematopoietic tumor cell lines K562 and HL60 or freshly frozen acute myelocytic leukemia (AML) blast cells by LAK cells; (3) LAK cells inhibit the growth of K562 and HL60 to more than 90% in clonogenic assays; (4) LAK cells have no inhibitory effect on hematopoietic progenitor growth in CFU-GM (colony-forming unit-granulocytes, macrophages), CFU-E (colony-forming unit-erythrocytes), BFU-E (burst-forming units-erythrocytes), or CFU-GEMM (colony-forming unit-granulocytes, erythrocytes, macrophages, megakaryocytes) assays. These results indicate that LAK cells have low toxicity for normal bone marrow and that LAK activity against tumor cells is not adversely affected by the presence of normal bone marrow cells. The differences in cytology and growth inhibition of neoplastic hematopoietic cells and hematopoietic progenitor cells by LAK cells in vitro could create a therapeutic index that might allow the use of LAK cells for cleansing of the autologous bone marrow graft and for adjuvant therapy in combination with autologous bone marrow transplantation without compromising the reconstitution of the bone marrow in the host.

ADOPTIVE IMMUNOTHERAPY with lymphokine-activated killer (LAK) cells and interleukin-2 (IL-2) represents a new approach in the treatment of cancer. LAK cells are functionally defined as lymphocytes capable, after culturing in IL-2, of lysing natural killer (NK)-resistant fresh tumor cells in vitro and have been proven useful in the control of metastatic tumors in animal models and in some human cancer patients. The majority of the LAK precursors are lymphocytes with large granular lymphocyte (LGL)/NK characteristics on the basis of phenotype (CD3-, CD5-, CD16+, and NKH-1+) and LGL morphology.

Since effective bone marrow cryopreservation techniques have become available, the use of high-dose chemotherapy followed by autologous bone marrow transplantation (ABMT) has been studied in patients with solid tumors or tumors of hematopoietic origin. ABMT would obviate two major limitations of syngeneic and allogeneic grafts, namely that only 25% to 40% of all patients have suitable donors, and the major problem of graft-versus-host disease.

To eliminate the residual malignant cells in the autologous bone marrow graft, several methods of purging are used: antibody-mediated techniques (in combination with comple-ment, magnetic beads or toxins), drugs (such as 4-hydroperoxycyclophosphamide and bleomycin), photosensitization, culture of bone marrow, and combinations of drugs and antibodies.

It has been shown in animal and human studies that most hematopoietic tumors are sensitive to the cytolytic activity by autologous and allogeneic LAK cells in vitro and that proliferating and cytotoxic LAK cells can be generated from most patients with such hematopoietic tumors. Therefore, treatment with LAK cells in combination with ABMT offers a new and interesting possibility for the cleansing of malignant cells from the autologous bone marrow and as adjuvant therapy to treat residual cancer after transplantation in patients with hematopoietic malignancies.

The use of LAK cells for purging of the autologous bone marrow graft depends upon the ability of LAK cells to selectively kill neoplastic cells without harming the ability of the treated graft to reconstitute the recipient.

Conflicting reports exist regarding the role of NK cells in bone marrow graft rejection and the regulation of hematopoiesis. NK cells have been reported to have lytic effects against normal bone marrow cells in vitro and in vivo through direct cell-to-cell contact or through the production of lymphokines, such as interferon and tumor necrosis factor. Because LAK activity is derived primarily from IL-2-activated LGL/NK cells, there is the potential that LAK cells may cause adverse effects on normal hematopoietic progenitor cells. Therefore, limitations to the use of LAK cells in combination with ABMT might be the toxicity of LAK cells for hematopoietic progenitor cells and decreased lytic activity of LAK cells against tumor cells in the presence of normal bone marrow.

To investigate these possible limitations, the following in vitro studies were performed: cytolytic activity of LAK cells against bone marrow cells, cold target competition with unlabeled bone marrow cells for the killing of hematopoietic tumor cells by LAK cells, and the effect of LAK cells on colony growth of hematopoietic progenitor and tumor cells.
This report shows the effectiveness of LAK cells in discriminating between normal bone marrow cells and neoplastic hematopoietic cells in vitro.

MATERIALS AND METHODS

**Tumor cells.** The NK-sensitive erythroleukemia line K562 and the NK-resistant promyelocyte line HL60 were grown in HEPES-buffered RPMI 1640 with 10% fetal calf serum (FCS, Gibco, Grand Island, NY), 10 mmol/L L-glutamine and 0.1% gentamicin. Blood samples of acute myeloid leukemia (AML) patients in blast crisis were collected. After separation by Ficoll-Hypaque, cells were cryopreserved in 20% human AB serum and 10% dimethyl sulfoxide. For use as tumor targets in cytotoxicity assays, the cryopreserved tumor cells were thawed rapidly in a 37°C water bath, resuspended slowly in RPMI medium, and washed twice. Only last preparatations of at least 80% viability were used in cytotoxicity assays.

**Collection and preparation of bone marrow.** Collection of normal human bone marrow from donors for bone marrow transplantation was performed after informed consent, as previously described. Briefly, after aspiration from the posterior iliac crest, cells were collected in Hank’s balanced salt solution (HBSS) with 100 U/mL of preservative-free heparin, and then diluted in RPMI 1640 with 5% FCS. The bone marrow cells were then depleted of monocytes by incubation with carbonyl-iron particles (45 minutes, 37°C) and subsequently centrifuged over Ficoll-Isoapaue (1,000 g, 30 minutes, 20°C). The interphase cells were then collected and incubated with 2-aminoethylisothioauroniumbromide-pretreated sheep RBC and centrifuged over Ficoll-Isoapaue to deplete T lymphocytes. The interphase cells, containing partially purified bone marrow cells, were collected, washed twice, and cryopreserved at a concentration of 10^7 cells/mL in a medium consisting of 70% RPMI, 20% FCS, and 10% dimethylsulfoxide with a computer-controlled freezer (Cryoson, Middenbeemster, The Netherlands). The nonresetting, monocyte-depleted interphase cells contained <2% T lymphocytes, as determined with indirect immunofluorescence microscopy using monoclonal antibody Leu 4 (anti-CD3, Becton Dickinson, Mountain View, CA) and fluorescein isothiocyanate (FITC)-labeled antinmouse immunoglobulin antisera (Nordic Immunological Laboratories, Tilburg, The Netherlands) and <2% mononuclear phagocytes, as determined with an-naphthylbutyrate esterase staining.

Immediately before use, cells were thawed, resuspended in RPMI plus 20% FCS, and washed twice.

**Preparation of LAK cells.** LAK cells were prepared as described by Rosenbaum et al with slight modifications. Lymphocytes from buffy coats of normal blood bank donors were separated by centrifugation over Ficoll-Isoapaue (1,077 g/mL, 1,000 g, 25 minutes, 20°C), washed three times, and cultured for four to five days at a concentration of 0.5 x 10^6 cells/mL in 4 mL cluster wells with HEPES-buffered RPMI 1640 plus 10% heat-inactivated pre-screened pooled human AB serum, 10 mmol/L L-glutamine, 0.1% gentamicin, and 500 u/mL recombinant IL-2 (a gift from Ortho Diagnostic Systems, Raritan, NJ) at 37°C in an atmosphere of 5% CO2 for one hour at 37°C. Previous experiments revealed that 500 U/mL IL-2 induced optimal LAK activity. Unactivated peripheral blood mononuclear cells (PB-MNCs) were obtained and cultured in the same way without IL-2.

**Monoclonal antibodies.** The following monoclonal antibodies used were: OKT 3 (CD3, Ortho), Leu 3A (CD4, Becton-Dickinson), Leu 2A (CD8, Becton-Dickinson), Leu 11B (CD16, Becton-Dickinson), Leu 19 (recognizes a 220 kD antigen present on NK cells and cytotoxic T-cell subsets, Becton-Dickinson), anti-IL-2 receptor (CD25, Becton-Dickinson), anti-HLA-DR (Becton-Dickinson), B1 (CD20, Coulter Immunology, Hialeah, FL), and OKM 1 (CD11, Ortho).

As a second step, reagent FITC-labeled goat antimouse immunoglobulin G (IgG) antibody (Cappel, Turnhout, Belgium) was used.

**Flow cytometry.** For surface marker analysis, 5 x 10^5 lymphocytes (in 50 μL; 1% bovine serum albumen/phosphate-buffered saline [BSA/PBS]) were incubated with 20 μL of the primary antibody (in optimal dilution) for 30 minutes at 4°C. The cells were washed, incubated with 20 μL FITC-labeled goat antimouse IgG for 30 minutes at 4°C, washed again, and resuspended in 1% paraformaldehyde.

Analysis for fluorescence was performed with a fluorescence-activated cell sorter (FACS) analyzer flow cytometer (Becton-Dickinson).

**Cytotoxicity assay.** Cytotoxicity was measured in a standard four-hour 1Cr-release microcytotoxicity assay using 96-well, U-bottomed microtiter plates (Costar, Cambridge, MA). The target cells were labeled with 100 μCi of Na2CrO4 for one hour at 37°C, washed three times, and seeded into 96-well microplates at a concentration of 5 x 10^4 cells/well mixed with a variable number of effector cells to a final volume of 200 μL. Following centrifugation (1,000 g for one minute), the plates were incubated for four hours at 37°C. The supernates were harvested by using the Skatron harvesting system (Skatron, Lier, Norway) and counted in a γ counter (Packard Multi-Prias, Packard IC, Downers Grove, IL) to determine experimental release (ER). Spontaneous release (SR) and maximum release (MR) were determined by incubating targets in medium only or in Zaponin (Coulter Electronics, Luton, Beds, England). The SR never exceeded 20% of the MR. Each assay was set up in duplicate, and cytotoxicity values were calculated by using the following formula:

\[
\text{percent cytotoxicity} = \frac{\text{ER} - \text{SR}}{\text{MR} - \text{SR}} \times 100
\]

In cold target competition studies, unlabeled target cells in different concentrations were mixed with 5 x 10^6 labeled targets before incubation. Percentage inhibition of lysis was calculated as follows:

\[
\% \text{ inhibition of lysis} = \frac{(\% \text{ lysis without cold target}) - (\% \text{ lysis with cold target})}{\% \text{ lysis without cold target}} \times 100
\]

**Cell-mediated inhibition of CFU.** A quantity of 3 or 5 x 10^4 bone marrow cells or 10^5 tumor cells in 0.25 mL RPMI plus 15% human serum was mixed in 45 mL round bottom tubes with an equal volume of this medium containing LAK cells at an E/T ratio of 10:1. After centrifugation (1,000 g, 15 seconds) to allow cell-to-cell contact, the cell mixture was incubated for 18 hours (5% CO2, 37°C), washed once, resuspended in α-modified Eagle’s minimal essential medium (α-MEM, Flow Laboratories, Irvine, Scotland) with 20% FCS and subsequently cultured for CFU-GM (colony-forming unit-granulocytes, macrophages), BFU-E (burst-forming unit-erythrocytes), CFU-GEMM (colony-forming units-granulocytes, erythrocytes, macrophages, megakaryocytes).

**CFU-GM.** A quantity of 5 x 10^4 bone marrow cells was cultured in 1 mL medium containing 20% FCS, 20% leukocyte-conditioned medium MEM or 20% Iscove’s modified Dulbecco’s medium (IMDM) with 50 U rGM-CSF (recombinant granulocyte-monocyte colony stimulating factor, Genetic Institute, Boston), 20% α-MEM, and 40% methylcellulose 2.25% in 35-mm plastic dishes (5% CO2, 37°C). CFU-GM colonies, defined as granulocytic, monocytic, or eosinophilic aggregates of more than 20 cells, were scored on day 10 under an inverted microscope.
CFU-E/BFU-E. A quantity of 3 or 5 × 10⁴ bone marrow cells was cultured in 1 mL of medium containing 20% FCS, 20% leukocyte-conditioned medium, or 20% IMDM with 50 U rGM-CSF, 5% 10⁻³ mol/L 2-mercaptoethanol, 5% IMDM with 1 U erythropoietin (Connaught Step III, Toronto, Canada), 5% deionized bovine serum albumin (Sigma, St Louis), 5% human transferrin, and 40% methylcellulose 2.8% in 35 mm plastic dishes (5% CO₂, 37°C). CFU-E, defined as clusters of eight to 64 hemoglobinized cells, were scored on day 7. The number of BFU-E was scored on day 14.

CFU-GEMM. A quantity of 3 or 5 × 10⁴ bone marrow cells was cultured in 1 mL medium containing 30% ABO-compatible human heparin plasma, 7.5% phytohemagglutinin-leukocyte-conditioned medium or 7.5% IMDM with 25 U IL-3 (Genzyme, Boston), 5% 10⁻¹ mol/L 2-mercaptoethanol, 5% deionized bovine serum albumin, 5% human transferrin, 7.5% IMDM with 1 U of erythropoietin, and 40% methylcellulose 2.8% in 35 mm plastic dishes (5% CO₂, 37°C). CFU-GEMM colonies, defined as colonies containing at least both erythroid and myeloid cells, were scored on days 14 through 18.

Tumor cell colonies. A quantity of 10³ tumor cells (K562 or HL60) was cultured in 1 mL medium containing 30% ABO-compatible human heparin plasma, 7.5% phytohemagglutinin-leukocyte-conditioned medium or 7.5% IMDM with 25 U IL-3 (Genzyme, Boston), 5% 10⁻¹ mol/L 2-mercaptoethanol, 5% deionized bovine serum albumin, 5% human transferrin, 7.5% IMDM with 1 U of erythropoietin, and 40% methylcellulose 2.8% in 35 mm plastic dishes (5% CO₂, 37°C). Tumor cell colonies, containing at least 20 cells were counted on day 7.

Normal values and calculations for cell-mediated inhibition of CFU. One hundred percent growth of hematopoietic progenitor cells was defined as the number of colonies cultured from 1 × 10⁷ untreated enriched bone marrow cells. Normal values for colony growth per 1 × 10⁴ cells plated (mean ± SE): 27 ± 1 for CFU-GM, 23 ± 3 for CFU-E, 25 ± 2 for BFU-E, and 1.6 ± 0.3 for CFU-GEMM. Growth of tumor colonies (mean ± SE) from 10⁴ cells in untreated control cultures were 588 ± 95 for K562 and 818 ± 77 for HL60. In the experiments, the percentage of surviving progenitor cells or tumor cells were calculated by dividing the total number of colonies by the number of colonies in the untreated control cultures after correction for the number of plated cells.

Statistics. Statistical analysis was performed using Student’s t test.

RESULTS

Proliferation and surface marker analysis of LAK cells. Cell recovery after four days of culture was 80 ± 33% for unactivated PB-MNCs and 104 ± 42% for LAK cells. Comparison of surface markers (data not shown) showed no difference in expression of T cell, NK cell, B cell or monocyte markers between unactivated PB-MNCs and LAK cells, except for the expression of the IL-2 receptor (CD25) and HLA-DR, which are both associated with lymphocyte activation. CD25 was expressed in 4 ± 1% of the unactivated PB-MNCs and 25 ± 7% of the LAK cells (P < .001). HLA-DR was expressed in 13 ± 4% and 29 ± 8% (P < .002), respectively.

Cytotoxicity of unactivated PB-MNCs and LAK cells against NK-sensitive and NK-resistant tumor cells and normal bone marrow cells. Cytolytic activity of unactivated PB-MNCs and LAK cells in a four-hour ⁵¹Cr-release assay against the NK-sensitive tumor cell line K562, the NK-resistant tumor cell line HL60, and normal bone marrow cells is shown in Fig 1. Unactivated PB-MNCs displayed some NK activity, as demonstrated by killing of K562, but the cytolytic activity against K562 by LAK cells was considerably higher. LAK cells also exhibited high levels of lysis against HL60, in contrast to the unactivated PB-MNCs. LAK cells and unactivated PB-MNCs did not differ in their cytolytic activity against bone marrow cells, which was very low. The data in Fig 1B clearly show substantial differences in susceptibility to LAK cell lysis between the tumor cell lines K562 and HL60 and the normal bone marrow cells.

Cold target competition with tumor cell lines K562 and HL60 and bone marrow cells. To determine the influence of normal bone marrow cells on the cytolytic activity of LAK cells against tumor cells, cold target competition experiments with K562, HL60, and normal bone marrow cells were performed. As shown in Fig 2, normal bone marrow cells could not cold target compete against K562 or HL60 at cold/hot target ratios of 10:1 or 1:1. At higher ratios (25 to 100:1) normal bone marrow cells were able to cold target competition up to a mean of 22%. In one experiment (data not shown), normal allogeneic PB-MNCs and erythrocytes were used as cold targets v labeled HL60. At a 100:1 cold/hot ratio, an inhibition of 20% with unlabeled erythrocytes and of 33% with unlabeled PB-MNCs was found.
Cold target competition with fresh AML blast cells and bone marrow cells. Since fresh tumor targets are in general less sensitive to LAK lysis than cultured tumor cells, cold target competition experiments were repeated with freshly frozen AML blast cells (Fig 3). Cold target competition with normal bone marrow cells even at a cold/hot ratio of 100:1 did not have any significant effect on the LAK lysis of AML 1 blast cells. In the case of AML 2, normal bone marrow cells could cold target compete weakly up to a mean of 21%.

Growth of colony-forming cells after incubation with LAK cells. Because some cytolytic activity of LAK cells against normal bone marrow was found and bone marrow cells had shown to be capable of cold target inhibition to a low extent with tumor cells, the direct effect of LAK cells on the growth of different hematopoietic progenitors in vitro was studied. Normal bone marrow cells were incubated for 18 hours with LAK cells at an E/T ratio of 10:1 and subsequently cultured for CFU-GM, CFU-E, BFU-E, and CFU-GEMM. Table 1 shows that the growth of CFU-GM, CFU-E, BFU-E, and CFU-GEMM from four normal bone marrow donors was not inhibited by day 5 LAK cells from four different normal donors (A-D). The growth of CFU-E, BFU-E, and CFU-GEMM seemed even to be slightly increased after incubation with LAK cells as compared to untreated controls.

To rule out any influence of the leukocyte-conditioned medium on the cell-mediated inhibition of CFU by LAK cells, additional experiments with LAK cells from donors E and F were carried out in the presence of recombinant hematopoietic growth factors instead of leukocyte-conditioned medium. No differences were observed comparing the two CFU culture protocols.

In contrast, growth of colony-forming cells of K562 and HL60 was almost completely inhibited after incubation with LAK cells. Culturing of LAK cells only in any of the colony-forming assays did not lead to colony growth.

DISCUSSION

In this study, the feasibility of exposing normal bone marrow cells to the cytolytic activity of LAK cells and the effectiveness of LAK cells to eliminate malignant cells from normal bone marrow was evaluated in vitro.

Considerable controversy exists about the role of NK cells in the regulation of hematopoiesis. It has been reported that NK cells inhibit,^{16,20,22,26} do not effect,^{21,32,33} or even stimulate^{32,33} the growth of various hematopoietic stem cells. Even differences in susceptibility to inhibitory effects by resting and IL-2-activated NK cells between bone marrow- and peripheral blood-derived hematopoietic progenitor cells have been described.^{14} Much of the controversy in these various reports may be related to the definition of the effector cells used, the purity of the NK population, phenotypic and functional heterogeneity of NK cells,^{34,36} differences in the activation state of NK cells,^{32,34,37} differences in preparation of bone marrow cells, or other differences in techniques. Nevertheless, because LAK activity is mainly generated from IL-2-activated LGL/NK cells, it was first investigated whether LAK cells could express detectable toxicity for normal bone marrow cells.

Only little cytolytic activity of LAK cells against normal bone marrow was found, which is in agreement with previous studies in rats^{18} and in humans.^{39} The mean lysis was below 10% and did not differ from the lysis of bone marrow cells by unactivated PB-MNCs. Unlabeled bone marrow cells weakly competed with labeled cultured tumor cells but only at high ratios (25 to 100:1). However, a comparable level of cold target inhibition could be demonstrated with unlabeled erythrocytes or allogeneic PB-MNCs. This suggests that the cold target inhibition found at high cold/hot ratios might be caused nonspecifically by the high number of unlabeled target cells instead of by a specific interaction between the unlabeled target cells and the LAK effectors. The experiments with freshly frozen AML cells confirmed the results with cultured tumor cell lines. In the case of AML 1, unlabeled bone marrow cells did not interfere with LAK lysis even at a cold/hot ratio of 100:1. The lysis of AML 2 blast cells by LAK cells was only inhibited up to a mean of 21%.
Fig 3. Cold target competition in four-hour $^{51}$Cr-release assays with as effectors day 5 LAK cells at an E/T ratio of 50:1 and as targets freshly frozen AML 1 (A) and AML 2 (B) blast cells and normal bone marrow cells. This figure shows mean percentages (and SE) of lysis of AML blast cells by LAK cells (from four different normal donors) in the presence of unlabeled bone marrow cells (□) or unlabeled AML cells (■) at different cold/hot target ratios.

Nevertheless, the low-level lysis of bone marrow and the ability of bone marrow cells to cold target compete at high cold/hot ratios could also be explained by the presence in the bone marrow of a rare cell type, which is susceptible to lysis by LAK cells.

To critically analyze the possible minimal effects that LAK cells may have on bone marrow, it was studied whether LAK cells were capable of inhibiting the growth of different hematopoietic progenitor cells in colony-forming assays. After an 18-hour preincubation with LAK cells at an E/T ratio of 10:1, normal human bone marrow was cultured for CFU-GM, CFU-E, BFU-E, and CFU-GEMM. Previously, it was demonstrated that human hematopoietic progenitor cells are sensitive to cytotoxic T-cell-mediated lysis and growth inhibition in colony-forming assays, provided they express the relevant antigenic determinant, as was shown for HLA-antigens and for minor transplantation antigens such as H-Y and other minor transplantation antigens.

No inhibition of any colony-forming assay by LAK treatment of bone marrow was found regardless if leukocyte-conditioned medium or recombinant hematopoietic growth factors were used. However, the growth of tumor cells was almost completely inhibited by LAK treatment. In accordance with these data, no inhibition of any colony-forming assay for human hematopoietic progenitors by non–major histocompatibility complex (MHC)-restricted lysis using CD3$^+$ NK clones or CD3$^+$ γ-δ T-cell clones was found. These data are further supported by recent findings in rats, which showed that bone marrow grafts pretreated with LAK cells retained the ability to reconstitute lethally conditioned recipients. LAK cells were even capable of efficiently purging up to 10⁷ leukemia cells from the bone marrow. In contrast to these findings, Fujimori et al. reported suppression of colony formation from CFU-E, CFU-GM, and CFU-Mix by LAK cells. These conflicting data might be explained by differences in techniques for colony-forming assays and preparation of LAK cells. Also, different effects of various lymphokines produced by LAK cells could play a role. NK cells are known to produce various cytokines, such as interferons, interleukins, and cytotoxic factors (ie, tumor necrosis

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<th>LAK Cells</th>
<th>Normal Hematopoietic Progenitor Cells</th>
<th>Tumor Cells</th>
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<tr>
<td></td>
<td>CFU-GM</td>
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<td>Donor A</td>
<td>99 ± 10*</td>
<td>109 ± 30</td>
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<td>Donor B</td>
<td>90 ± 8</td>
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<td>Donor F</td>
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In all experiments with LAK cells from donors, A through D, leukocyte-conditioned medium was used in the CFU/BFU assays. In the experiments with LAK cells from donors E and F, the leukocyte-conditioned medium in CFU/BFU assays was replaced by different recombinant hematopoietic growth factors. See Materials and Methods section for procedure.

Abbreviation: NT, not tested.

*Values are mean percentages ± SE of four experiments, as compared to mean growth of untreated controls.
†Values are mean percentages ± SE of three experiments, as compared to mean growth of untreated controls.
‡Values are mean percentages of two experiments, as compared to mean growth of untreated controls.
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factor,45-47 which can have variable effects on hematopoietic stem cell growth in CFU assays.22,24,48,49

In conclusion, even if LAK cells do have a certain cytolytic activity against normal bone marrow cells, the in vitro data presented in this report indicate that the difference in susceptibility of bone marrow cells and malignant hematopoietic cells to lysis and growth inhibition by LAK cells seems to constitute a therapeutic index, which might allow the use of LAK cells for bone marrow cleansing and adjuvant therapy in combination with ABMT.

ACKNOWLEDGMENT

The authors would like to thank Drs Dick van der Harst for his assistance in some of the experiments, Catherine Fekete for her expertise in typing this manuscript, and Dr John C. Hiserodt for helpful discussions.

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