

# Taurine Attenuates Recombinant Interleukin-2–Activated, Lymphocyte-Mediated Endothelial Cell Injury

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**BACKGROUND.** Recombinant interleukin-2 (rIL-2) immunotherapy is limited by microvascular endothelial cell (EC)-targeted injury. The interaction between rIL-2-activated lymphoid cells and EC is a possible mechanism of this systemic toxicity. Taurine, a  $\beta$ -amino acid, is known to have several physiologic actions, including the modulation of calcium homeostasis. The aims of this study were to analyze the effects of taurine on rIL-2-activated, lymphocyte-mediated EC and tumor cell cytotoxicity and to investigate the mechanisms of its action.

**METHODS.** IL-2-activated cytotoxicity, mediated by peripheral blood mononuclear cells, against susceptible tumor cell lines and against EC (fresh EC and an EC cell line) in the presence of taurine was assessed. The effects of taurine on lymphocyte  $[Ca^{2+}]_i$  were assessed by flow cytometry, and the effects of taurine on granzyme activity were assessed by spectrophotometry.

**RESULTS.** The authors' findings indicated that the addition of taurine significantly reduced rIL-2-activated EC cytotoxicity mediated by natural killer cells, without reducing antitumor response. Taurine was also shown to reduce significantly EC lysis mediated by lymphokine-activated killer (LAK) cells, while also significantly increasing tumor cytotoxicity. The authors demonstrated the importance of calcium in the role played by taurine in lymphocyte-mediated cytotoxicity and found that LAK  $[Ca^{2+}]_i$  following conjugation to EC was enhanced by taurine. They also found that taurine enhanced  $Ca^{2+}$ -dependent granzyme exocytosis from LAK cells.

**CONCLUSIONS.** These findings indicate that taurine may play a dual role in rIL-2 immunotherapy, due to its ability to reduce the vascular injury associated with this therapy while enhancing its antineoplastic activity. *Cancer* 1998;82:186–99. © 1998 American Cancer Society.

**KEYWORDS:** taurine, interleukin-2, immunotherapy, endothelial cells, natural killer cells, lymphokine-activated killer cells, cytokines, cytotoxicity.

Interleukin-2 is a potent antineoplastic agent that has shown promise in the treatment of patients with advanced cancer who have failed to respond to conventional therapy. Marked tumor regression has been observed in murine models and in some human cancers after administration of lymphokine-activated killer (LAK) lymphocytes and recombinant interleukin (rIL)-2.<sup>1</sup> Adoptive immunotherapy with tumor-infiltrating lymphocytes (TILs) has produced response rates of up to 55% in the treatment of metastatic malignant melanoma.<sup>2</sup> The antitumor function of rIL-2 is due to its ability to stimulate profound responses in lymphoid cells, specifically in B and T lymphocytes and large granular lymphocytes.

However, the therapeutic value of rIL-2 is limited by the development of an associated "vascular leak syndrome" (VLS),<sup>1,3</sup> in which

the normal barrier function of the endothelium is deranged, with resultant transmigration of fluid, neutrophils, and proteins into surrounding tissue. The clinical manifestations of this loss of endothelial permeability include hypotensive reactions, multiorgan system dysfunction, and generalized fluid extravasation, with the result that rIL-2 administration must be withheld or otherwise limited. To date, strategies addressing this complication involve either reduction of dose schedule or administration of agents that suppress the activity of secondary cytokines, such as TNF and IL-1, with a resultant reduction in associated side effects.<sup>4</sup> The underlying molecular mechanisms of the VLS are not fully understood, and current treatment modifications to reduce this side effect often compromise the therapeutic efficacy of rIL-2.

The precise mechanisms of rIL-2-induced endothelial cell (EC) damage have not yet been fully elucidated. It has been well documented that the VLS is a consequence of EC damage<sup>5-7</sup> and is not mediated by the direct action of rIL-2<sup>8</sup> or by the generation of cytokines<sup>9</sup> or toxic radical species.<sup>5,10</sup> It has also been suggested that the VLS may be a result of direct EC injury mediated by activated natural killer (NK) cells, eosinophils, or other lymphocytes. rIL-2-induced vascular leak has been shown to be less severe in nude mice that have undergone whole body irradiation.<sup>11,12</sup> LAK cells have been shown to adhere avidly to and effectively lyse cultured EC,<sup>13</sup> and in vivo studies have demonstrated direct histologic evidence<sup>7</sup> of LAK cell-induced endothelial damage. It has been demonstrated that LAK cells of the CD16<sup>+</sup>(NK) cell phenotype mainly lyse autologous EC after specific recognition of a certain molecule present on these targets.<sup>14</sup> Normal, circulating NK cells recognize EC as "self" and thus do not regard them as targets for lysis.<sup>5,15</sup> However, subsequent to contact with rIL-2, these cells undergo alterations in function, resulting in their destruction of EC.

Taurine is the most abundant free amino acid in lymphocytes (44%).<sup>16</sup> Although to date little is known about its effects on the immune system, taurine has been shown to stimulate T and B lymphocytes in response to mitogens and to increase cytoplasmic Ca<sup>2+</sup> concentrations in these cells.<sup>17</sup> Because regulation of intracellular calcium levels [Ca<sup>2+</sup>]<sub>i</sub> is intimately related to lymphocyte activation<sup>18,19</sup> and cytolysis,<sup>5</sup> and because lymphocytes are fundamental to the process of rIL-2 immunotherapy, we hypothesized that taurine would play a valuable role in enhancing the efficacy of this therapy.

The current study was designed to evaluate the hypothesis that taurine may play a beneficial role in IL-2 immunotherapy by regulating NK and LAK cell

cytosolic Ca<sup>2+</sup> levels, and, if this proved to be correct, to evaluate the mechanism(s) by which taurine modulates lymphocyte function.

## MATERIALS AND METHODS

### Reagents

Cells were maintained in culture medium (CM; Dulbecco-modified Eagle medium, RPMI 1640, or Medium 199 supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate) at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere. Taurine, taurine analogues (N-[2 Hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid], or HEPES; 2-[N-Cyclohexylamino] ethanesulfonic acid, or CHES), superoxide dismutase (SOD), verapamil hydrochloride, and BAPTA (1,2-bis(2 aminophenoxy) ethane N,N,N',N'-tetracetic acid) were purchased from Sigma Chemical Co. (St. Louis, MO). Human recombinant rIL-2 (Proleukin, Eurocetus B.V., Amsterdam, the Netherlands) was kindly donated by Chiron U.K. Ltd.

### Separation of Peripheral Blood Mononuclear Cells

Venous blood was collected from random normal human donors at our institution and anticoagulated with heparin. Peripheral blood mononuclear cells (PBMCs) were isolated by Histopaque (Sigma Diagnostics, St. Louis, MO) density gradient centrifugation and depleted of adherent mononuclear cells by plastic adherence for 1 hour in CM in 25 cm<sup>2</sup> flasks (Nunclon, Roskilde, Denmark). Nonadherent PBMCs were recovered, washed, and resuspended in CM.

### Generation of rIL-2-Activated Cells

To activate precursors of cytolytic cells, PBMCs were suspended at a concentration of 1 × 10<sup>6</sup> cells/mL in CM with or without human recombinant rIL-2 (1000 units/mL). To prevent lymphocyte adherence, cells were cultured for 18 hours or for 72 hours in 17 × 10 mm round-bottomed culture tubes (No. 2059; Falcon, Becton Dickinson Labware, Lincoln Park, NJ) at 37 °C in humidified 5% CO<sub>2</sub>. After incubation, cells were washed, adjusted to a concentration of 2.5 × 10<sup>6</sup> cells/mL in CM containing rIL-2, and analyzed for cytolytic activity against target cells. For experiments examining the effects of taurine on lymphocyte activity, varying concentrations of taurine (0.1–1.0 mg/mL) were added to the lymphocyte suspension at the same time as IL-2.

### Cultured Cells

Human umbilical vein endothelial cells (HUVECs) were isolated by collagenase treatment of the umbilical vein and cultured on a 2% gelatin-coated culture

flask (Falcon, Lincoln Park, NJ) in complete Medium 199 supplemented with 20% FCS, penicillin (100 U/mL), streptomycin sulfate (100  $\mu\text{g}/\text{mL}$ ), fungizone (0.25  $\mu\text{g}/\text{mL}$ ), heparin (16 U/mL), EC growth supplement (75  $\mu\text{g}/\text{mL}$ ), and 2 mM glutamine, as previously described.<sup>20,21</sup> Cells were grown at 37°C in a humidified 5% CO<sub>2</sub> condition and subcultured by trypsinization with 0.05% trypsin-0.02% ethylene diamine tetraacetic acid when confluent monolayers were reached. Endothelial cells were identified by typical phase contrast "cobblestone" morphology and by the presence of von Willebrand factor antigen using immunofluorescence technique. In all experiments reported herein, HUVECs were used as individual isolates between passages 3 and 5.

The human EC line ECV-304, an established cultured cell line derived from human umbilical vein, and the K562 erythromyeloid leukemia cell line were obtained from the American Type Culture Collection (ATCC, Bethesda, MD). The ECV-304 cell line has been previously characterized and compared with HUVEC in relation to intercellular adhesion molecule-1 (ICAM-1) expression and cellular injury induced by hypoxic stress<sup>22</sup> and employed to mimic HUVEC in several studies.<sup>23</sup> The Daudi Burkitt's lymphoma-derived cell line was obtained from the European Collection of Animal Cell Cultures (ECACC, Wilts, UK).

#### Chromium-51 Labeling of Cells

After washing with CM, target cells were resuspended in CM at  $5 \times 10^6$  cells/0.2 mL, to which 250  $\mu\text{Ci}$  Na<sub>2</sub><sup>51</sup>CrO<sub>4</sub> (Dupont De Nemours, Wilmington, DE) was added and incubated for 90 minutes at 37 °C with agitation. Cells were washed in Hanks' balanced salt solution and incubated at 37 °C for 30 minutes with agitation to remove unbound radioactivity. Labeled cells were then washed three times in CM to remove soluble label and dead cells. These labeled cells were used as targets in the cytotoxicity assay or in the EC adherence assay.

#### Assay for NK/LAK Activity and EC Cytotoxicity

A standard 4-hour <sup>51</sup>Cr release assay was used for the assessment of NK/LAK activity as described previously,<sup>24</sup> using the NK-sensitive K562 cell line and the LAK-sensitive cell line Daudi as the target cells, respectively. To determine EC cytotoxicity, confluent <sup>51</sup>Cr-labeled ECV-304 or HUVEC were cultured with effector cells for a 12-hour period, which was found to be necessary for EC lysis.<sup>5,25</sup>

The cytotoxicity assay was performed in triplicate in 96-well, flat-bottomed microtitre plates (Nunclon) by adding effector cells to  $5 \times 10^3$  target cells at effector-to-target (E:T) ratios ranging from 5:1 to 50:1 in

a final volume of 200  $\mu\text{L}$  of CM. An E:T ratio of 20:1 was found to be optimal for EC lysis by both NK and LAK effectors. The culture plate was centrifuged (100  $\times$  g, 3 minutes) and incubated for up to 12 hours at 37 °C. The plates were then centrifuged and supernatants from each well were removed and counted. The specific <sup>51</sup>Cr release was calculated as described previously.<sup>13</sup>

To determine calcium involvement in cytotoxicity, the Ca<sup>2+</sup> channel blocker verapamil hydrochloride and the Ca<sup>2+</sup> chelator BAPTA were added to the assay wells at the same time as the effector cells were being added to the target cells, and SOD was added to investigate the antioxidant role in lymphocyte cytotoxicity.

#### Lytic Units

Cytotoxic activity was expressed as lytic units (LU) per 10<sup>6</sup> effector cells as previously described,<sup>13</sup> and is defined as that entity capable of causing specific lysis of one target cell over a specified incubation period.

#### Monolayer Adhesion Assay

Confluent EC monolayers were established in 96-well, flat-bottomed microtitre plates and 100  $\mu\text{L}$  <sup>51</sup>Cr-labeled lymphocytes ( $1 \times 10^6$  cells/mL) were added to each well at E:T of 20:1. Cells were then incubated at 37 °C for 1 hour, after which the supernatant was removed from each well and placed in a sampling tube. Sodium dodecyl sulfate detergent was added to each well and the plate was incubated for 16 hours at 37 °C. The resulting lysed cell solution was removed to a sampling tube and counted. Percent adherence was determined using the following equation:

$$\% \text{ Adherence} = \frac{\text{Lysed cell solution (cpm)}}{\text{Lysed cell solution (cpm)} + \text{Supernatant (cpm)}} \times 100$$

For experiments examining the effects of taurine on lymphocyte adherence, a range of taurine concentrations (0.25–1.00 mg/mL) were added to the lymphocyte suspension at the same time as IL-2.

#### Determination of Intracellular Calcium Changes

The effect of taurine on [Ca<sup>2+</sup>]<sub>i</sub> in rIL-2-activated NK and LAK cells was determined using a flow cytometric assay with the calcium-sensitive fluorescent dye Fluo-3 (Molecular Probes, Eugene, OR), as previously described.<sup>26</sup> Briefly, Fluo-3 (FL1) was used to identify effector cells, and target cell populations were distinguished from the smaller effector cells by forward scatter. Lymphocyte [Ca<sup>2+</sup>]<sub>i</sub> was assessed after conjugate formation with either ECs or the Daudi cell line. Effectors and targets were mixed at a ratio of 1:1, centri-

fused to aid conjugate formation, and analyzed for 10 minutes after initiation of conjugation, at 2-minute intervals.  $[Ca^{2+}]_i$  was determined by measuring the ratio of median channel fluorescence (FLI) for conjugated to unconjugated lymphocytes. Controls consisted of cells that were combined (but not centrifuged, thus not allowing conjugate formation, i.e., unconjugated) and analyzed just before and after the 10-minute test period (one each for T = 0 and T = 10 minutes).

#### Determination of N-benzyloxycarboxy-L-lysine thiobenzyl (BLT) Esterase Exocytosis

A colorimetric assay described previously<sup>27</sup> was used to examine the effect of taurine on the exocytosis of BLT esterase, a granzyme present in lymphocyte cytotoxic granules, from IL-2-activated lymphocytes. LAK cells were first incubated with calcium antagonists TMB-8 and ethyleneglycoltetraacetic acid (EGTA) to establish whether there was a role played by  $Ca^{2+}$  in the exocytotic process, and were subsequently stimulated with A23187 and phorbol myristate acetate (PMA), which have been shown to potentiate LAK cell activity.<sup>28</sup> BLT esterase release from lymphocytes was assessed by spectrophotometry at  $\lambda = 412$  nm.

#### Assessment by Flow Cytometry of Percentages of Lymphocyte Subsets

The effects of taurine on levels of lymphocyte subsets within the heterogeneous LAK cell population was examined and compared with control LAK cells cultured in CM containing IL-2 only. Monoclonal antibodies CD16/56 (NK cell), CD3 (T cell), and CD19 (B cell) (Becton Dickinson, San Jose, CA) were used to assess the relative percentage of each lymphocyte subset present in the total population and receptor expression was analyzed by FACScan (Becton Dickinson). CD69 and CD25 (IL-2 receptor) (DAKO, Carpinteria, CA) levels were also assessed as indicators of lymphocyte activation levels.

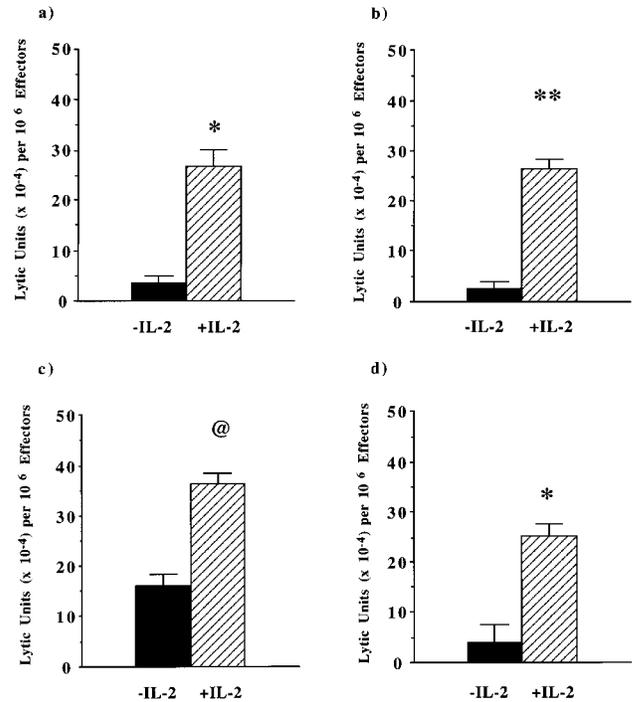
#### Statistical Analysis

The data are presented as mean  $\pm$  standard error of mean. Significance was computed using the DataDesk computer software program on a Macintosh LC 475 and determined by analysis of variance with Scheffe post hoc correction. *P* values of  $\leq 0.05$  were regarded as statistically significant.

## RESULTS

#### Cytotoxicity of rIL-2-Activated Cells

The ability of rIL-2-activated lymphocytes to lyse tumor cell lines and EC targets was first examined using the standard <sup>51</sup>Cr release cytotoxicity assay. PBMCs



**FIGURE 1.** rIL-2-activated, lymphocyte-mediated EC cytotoxicity (a and b) and tumor cytotoxicity (c and d) are shown. PBMCs were cultured in control medium or in medium containing rIL-2 (1000 units/mL) for 18 hours (a) or 72 hours (b), then added to ECV-304 or cultured for 18 hours (c) or 72 hours (d), and finally added to tumor target cells (K562 or Daudi, respectively). Specific <sup>51</sup>Cr release was measured after 12 hours (a and b) or 4 hours (c and d). Results represent the mean  $\pm$  standard error of mean of 6 experiments performed in triplicate. \**P* < 0.00005 vs. control; \*\**P* < 0.000001 vs. control; @ *P* < 0.005 vs. control.

were cultured for 18 hours or for 72 hours in CM with rIL-2 (1000 units/mL). It has been established that these incubation periods with IL-2 are required for the generation of activated NK cells<sup>29,30</sup> and of LAK cells,<sup>13,31</sup> respectively.

That NK cells were activated after incubation with IL-2 (18 hours) is confirmed in Figure 1c, in which these cells (+IL-2) mediated significantly increased lysis of the NK "sensitive" K562 tumor cell line after a standard 4-hour coculture compared with cells cultured in IL-2 free CM (-IL-2). Similarly, the formation of cells bearing LAK activity after a longer incubation with IL-2 (72 hours) is confirmed in Figure 1d, in which these effectors (+IL-2) mediated significantly increased lysis of the LAK "sensitive" Daudi tumor cell line after 4-hour coculture compared with cells cultured in IL-2 free CM (-IL-2). The greater lysis of tumor cells by NK cells in the absence of -IL-2, observed in Figure 1c, as compared with LAK cells (-IL-2) in Figure 1d, is probably due to the fact that the K562

tumor cell line is NK sensitive even without IL-2 activation, whereas the Daudi cell is exclusively LAK sensitive and requires prior activation with IL-2.

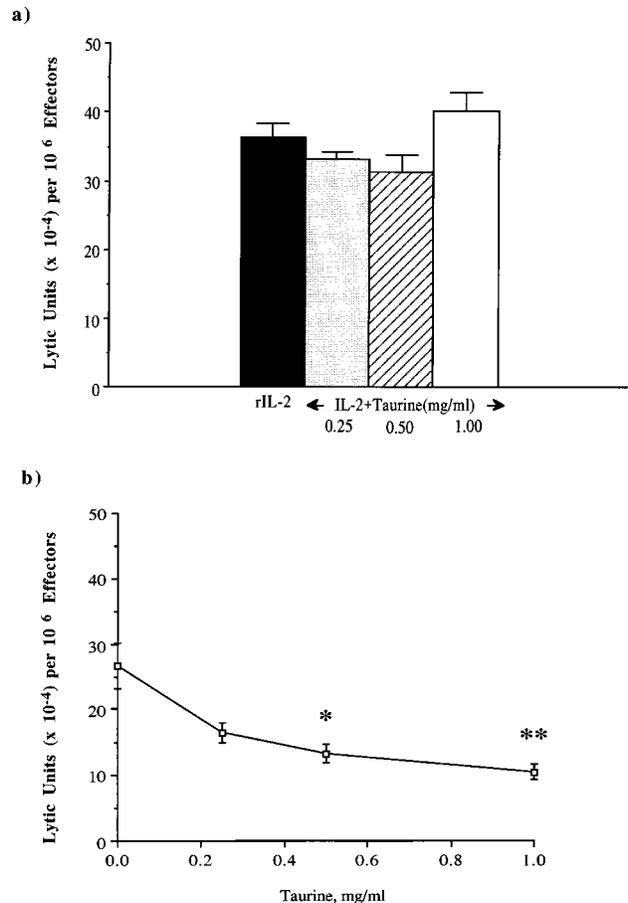
IL-2-activated NK and LAK effectors were added to EC cell line targets (ECV-304) at an E:T ratio of 20:1, which was established as the optimal ratio for target lysis by these effectors. Lysis was observed after 12 hours of coculture. In contrast, PBMC cultured in CM only (-IL-2) and then added to EC caused negligible lysis after 12 hours (Figs. 1a and b). This finding indicated that IL-2 stimulated the lymphocyte to become cytotoxic towards a cell line that it had previously tolerated, and it was this finding that led us to regard the IL-2-activated NK cell/LAK cell as a possible mediator of the vascular injury associated with IL-2 therapy.

To evaluate the direct effect of rIL-2 on EC and the tumor cell lines used, the cytokine was added to EC and K562/Daudi cultures at concentrations ranging from 100 to 10,000 IU/mL. No significant toxicity was observed against either the EC line, as has been previously reported,<sup>5,8</sup> or against the tumor targets (data not shown), even at the highest concentrations of IL-2 examined.

#### Taurine Protects EC Targets from Lysis by NK Cells

The effect of different taurine concentrations on NK cell-mediated lysis of both EC and tumor cell targets was assessed. In mammals, taurine is present in abundant quantities, and it is in the high  $\mu\text{M}$  range in human plasma and cells of the immune system. The concentrations of taurine used throughout this study were in the pharmacologic range and were not found to be directly toxic to the cells studied, even at the highest concentrations examined. PBMCs were pretreated for 18 hours (activated NK cells) with rIL-2 (1000 units/mL)  $\pm$  taurine (0.25–1.00 mg/mL), and the sensitivity of tumor targets and EC targets to lysis mediated by these cells was tested (Figs. 2a and b, respectively). Taurine and rIL-2 were not removed from the culture medium when effector cells were added to target cells.

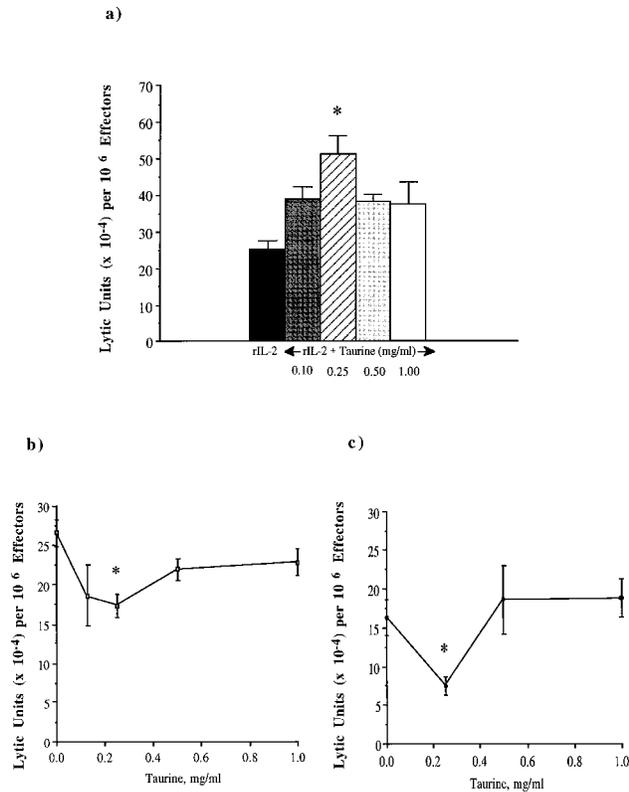
Incubation of activated NK cells with taurine did not lead to a significant alteration in the lysis of K562 tumor cells (Fig. 2a). A reduction in EC sensitivity to activated NK cell-mediated cytotoxicity by combination with taurine treatment was observed; significant reductions were observed at taurine concentrations of 0.5 mg/mL and 1 mg/mL ( $P < 0.05$  and  $P < 0.005$ , respectively) (Fig. 2b). The further enhancement of effector cell function by raising concentrations of taurine to levels exceeding 1 mg/mL was not assessed, as this would bring concentrations into the superpharmacologic range, which would be associated with solubility problems and would not be clinically viable.



**FIGURE 2.** rIL-2-activated, NK cell-mediated (a) tumor cytotoxicity and (b) EC lysis are shown. PBMCs were cultured with rIL-2 (1000 units/mL) alone or in combination with taurine (0.25–1.00 mg/mL) for 18 hours and added to (a) tumor cells for 4 hours of coculture or (b) ECV-304 for 12 hours of coculture. Specific  $^{51}\text{Cr}$  release was measured after (a) 4 hours and (b) 12 hours. Results represent the mean  $\pm$  standard error of mean of 6 experiments performed in triplicate. \* $P < 0.05$  vs. rIL-2; \*\* $P < 0.005$  vs. rIL-2.

#### The Effects of Taurine on LAK Cell-Mediated Tumor Cell and EC (Fresh and EC Cell Line) Lysis

In a parallel study with LAK cells, the effects of different taurine concentrations on LAK cell-mediated EC lysis was assessed using both an EC cell line (ECV-304) and fresh HUVEC, whereas the effects of taurine on tumor cytotoxicity was examined using the Daudi cell line. PBMCs were cultured for 72 hours (LAK cells) with rIL-2 (1000 units/mL)  $\pm$  taurine (0.1–1.0 mg/mL). The sensitivity of Daudi cells to lysis by these effector cells was found to be significantly ( $P < 0.05$ ) enhanced when they were cultured with taurine at a concentration of 0.25 mg/mL (Fig. 3a). This same concentration was associated with significant ( $P < 0.05$ ) EC protection for both the endothelial cell line ECV-304 and for



**FIGURE 3.** Lymphokine-activated killer cell-mediated tumor cytotoxicity and endothelial cell lysis are shown. Peripheral blood mononuclear cells were cultured with recombinant interleukin-2 (rIL-2) (1000 units/mL) alone or in combination with taurine (0.1–1.0 mg/mL) for 72 hours and added to (a) tumor cells, (b) ECV-304, or (c) human umbilical vein endothelial cells. Specific <sup>51</sup>Cr release was measured after (a) 4 hours or (b and c) 12 hours. Results represent the mean ± standard error of mean of 6 experiments performed in triplicate. \**P* < 0.05 vs. IL-2.

HUVEC (Figs. 3b and 3c, respectively). Lower levels of taurine were assessed in these experiments with LAK cells, as it was at these concentrations that alterations in effector cell function were observed, whereas higher concentrations were found to be more effective at mediating alterations in NK cell function.

The dose-response curve of LAK-mediated EC lysis by taurine is unusual in that the lower doses of taurine are more effective at inhibiting target lysis than the higher doses. This observation that taurine is more effective at lower doses is similar to a finding in an animal study by Banks et al.,<sup>32</sup> in which it was observed that protection against ozone-induced alveolar pneumocyte damage was less obvious at pharmacologic concentrations of taurine (250–500 μM) compared with its efficacy at a lower physiologic concentration (100 μM). The lowest doses of taurine used in this in vitro assay were still within the pharmacologic range.

**TABLE 1**  
rIL-2-Activated, NK Cell-Mediated Tumor and EC Cytotoxicity with Taurine Analogues HEPES and CHES

Treatment	rIL-2-activated NK cells with the following lytic units (10 <sup>-4</sup> ) per 10 <sup>6</sup> effectors <sup>a</sup>	
	vs. ECV-304	vs. K562
Control	26.7 ± 3.32	36.4 ± 2.09
Taurine 0.25 mg/mL	16.5 ± 1.52	33.2 ± 1.20
Taurine 0.50 mg/mL	13.3 ± 1.32 <sup>b</sup>	31.4 ± 2.50
Taurine 1.00 mg/mL	10.4 ± 1.26 <sup>c</sup>	40.2 ± 2.76
HEPES 10 mM	14.7 ± 7.60 <sup>c</sup>	39.0 ± 7.70
CHES 10 mM	21.0 ± 5.50	37.0 ± 5.30

rIL-2: recombinant interleukin-2; NK: natural killer; EC: endothelial cells.

<sup>a</sup> PBMCs were cultured with rIL-2 (1000 units/mL) alone or in combination with taurine (0.25–1.00 mg/mL) or its analogues, HEPES and CHES (10 mM) for 18 hours and added to tumor cells. Specific <sup>51</sup>Cr release was measured after 12 hours (ECV-304) or 4 hours (K562) coculture. Results represent the mean ± standard error of mean of 6 experiments, performed in triplicate.

<sup>b</sup> *P* < 0.05 vs. control.

<sup>c</sup> *P* < 0.005 vs. control.

**TABLE 2**  
rIL-2-Activated, LAK Cell-Mediated Tumor and EC Cytotoxicity with Taurine Analogues HEPES and CHES

Treatment	LAK cells with the following lytic units (10 <sup>-4</sup> ) per 10 <sup>6</sup> effectors <sup>a</sup>	
	vs. ECV-304	vs. Daudi
Control	26.6 ± 1.73	25.4 ± 2.23
Taurine 0.25 mg/mL	17.3 ± 1.55 <sup>b</sup>	51.4 ± 4.80 <sup>b</sup>
Taurine 0.50 mg/mL	21.9 ± 1.43	38.1 ± 2.42
Taurine 1.00 mg/mL	22.8 ± 1.76	37.7 ± 5.89
HEPES 10 mM	25.6 ± 7.40	39.9 ± 0.60 <sup>b</sup>
CHES 10 mM	30.0 ± 4.00	40.2 ± 4.80

rIL-2: recombinant interleukin-2; LAK: lymphokine-activated killer; EC: endothelial cell.

<sup>a</sup> PBMCs were cultured with rIL-2 (1000 units/mL) alone or in combination with taurine (0.25–1.00 mg/mL) or its analogues, HEPES and CHES (10 mM), for 72 hours and added to tumor cells. Specific <sup>51</sup>Cr release was measured after 12 hours (ECV-304) or 4 hours (Daudi) coculture. Results represent the mean ± standard error of mean of 6 experiments, performed in triplicate.

<sup>b</sup> *P* < 0.05 vs. control.

### Taurine Analogues

An identical series of experiments were repeated with the substitution of taurine with its analogues, HEPES and CHES (Tables 1 and 2), to assess whether they possessed similar properties to taurine. HEPES was found to reduce significantly (*P* < 0.05 vs. rIL-2) activated NK cell-mediated EC lysis to a degree similar to that observed with taurine, but neither analogue caused an alteration in K562 cytotoxicity (Table 1). HEPES was also found to increase significantly (*P* < 0.05) the antitumor efficacy of LAK cells against the Daudi cell line (Table 2), whereas neither

HEPES nor CHES modulated EC lysis in the same manner as their analogue, taurine. Thus, despite the finding that the more structurally similar analogue, HEPES, had some similar functions to taurine, in some cases the analogues did not have the same effects. This may have been due to the relatively high concentrations used, whereas the analogues, like taurine, may be more effective at lower levels. Also, although these analogues have an ethanesulfonic acid portion similar to taurine, they are otherwise structurally different, and this may endow them with different metabolic properties from those of taurine.

#### Endothelial Cell Protection by Taurine Is Effector Cell-dependent

As taurine was not removed from the culture medium when the effector cells were added to target cells, it was not known whether its protective effects were mediated through its modulation of the effector cell (lymphocyte) or of the target cell (EC). Experiments were thus undertaken in which taurine was confined to one of two culture periods: 1) EC (ECV-304) were incubated with taurine for 12 hours, washed to remove taurine, and exposed to rIL-2-activated NK cells/LAK cells; or 2) lymphocytes were incubated with taurine and IL-2 (18 and 72 hours), washed to remove taurine, and cocultured with EC.

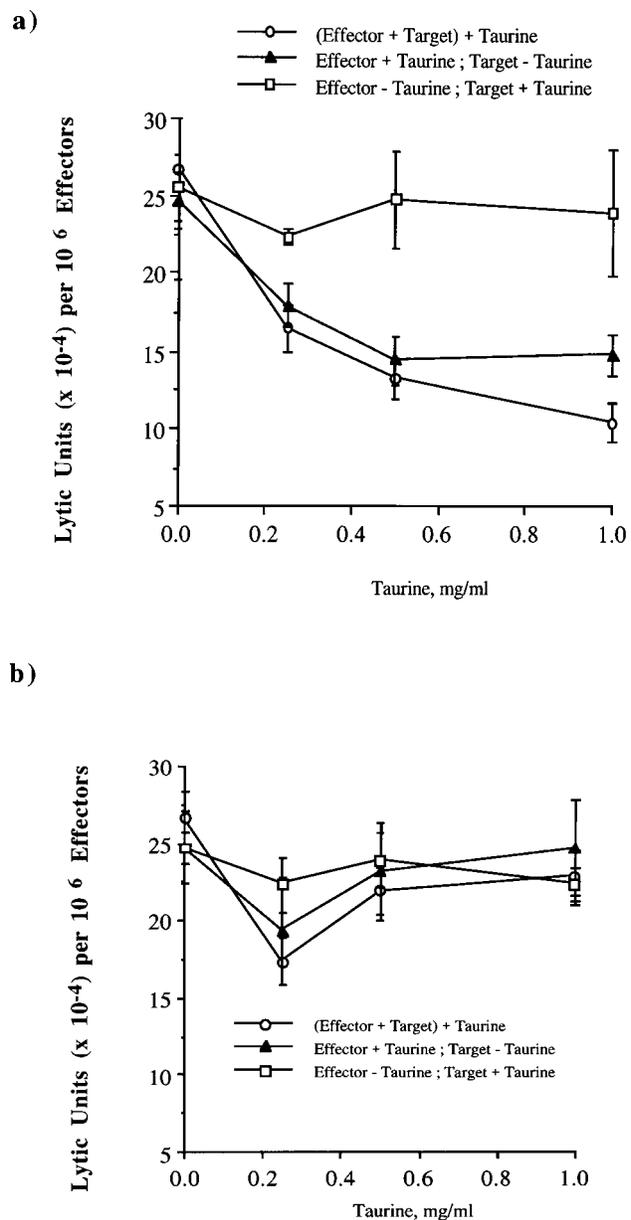
The results, shown in Figure 4 (a and b), indicated that the protective effect of taurine was due to its action on the lymphocyte effector cell rather than on the EC because incubation of NK/LAK cells alone with taurine prior to coculture with EC provided protection to the same extent as when both PBMC and EC were cultured with taurine.

#### Mechanism(s) of EC Protection by Taurine

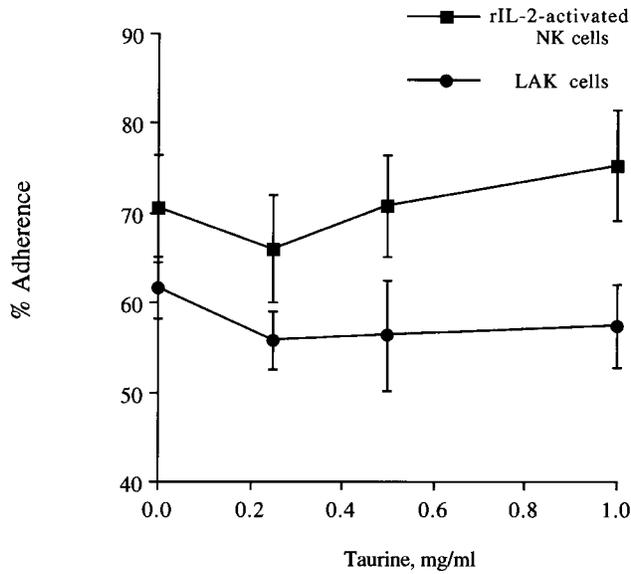
After our *in vitro* findings, we next tried to establish the mechanism(s) by which taurine was mediating EC protection against lymphocyte-mediated cytotoxicity while enhancing the antitumor activity of the same lymphocyte. We first needed to establish whether taurine was mediating its protective effect by limiting effector-target binding, or whether its effect was a post-binding event; thus, we next assessed lymphocyte-target interactions in the presence of taurine.

#### Adherence Modulation

The possibility that taurine might alter rIL-2-activated NK cell or LAK cell adherence to endothelium, and in doing so be responsible for reduced EC lysis, was examined. It was found that, across the taurine concentrations administered to lymphocytes, the percentage of adherence between NK/LAK cells and EC remained unchanged (Fig. 5), leading to the conclusion that the mechanism by which taurine mediated EC



**FIGURE 4.** The role of taurine in rIL-2-activated NK cell-mediated and LAK cell-mediated EC lysis is shown. PBMCs were cultured with rIL-2 (1000 units/mL) alone or in combination with taurine (0.25–1.00 mg/mL) for 18 hours (activated NK cells) (a) or 72 hours (LAK cells) (b). In one group, both ECs (targets) and NK cells (effectors) were incubated with taurine (circles). In a second group, effector cells were first incubated with taurine, which was then removed by washing, and these effectors were added to ECs (triangles). In a third group, ECs alone were incubated with taurine for 12 hours, washed, and exposed to rIL-2-activated NK cells (squares). Specific  $^{51}\text{Cr}$  release was measured after 12 hours. Results represent the mean  $\pm$  standard error of mean of three experiments performed in triplicate.



**FIGURE 5.** rIL-2-activated NK/LAK cell adherence to EC is shown. PBMCs derived from culture with rIL-2 (1000 units/mL) in the absence or presence of taurine (0.25–1.00 mg/mL) were labeled with  $^{51}\text{Cr}$ , after which their percentage adherence to ECV-304 was measured. Results represent the mean  $\pm$  standard error of mean of 3 experiments performed in triplicate.

protection occurred after lymphocyte-EC conjugation and thus was a postbinding event. The physiologic property by which taurine influenced the lymphocyte was next determined. The known physiologic properties of taurine include its antioxidant, antitoxic, membrane-stabilizing, and  $\text{Ca}^{2+}$ -modulatory actions, and it was these mechanisms that we undertook to examine in our model.

#### Antioxidation

The possibility that taurine functioned as an extracellular antioxidant was first assessed. We found, however, that specific cytolysis of EC by rIL-2-activated NK/LAK cells at the time periods examined was not significantly altered (data not shown) in the presence of SOD (6 units/well), a known extracellular reactive oxygen intermediate inhibitor. This suggested that EC was resistant to lymphocyte lysis by a mechanism independent of the respiratory burst product, superoxide anion.

#### Calcium Modulation

Because several of the steps involved in lymphocyte-mediated lysis are known to be  $\text{Ca}^{2+}$ -dependent, and as taurine is a known modulator of  $\text{Ca}^{2+}$  fluxes, it was hypothesized that taurine could mediate EC protection against lymphocyte lysis through its influence on

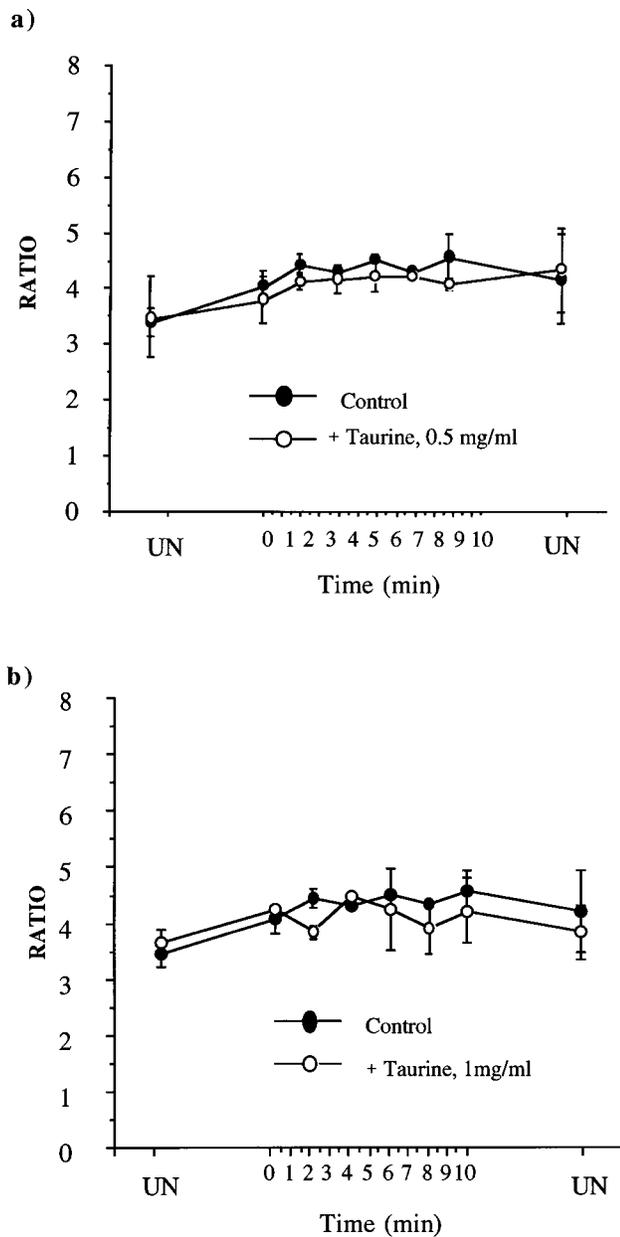
this divalent cation. To approach this question, we first needed to establish whether  $\text{Ca}^{2+}$  was involved in lymphocyte-mediated EC lysis; we did this by using the  $\text{Ca}^{2+}$  chelator BAPTA (2.5 and 10  $\mu\text{M}$ )<sup>33</sup> and the calcium channel blocker verapamil hydrochloride at a concentration not directly toxic to EC (0.2 mM). The addition of verapamil was found to inhibit lymphocyte-mediated cytotoxicity towards EC almost completely (data not shown). This indicated indirectly that the mechanism of lymphocyte-mediated cytotoxicity was  $\text{Ca}^{2+}$ -dependent. The  $\text{Ca}^{2+}$  chelator BAPTA caused a reduction in activated NK/LAK cell-mediated EC cytotoxicity similar to that caused by taurine (not shown); and when both agents were used together, there was no additive reduction in lysis observed, suggesting that both were working in a similar manner to protect endothelium.

#### The Effect of Taurine on Intracellular Calcium Levels in NK Cells Conjugated to Target Cells

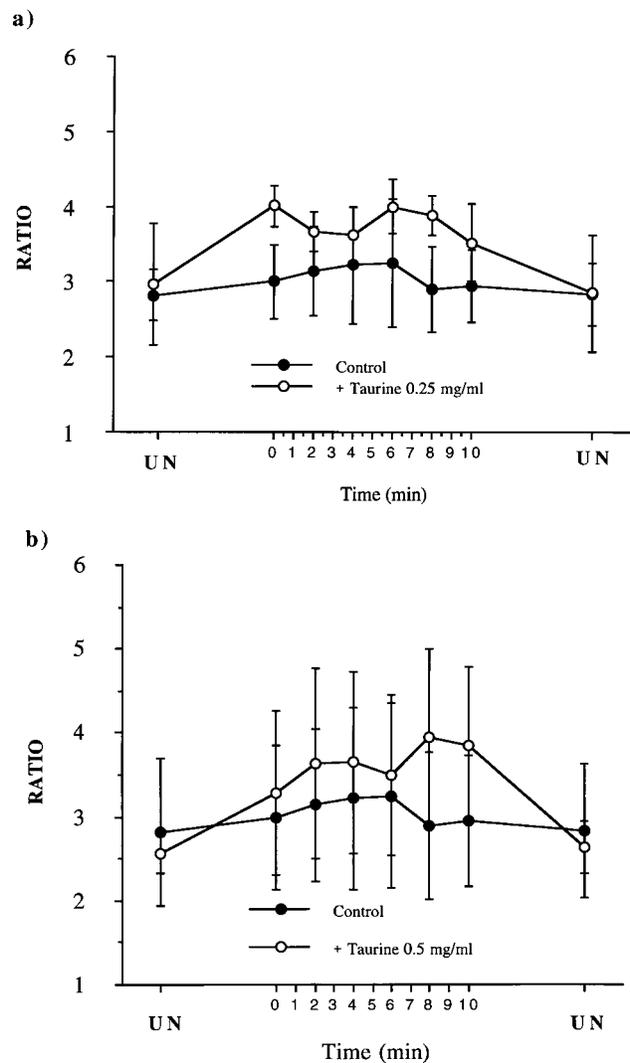
Having established that the observed beneficial properties of taurine in rIL-2 immunotherapy were at least partly calcium-dependent, we next directly assessed  $[\text{Ca}^{2+}]_i$  using a flow cytometric assay. To examine whether the observed reduction by taurine of NK cell-mediated EC lysis was associated with postconjugational changes in effector  $[\text{Ca}^{2+}]_i$  levels, activated NK cells ( $\pm$  taurine) and EC were allowed to conjugate. Results (Fig. 6) show an apparent rise in conjugated NK cells  $[\text{Ca}^{2+}]_i$  compared with unconjugated levels for both the control group and the groups incubated with 0.5 mg/mL taurine (Fig. 6a) or 1 mg/mL taurine (Fig. 6b); however, within the 10-minute test period, there was no significant difference between these groups.

Identical conjugation experiments were carried out using LAK cells ( $\pm$  taurine) as effectors and either EC or tumor cells (Daudi) as targets. Fig. 7a illustrates an overall increase, within the 10-minute test period, in  $[\text{Ca}^{2+}]_i$  levels of LAK cells incubated with taurine (0.25 mg/mL) prior to conjugation with EC. The rapidity of the rise from unconjugated levels to the first time point ( $T = 0$  minutes) was also greater for the group cultured with taurine and remained at a higher level ( $P < 0.065$  approached statistical significance) throughout the test period. Although there were no statistical differences within the 10-minute time frame between  $[\text{Ca}^{2+}]_i$  of control cells and cells incubated with taurine at concentrations of either 0.25 or 0.5 mg/mL, considerable variations in FL1 ratios between conjugated and unconjugated NK cells were observed between individuals, a finding also noted by others using this method of  $[\text{Ca}^{2+}]_i$  evaluation,<sup>26</sup> thus making statistical differences difficult to achieve.

The ratio of Fluo-3 intensity in conjugated-to-unconjugated effectors with Daudi cells as targets was



**FIGURE 6.** The effect of taurine on IL-2-activated NK cell  $[Ca^{2+}]_i$  after conjugation with EC targets is shown. Effectors (18 hours recombinant rIL-2-activated NK cells)  $\pm$  taurine 0.5 mg/mL (a) and 1.0 mg/mL (b) and EC targets (ECV-304) in RPMI 1640 + 10 mM HEPES were mixed at a ratio of 1:1, centrifuged, and incubated for the times indicated. Following resuspension, conjugates were analyzed and FL-1 median channel fluorescence determined for conjugated and unconjugated effectors. The ratios of these values are plotted against time, with control values for unconjugated peripheral blood mononuclear cells at T = 0 and T = 10 (UN). Results represent mean  $\pm$  standard error of 3 experiments.



**FIGURE 7.** The effect of taurine on LAK cell  $[Ca^{2+}]_i$  after conjugation with EC targets is shown. Effectors (LAK cells)  $\pm$  taurine 0.25 mg/mL (a) and 0.5 mg/mL (b) and (targets ECV-304) in RPMI 1640 + 10 mM HEPES were mixed at a ratio of 1:1, centrifuged, and incubated for the times indicated. After resuspension, conjugates were analyzed and FL-1 median channel fluorescence determined for conjugated and unconjugated effectors. The ratios of these values are plotted against time, with control values for unconjugated peripheral blood mononuclear cells at T = 0 and T = 10 (UN). Results represent mean  $\pm$  standard error of 3 experiments.

assessed, and no significant difference between either the control or the taurine-treated groups during the 10-minute test period was noted (data not shown). Thus, it appears that the effect that taurine has on  $[Ca^{2+}]_i$  in the IL-2-activated lymphocyte depends on the target cell to which it is conjugated, such that the same LAK cell responds differently with regard to its  $[Ca^{2+}]_i$  depending on whether it is conjugated to an endothelial cell or to a tumor target.

### The Effect of Taurine on BLT Esterase Levels in LAK Cells after Stimulation

It has so far been established that the cytolytic activity of LAK cells against tumor targets, Daudi, was significantly enhanced by prior culture with taurine, and that taurine did not cause these cells to experience significant alterations in  $[Ca^{2+}]_i$  after conjugation with tumor targets. The effect of taurine on lymphocyte cytolytic granule exocytosis was next determined. BLT esterase (Granzyme A) activity in a LAK cell population was measured and the role played by calcium in LAK cell exocytosis was examined.

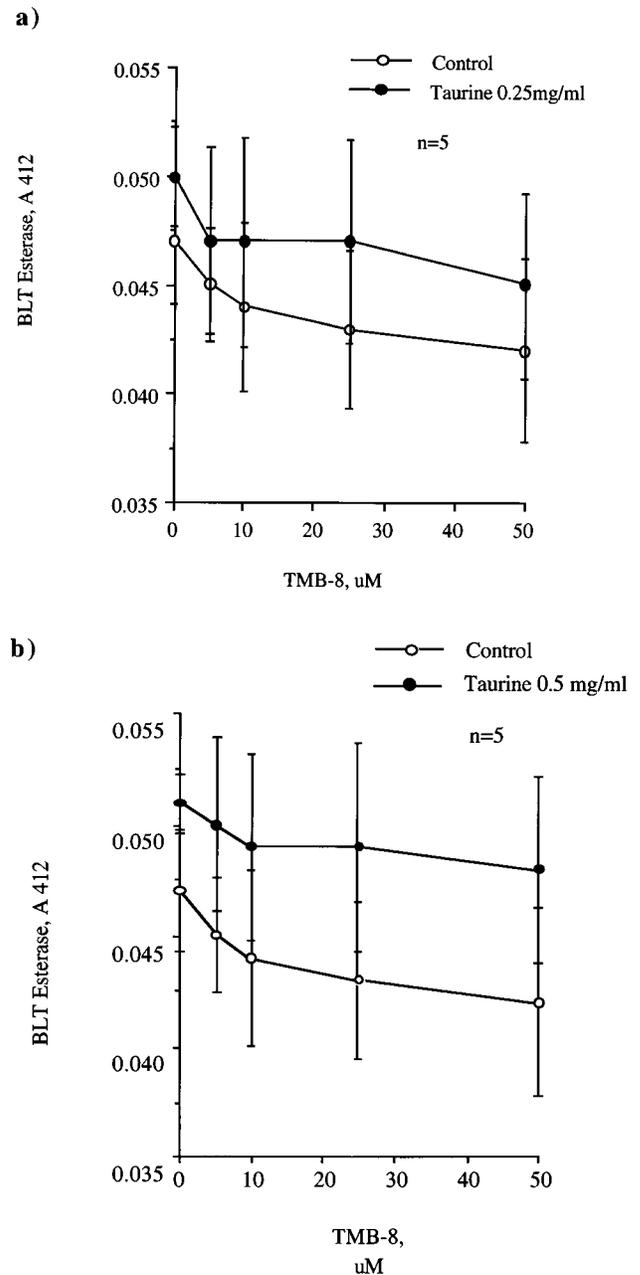
NK cells secrete granular enzymes such as BLT esterase in response to contact with susceptible target cells. We examined the effects of taurine on BLT esterase secretion from LAK cells in response to stimulation by PMA and A23187 and also looked at the effects of the intracellular  $Ca^{2+}$  antagonists TMB-8 and EGTA on BLT esterase secretion. LAK cells that had been cultured with taurine were found to have raised levels of BLT esterase activity compared with control LAK cells, and these levels were significantly ( $P < 0.05$  vs. control) elevated after culture with taurine at a concentration of 0.5 mg/mL (data not shown).

EGTA was found to inhibit BLT esterase secretion in response to PMA/A23187 at a concentration exceeding 0.5 mM (data not shown). TMB-8 also inhibited BLT esterase secretion from LAK cells in response to PMA/A23187 in a concentration-dependent manner (Figs. 8a and b), and again it was found that granzyme exocytosis was enhanced in the LAK cell population cultured with taurine to levels above those secreted by control LAK cells.

### The Effect of Taurine on Receptor Expression in a Lymphocyte Population

The effect of taurine on receptor expression within an LAK cell population was examined (Table 3) to assess whether taurine may influence lymphocyte function by altering the percentage of representation of a lymphocyte subset, e.g., by altering proliferation rates. Using flow cytometry, the effect of taurine on the percentage of receptor expression within the total LAK cell population was examined.  $CD16^+/56^+$  (NK cells),  $CD3^+$  (pan T cell marker), and  $CD19^+$  (pan B cell marker) were used to assess the percentage of each lymphocyte subset in the total population, and no significant differences were observed between the control population and the population with taurine.

A marker for NK activity, CD69, was also used for this population, and a small but nonsignificant increase in expression of this marker was observed in the groups incubated with taurine. Also, the marker for expression of the IL-2 receptor CD25 was used, and it was noted



**FIGURE 8.** The effect of taurine on BLT esterase production from LAK cells following stimulation with A23187 and PMA. Effectors (LAK cells)  $\pm$  taurine 0.25 mg/mL (a) and 0.5 mg/mL (b) were treated with the calcium antagonist TMB-8 before stimulation with PMA and A23187. BLT esterase exocytosis from LAK cells was then measured by spectrophotometry.

that its expression did not differ between the control group and the groups cultured with taurine.

### DISCUSSION

Use of the cytokine IL-2 as a single immunotherapeutic agent is hampered by its associated toxicity, which

**TABLE 3**  
**The Effect of Taurine on Receptor Expression in a LAK Cell Population**

Treatment	% Receptor expression of total population <sup>a</sup>				
	CD 16 <sup>+</sup> /56 <sup>+</sup>	CD 3 <sup>+</sup>	CD 19 <sup>+</sup>	CD 25 <sup>+</sup>	CD 69 <sup>+</sup>
Control	13.1 ± 2.86	77.4 ± 4.41	8.7 ± 3.74	10.5 ± 6.93	25.1 ± 8.18
Taurine 0.25 mg/mL	13.7 ± 4.38	80.1 ± 4.47	6.6 ± 2.64	11.6 ± 6.93	31.6 ± 7.79
Taurine 0.5 mg/mL	13.7 ± 2.38	77.7 ± 1.65	8.2 ± 3.07	6.4 ± 3.00	30.5 ± 9.05

LAK: lymphokine-activated killer; NK: natural killer; IL-2: interleukin-2; IL-2R: interleukin-2 receptor.

<sup>a</sup> Percentage of expression of lymphocyte markers for NK cells (CD16<sup>+</sup>/56<sup>+</sup>), T cells (CD3<sup>+</sup>), B (CD19<sup>+</sup>) cells, and IL-2R (CD25<sup>+</sup>) and NK activation (CD69<sup>+</sup>) were assessed for a population of PBMCs activated by IL-2 (1000 IU/mL) (72 hours) ± taurine.

develops within 24 hours of infusion. This toxicity is due to a diffuse vascular injury termed VLS,<sup>12,34</sup> which has prevented a more widespread application of this form of therapy. rIL-2 stimulates human lymphoid cells to proliferate and induces their differentiation into cytotoxic effector cells.<sup>24,35</sup> These LAK cells demonstrate a broad cytotoxic reactivity directed at a wide spectrum of tumor cells in a non-major histocompatibility complex (MHC) restricted lysis pattern,<sup>31</sup> resulting in intensive investigations of their efficacy in the experimental therapy of human malignancy. In this *in vitro* study, we hypothesized that the amino acid taurine could attenuate rIL-2-mediated EC injury by modulating lymphocyte function, a cell held partly responsible for IL-2-induced vascular injury, through a calcium-dependent mechanism.

In this study we demonstrated that rIL-2-activated NK cells and LAK cells mediated EC lysis and that taurine was capable of reducing this injury. As it was important to establish that taurine was not at the same time reducing the antitumor efficacy of IL-2, lymphocyte antitumor function in the presence of this amino acid was assessed. Taurine did not alter activated NK cell-mediated antitumor function, whereas it significantly enhanced LAK cell activity. We demonstrated that the protective effect of taurine against IL-2-mediated EC injury was conferred on the effector lymphocyte prior to coculture with the target endothelial cell through a Ca<sup>2+</sup>-dependent mechanism. Taurine was found to induce an increase in [Ca<sup>2+</sup>]<sub>i</sub> levels in LAK cells after their conjugation to EC and to maintain higher levels of Ca<sup>2+</sup>-dependent BLT esterase activity in their cytolytic granules.

In agreement with our findings, studies have shown that incubation of lymphocytes with rIL-2 for periods as short as 18 hours can result in the generation of LAK cell activity<sup>31</sup>; and because the symptoms of the VLS first appear within 24 hours of infusion of rIL-2, it is conceivable that toxicity could be mediated by these cells at this early stage. The finding that lym-

phocytes activated with rIL-2 are cytotoxic to a variety of vascular endothelium *in vitro* has been observed by others,<sup>5-7,13,31</sup> and these results together suggest that lymphocytes are fundamental to the process of endothelial damage during rIL-2 therapy.

To establish the underlying mechanism(s) of EC protection by taurine against lysis by the IL-2-activated lymphocyte, effector-target binding was investigated to see whether it might be inhibited by taurine. It was found that, across the range of taurine concentrations administered, adherence was unaltered, which indicated that EC protection by taurine was a postbinding effect and was not mediated by reduced target recognition.

Although taurine is a known antioxidant, it is unlikely that the mechanism(s) by which it acted to reduce EC lysis by LAK cells involved its antioxidant function, as previous findings have demonstrated that the lymphocyte cytotoxic mechanism does not involve the generation of toxic oxygen species.<sup>5,10,36</sup>

Taurine, in combination with mitogens, is known to affect proliferation and differentiation of T lymphocytes in culture,<sup>17</sup> and so it is not entirely unexpected that it should alter the function of the activated lymphocyte. We proposed that taurine influenced lymphocyte-mediated EC and tumor cell cytotoxicity through modulation of intracellular Ca<sup>2+</sup> movements. Ca<sup>2+</sup> is known to be central to the process of lymphocyte-mediated cytotoxicity<sup>18,36,37</sup> and taurine is unique in its ability to modulate Ca<sup>2+</sup> fluxes; it has been shown to stimulate the pumping rate of Ca<sup>2+</sup>-activated ATPase pumps, to decrease passive diffusion of Ca<sup>2+</sup>, and to modify Ca<sup>2+</sup> delivery to the channel as well as alter the kinetics of channel opening and closing.<sup>38</sup>

We undertook to determine the effects of calcium channel blockade on *in vitro* cell-mediated cytotoxicity and found that NK and LAK cell-mediated EC lysis were abrogated, indicating a role for Ca<sup>2+</sup> in our experimental system. A Ca<sup>2+</sup> chelator (BAPTA) attenuated lysis in a manner similar to taurine, and no additive

reduction in lysis was observed when both taurine and chelator were used together.

That rIL-2-induced injury of human endothelium is lymphocyte-mediated and  $\text{Ca}^{2+}$ -dependent is in agreement with the findings of Kotasek et al.<sup>5</sup> Several of the steps leading to lymphocyte-mediated lysis are known to be dependent on the presence of divalent cations, particularly  $\text{Ca}^{2+}$ . Extracellular  $\text{Ca}^{2+}$  is required for the regulated secretion of granule constituents<sup>39</sup> and for the polymerization of perforin to its active form polyperforin,<sup>40</sup> and intracellular  $\text{Ca}^{2+}$  also plays a major role in NK activation, proliferation,<sup>18</sup> and cytotoxicity.<sup>37</sup>

A series of experiments were undertaken to examine directly the effect of taurine on intracellular calcium levels in lymphocytes conjugated to EC and tumor targets. Results showed that  $[\text{Ca}^{2+}]_i$  levels in LAK cells cultured in taurine rose more rapidly after conjugation to EC and remained at a higher level throughout the study period than those observed with a control group. It thus appears that reduced EC lysis by these cells is associated with enhanced intracellular calcium levels, a finding similar to that reported by other authors.<sup>18,26</sup>

The LAK cell population is an ill-defined group of lymphocytes that appears to be heterogeneous<sup>41</sup>; some authors contend that LAK cells are primarily composed of stimulated NK cells, whereas others maintain that LAK cells are a subpopulation of lymphocytes proliferating from stem cell lymphocytes.<sup>42</sup> In contrast to our findings with LAK cells, experiments with activated NK cells have revealed no similar alteration in  $[\text{Ca}^{2+}]_i$  after conjugation with EC, perhaps providing a reason for the differences in sensitivity to taurine levels and subsequent EC lysis observed between NK and LAK effector cells. The possibility that aspects of the activity of NK and LAK cells are distinct even though their lytic mechanisms may be similar, has been observed before.<sup>43</sup> It is noteworthy that when LAK cells were used in conjugation experiments with tumor targets,  $[\text{Ca}^{2+}]_i$  was not significantly different between a control group and the group incubated with taurine. Thus, it appears that taurine can modulate LAK cell  $[\text{Ca}^{2+}]_i$  in a manner that is dependent on the target cell to which it is conjugated.

Given the importance of calcium in lymphocyte lytic activity, this difference may explain the ability of the same effector cells to mediate enhanced lysis of one target cell (tumor cell) and yet attenuate lysis of another (endothelial cell). A study looking at lymphocyte-EC interactions has suggested that the IP/ $\text{Ca}^{2+}$  second messenger pathway may play a role in EC functional alteration induced by lymphocyte adhesion.<sup>44</sup> By altering the levels of intracellular calcium, taurine

may be controlling part of the signaling process by which the lymphocyte "lytic hit" is controlled. The whole area of lymphocyte activation, signal transduction, and cytolytic function in response to contact with susceptible targets still requires further elucidation, but with increasing knowledge in this field it may be possible to establish the exact mechanism of action of taurine with regard to lymphocyte function.

Changes in cytolytic granule constituents could clearly alter the potential effector molecules participating in lymphocyte-mediated cytotoxicity,<sup>45</sup> and thus the release of BLT esterase (Granzyme A) from stimulated LAK cells was measured and the effects of taurine on this secretion assessed. Lymphocyte BLT esterase activity is  $\text{Ca}^{2+}$ -dependent<sup>46</sup> and is associated with cellular degranulation and cytotoxicity. LAK cells cultured with taurine were shown to have higher levels of BLT esterase secretion than control cells. These levels were inhibited in a dose-dependent manner by the intracellular calcium antagonists TMB-8 and EGTA, suggesting that these cells were capable of calcium-dependent granule exocytosis. Our finding suggests that taurine may enhance LAK cell-mediated tumor cytotoxicity by a mechanism involving, at least in part, enhanced levels of cytotoxic mediators.

Phenotypic studies of IL-2-activated cells have shown that the proportions of the cell subtypes can undergo alterations. Such changes might have important implications for the antitumor response, and it was important to assess whether taurine could modulate these proportions within the LAK cell population. Taurine, however, did not appear to alter the different lymphocyte subsets within the LAK cell population when compared with control cells incubated without taurine.

Other agents that have been reported to reduce the toxicity associated with IL-2 immunotherapy are often associated with a concomitant reduction in tumor cytotoxicity,<sup>4</sup> and thus therapeutic index is compromised. In our experimental model, taurine appeared to have a dual benefit in that, together with its ability to reduce the dose-limiting toxic effects of rIL-2, it produced a more potent antitumor immune response than that obtained with rIL-2 alone. Thus, although taurine's ability to reduce the toxicity of this cytokine might not at first appear to be dramatic enough to be clinically effective, this property, taken together with its ability to enhance antitumor function, might prove to be a combination that would result in an enhanced therapeutic index for IL-2. This increase in antitumor function contrasts with that observed when rIL-2 is used in combination with other biologic agents, e.g., granulocyte-macrophage-colony stimulating factor, IL-6, and interferon- $\gamma$ .

Although LAK cells have been found to cause EC damage in vivo and may explain part of the clinical toxicity observed in cancer patients treated with high dose rIL-2/LAK therapy, extrapolation of our in vitro data to the clinical setting requires preclinical testing. Taurine has been used in a variety of clinical situations and no known serious toxicities have been reported. The concentrations of taurine used in this study are in the pharmacologic range, and whether their use is applicable to the clinical setting or not would need to be assessed by clinical trial. In a clinical trial by Milei et al.,<sup>47</sup> taurine was administered intravenously by rapid infusion at a level comparable to the highest used in these in vitro experiments, and no apparent adverse effects were recorded. The studies reported herein suggest that the therapeutic index of rIL-2 in the treatment of human cancers may be significantly increased by combination with taurine, and that the use of taurine with IL-2 immunotherapy merits clinical evaluation.

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