

Human Lymphokine-activated Killer Cells Are Cytotoxic against Cells Infected with *Toxoplasma gondii*

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Summary

Experiments were conducted to determine whether human lymphokine-activated killer (LAK) cells are cytotoxic against cells infected with *Toxoplasma gondii*. Nylon wool nonadherent (NWN) peripheral blood lymphocytes, as well as purified natural killer cell (NK) ($CD3^- CD16^+ CD56^+$) and T ($CD3^+ CD16^- CD56^-$) cells obtained from five healthy *T. gondii* seronegative volunteers exhibited minimal cytotoxic activity against *T. gondii*-infected cells. When standard LAK (S-LAK) cell preparations were induced by incubation of NWN cells with recombinant interleukin 2, induction of remarkable cytotoxic activity against *T. gondii*-infected cells was observed in LAK cell preparations from each of the volunteers. The phenotype of the LAK precursor and effector cells varied depending on the target cell used. Whereas the precursor and the effector cells of most of the LAK activity against K562 and Daudi cells were cells with NK phenotype, when *T. gondii*-infected cells were used as targets, both cells with NK and T cell phenotypes were precursors and effectors of the lysis. When cytotoxic activity of S-LAK cells was compared with the activity of adherent LAK (A-LAK) cells, A-LAK cells displayed higher cytotoxic activity against *T. gondii*-infected cells, as well as against K562 and Daudi cells. Cold target inhibition experiments suggested that there is a subset of LAK effector cells capable of lysing both *T. gondii*-infected cells and Daudi cells, whereas other subsets preferentially or exclusively lyse one of these target cells.

Toxoplasma gondii is an obligate intracellular protozoan that infects humans throughout the world. Although *T. gondii* is a relatively uncommon cause of disease in immunocompetent individuals, those with a deficiency in cell-mediated immunity (i.e., AIDS patients, those on high dosage of cytotoxic drugs or corticosteroids, newborns, and fetuses) may develop severe disease because of this parasite (1, 2).

Cell-mediated immunity plays a major role in protection against *T. gondii* (3–5). Although both $CD4^+$ and $CD8^+$ T lymphocytes are important in this resistance, $CD8^+$ T lymphocytes appear to be the paramount subset in the protective response (4, 5). $CD8^+$ T lymphocytes may provide protection through lysis of cells infected with the parasite (6–8) and/or secretion of cytokines (5).

The role in *T. gondii* infection of cells that do not require prior sensitization with antigen to respond remains controver-

sial. Whereas some investigators have reported that NK cells are cytotoxic against extracellular tachyzoites of *T. gondii* (9, 10), others have been unable to reproduce this observation (11, 12). Our previous observation that administration of IL-2 to mice resulted in significant survival of these animals after an ordinarily lethal challenge with *T. gondii* (13) raised the possibility that lymphokine-activated killer (LAK)¹ cells confer protection against this parasite.

LAK cells are effector cells that upon culture with IL-2 become cytotoxic for NK-resistant targets, including fresh autologous and allogeneic tumor cells and tumor cell lines (14, 15). LAK cells have been proven effective as adoptive immunotherapy in animal models of metastatic tumors and viral infection, as well as in patients with some forms of cancer (16–20). We considered it interesting to determine whether human LAK cells have cytotoxic activity against cells infected

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¹ Abbreviations used in this paper: A-LAK, adherent lymphokine-activated killer; CM, complete medium; DT, dye test; NWN, nylon wool nonadherent; S-LAK, standard lymphokine-activated killer.

with *T. gondii*. In addition, we studied the activity of LAK cells termed adherent LAK (A-LAK) cells, which have a high cytotoxic activity against tumor cells (21–24) and superior in vivo antimetastatic activity compared to standard (unseparated) LAK (S-LAK) cells (25).

Materials and Methods

Preparation of PBL. PBMC were obtained after signed consent from normal volunteers who were seronegative for *T. gondii* antibody in the agglutination test and Sabin-Feldman dye test (DT) (26, 27). PBMC were isolated from heparinized venous blood by centrifugation on Ficoll-Hypaque gradients (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). Cells were collected from the gradient interphase and washed three times with RPMI 1640 medium (Gibco Laboratories, Grand Island, NY). Cells were then resuspended at a concentration of 20×10^6 cells/ml in complete medium (CM) consisting of RPMI 1640 supplemented with 2 mM L-glutamine, 5×10^{-5} M 2-ME, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 10% heat-inactivated, DT-negative pooled human AB⁺ serum (Irvine Scientific, Santa Ana, CA), loaded onto a prewashed nylon wool column (Polysciences, Inc., Warrington, PA), and eluted from the column after a 1-h incubation at 37°C, 5% CO₂. Removal of monocytes and B cells was confirmed by FACS[®] analysis (Becton Dickinson & Co., San Jose, CA).

Isolation of NK and T Cells. Purification was performed following the methodology previously described (28). Briefly, nylon wool nonadherent (NANA) cells were incubated for 30 min on ice with either anti-CD3 mAb (Dako Corp., Carpinteria, CA) or anti-CD56 mAb (Becton Dickinson & Co.). After two washes, magnetic beads coated with goat anti-mouse IgG (Advanced Magnetics, Inc., Cambridge, MA) were added and incubated on ice for 30 min with frequent mixing. Rosetting cells were removed with a magnet (Dynal, Inc., Great Neck, NY). Addition of magnetic beads was repeated once to ensure adequacy of purification. This procedure resulted in populations purified for CD3⁻ CD16⁺ CD56⁺ NK cells (>96% by FACS[®] analysis) and CD3⁺ CD16⁻ CD56⁻ T cells (>98% by FACS[®] analysis).

Preparation of LAK Cells. NANA cells were cultured at 2×10^6 cells/ml in CM supplemented with 1,000 U/ml of human rIL-2 (generous gift from Chiron Corporation, Emeryville, CA) in a tissue culture flask (Corning Glass Works, Corning, NY) positioned on its flat side. A-LAK cells were obtained following the protocol of Melder et al. (22). After 24 h incubation at 37°C, 5% CO₂, nonadherent cells were removed and adherent cells washed five times with warm RPMI. Fresh CM supplemented with rIL-2 plus 50% (vol/vol) conditioned medium (supernatant obtained after centrifugation of nonadherent cells) was added to adherent cells. Cell concentration was kept below 2×10^6 cells/ml by adding fresh medium with rIL-2. In flasks containing standard (unseparated) LAK (S-LAK) cells, nonadherent cells were never removed. In experiments in which both types of cell populations were compared, cells were cultured for 8–12 d and fresh rIL-2 was added every 5 d. Before use in cytotoxicity assays, A-LAK cells as well as S-LAK cells were incubated with cold 5 mM EDTA in PBS for 10 min to detach adherent cells, and then washed three times. In some experiments, purified NK and T cells were incubated with 1,000 U/ml rIL-2 for 7 d and then used as effector cells in cytotoxicity assays.

Complement-mediated Depletion of Lymphocyte Subpopulations. S-LAK cells were incubated for 1 h at 4°C with optimal doses of either anti-CD5 (pan T) mAb (Becton Dickinson & Co.) or anti-

CD56 mAb (Coulter Cytometry, Hialeah, FL). This was followed by incubation with a 1:8 dilution of baby rabbit complement (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) for 1 h at 37°C. This treatment was repeated once and resulted in >90% depletion of the appropriate lymphocyte subset as demonstrated by cytofluorometric analysis.

Target Cells. Autologous PHA-induced blasts were obtained by incubating PBMC in RPMI 1640 with 10% FCS (HyClone Laboratories, Inc., Logan, UT) and 2 μ g/ml of PHA-P (Sigma Chemical Co., St. Louis, MO) for at least 4 d. Tachyzoites of the RH strain of *T. gondii* were exposed to 1,300 erg of UV light as previously described (6) and then used to infect PHA-induced blasts at a multiplicity of infection of 10 parasites per PHA-induced blast. Tachyzoites that remained extracellular were removed by Ficoll-Hypaque density gradient centrifugation (6). Cytocentrifuge preparations were made from these cell preparations and the percentage of infected cells was assessed by light microscopy (29). Rates of infection ranged from 60 to 80%. Tumor cell lines K562 (NK susceptible) and Daudi (NK resistant) (American Type Culture Collection, Rockville, MD) were maintained in RPMI 1640 with 10% FCS.

Cytotoxicity Assays. This was performed as previously described with minor modifications (6). Briefly, 5×10^3 target cells labeled with ⁵¹Cr were added to wells of 96-well U-bottomed plates (Costar Corp., Cambridge, MA) and incubated with different numbers of effector cells for 4 h at 37°C, 5% CO₂ in RPMI 1640 with 10% FCS. Supernatants were harvested and radioactivity counted using a gamma counter (model 5500 B Beckman Instruments, Palo Alto, CA). The percent specific ⁵¹Cr-release was calculated using the following formula: $100 \times [(\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})]$. Percent specific ⁵¹Cr-release represents the mean from triplicate wells. When cytotoxic activity was expressed in lytic units, lytic unit was defined as the number of effector cells required to lyse 30% of tumor target cells or 30% of the cells infected with *T. gondii*. Lytic units were calculated using the method of Bryant et al. (30).

In experiments in which supernatants from LAK cell preparations were tested for cytotoxic activity, 5×10^3 ⁵¹Cr-labeled target cells were resuspended in serial dilutions of the supernatant and incubated for 4 h at 37°C, 5% CO₂.

Cold Target Inhibition Assay. Different numbers of unlabeled target cells were added in quadruplicate to wells containing 5×10^3 ⁵¹Cr-labeled target cells before addition of effector cells. E/T ratios were chosen so that percent specific ⁵¹Cr-release was located in the linear portion of the cytotoxicity curve. Percent specific ⁵¹Cr-release was assayed as described above.

Cytofluorometric Analysis. Cells adjusted to a concentration of 10^7 cells/ml in PBS with 0.1% sodium azide were stained with the following mAbs: FITC-conjugated anti-CD3 (Becton Dickinson & Co.), FITC-conjugated anti-CD14 (Becton Dickinson & Co.), PE-conjugated anti-CD16 (Becton Dickinson & Co.), PE-conjugated anti-CD19 (Caltag Laboratories, South San Francisco, CA), and PE-conjugated anti-CD56 (Coulter Cytometry). Cells were then analyzed in a FACScan[®] cytofluorometer (Becton Dickinson & Co.).

Morphology. Cytocentrifuge preparations of effector cells were air-dried and fixed in methanol for 5 min. Cells were then stained with a 10% solution of Giemsa stain for 25 min and examined by light microscopy.

Statistical Analyses. Data were analyzed using Student's *t* test and Spearman's rank correlation coefficient.

Results

Cytotoxic Activity of Resting PBL. Resting Nwana cells from five donors displayed minimal cytotoxic activity against *T. gondii*-infected cells (Fig. 1 *a*). Cytotoxicity against uninfected cells was not observed in any case. NK and T cells were purified from resting Nwana cells and their cytotoxicity tested against *T. gondii*-infected cells, K562 cells, and Daudi cells. Whereas purified NK cells displayed higher cytotoxic activity against K562 cells than Nwana cell preparations, purified T cells had no lytic activity against these target cells (Fig. 1 *b*). In contrast, neither NK nor T cells displayed any significant cytotoxic activity against *T. gondii*-infected cells (Fig. 1 *a*). None of the cell populations were cytotoxic for Daudi cells (data not shown).

Effect of Incubation of PBL with rIL-2. For each of the donors, incubation of Nwana cells with rIL-2 to generate LAK activity (S-LAK cells) induced significant cytotoxicity against *T. gondii*-infected cells but not against uninfected cells (Fig. 2 *a*). Incubation with rIL-2 also resulted in increased cytotoxic activity against K562 cells, as well as induction of cytotoxic activity against Daudi cells (Fig. 2 *b*). Enhancement of the cytotoxic activity against *T. gondii*-infected cells and Daudi cells required the presence of rIL-2 since Nwana cells incubated in CM without rIL-2 did not exhibit significant cytotoxic activity against either of these targets (Fig. 2, *a* and *b*).

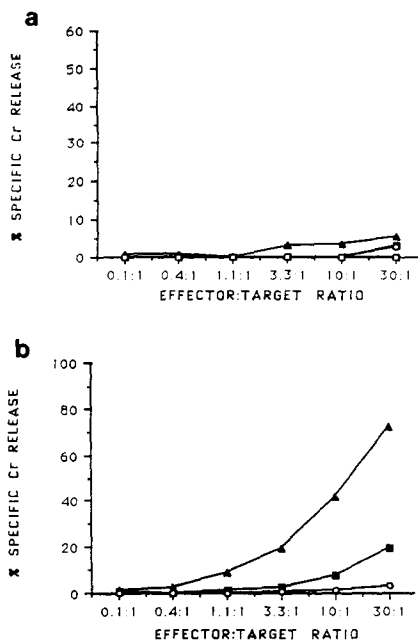


Figure 1. Cytotoxic activity of resting PBL against either *T. gondii*-infected or uninfected cells (*a*), and K562 cells (*b*). (*a*) Nwana cells against either *T. gondii*-infected (■) or uninfected (□) cells; NK (▲) and T cells (○) against *T. gondii*-infected cells. Results obtained with uninfected cells were similar for all the effector cells. (*b*) Nwana cells (■), NK cells (▲), and T cells (○). Results are representative of those obtained in six separate experiments.

Kinetics of induction of LAK activity was studied by incubating Nwana cells with 1,000 U/ml rIL-2 for 4, 7, and 12 d. As can be seen in Fig. 3, *a* and *b*, maximal cytotoxic activity against *T. gondii*-infected cells and tumor cell lines was observed after 7 d of culture. Prolonging duration of incubation resulted in a decrease in cytotoxic activity.

To determine whether LAK cell supernatants alone effect the cytotoxicity observed in our assays, uninfected and *T. gondii*-infected cells, as well as Daudi cells, were resuspended in concentrations of LAK cell supernatant that ranged from 100 to 1% (vol/vol) in CM. Significant cytotoxic activity was not observed at any of the concentrations of supernatants tested against any of the target cells (data not shown).

Characterization of Precursor Cells. Nwana cells as well as purified NK and T cells were incubated with 1,000 U/ml rIL-2 for 7 d. As shown in Fig. 4, *a* and *b*, whereas the precursor cells responsible for most of the cytotoxic activity against tumor cells had the NK phenotype, when *T. gondii*-infected cells were used as targets, both cells with NK and T cell phenotypes gave rise to effector cells with significant cytotoxic activity against these target cells.

Characterization of Effector Cells. Experiments were conducted to assess the cytotoxic activity of the lymphocyte subsets present in 7-d S-LAK preparations against *T. gondii*-infected cells. Treatment of effector cells with either anti-CD5 or anti-CD56 mAb and complement resulted in populations of cells purified (>90% by FACS[®] analysis) for CD3⁻ CD56⁺ and CD3⁺ CD56⁻ cells, respectively. Fig. 5, *a* and *b* demon-

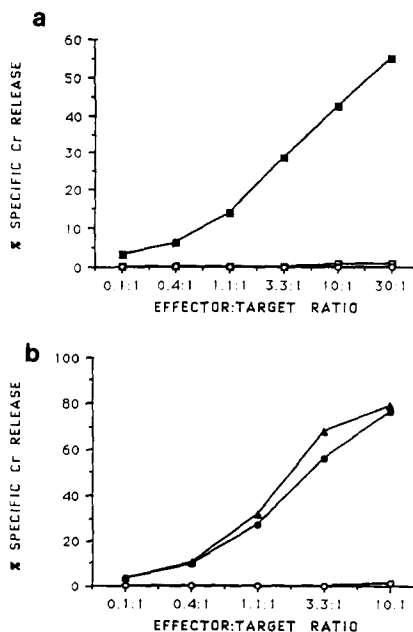


Figure 2. Cytotoxic activity of S-LAK cells after 7 d of incubation with rIL-2. *T. gondii*-infected cells (■) and uninfected cells (□) (*a*); K562 cells (▲) and Daudi cells (●) (*b*). Cells incubated in CM without rIL-2 for 7 d were also tested against *T. gondii*-infected cells (○) (*a*) and against Daudi cells (○) (*b*). Results are representative of those obtained in seven separate experiments.

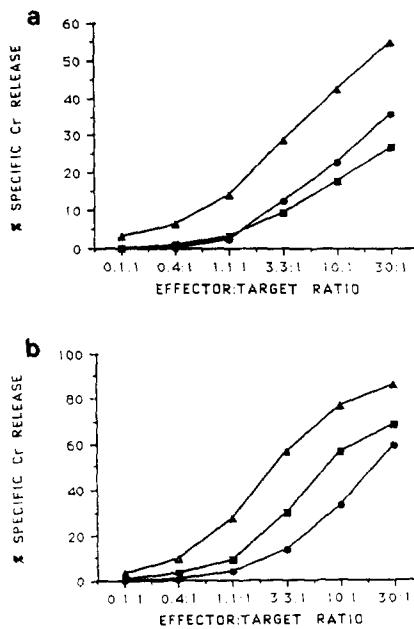


Figure 3. Kinetics for induction of cytotoxic activity against *T. gondii*-infected cells (a) and Daudi cells (b). Cells were incubated with rIL-2 for 4 (■), 7 (▲), or 12 (●) d. Results are representative of those obtained in two separate experiments.

strates that cells purified for CD3⁺ CD56⁻ lymphocytes exhibited a significantly lower cytotoxic activity against tumor cells than cells purified for CD3⁻ CD56⁺ lymphocytes. When *T. gondii*-infected cells were used as targets, both groups of effector cells exhibited significant cytotoxicity.

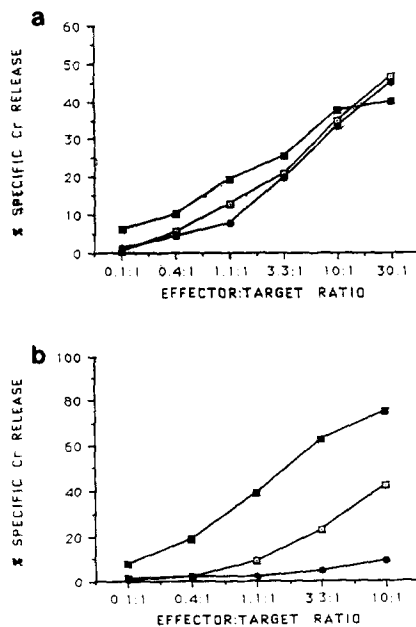


Figure 4. Characterization of precursor cells for lytic activity against *T. gondii*-infected cells (a) and tumor cells (b shows results obtained with Daudi cells; similar results were observed with K562 cells). Either NWN cells (□), NK cells (■), or T cells (●) were incubated with IL-2 for 7 d. Results are representative of those obtained in four separate experiments.

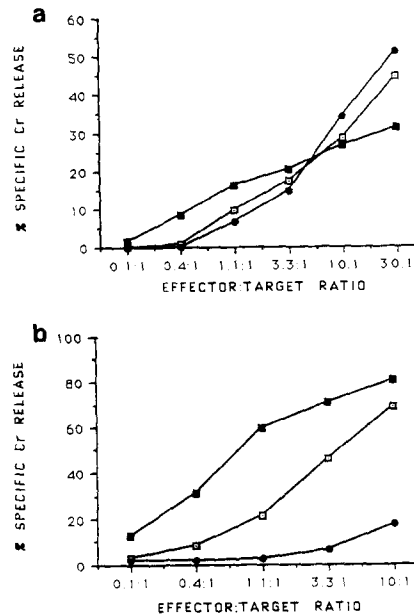


Figure 5. Characterization of effector cells responsible for lysis of *T. gondii*-infected cells (a) and tumor cells (b shows results obtained with Daudi cells; similar results were observed with K562 cells). Before cytotoxicity assays, effector cells were treated with complement alone (□), anti-CD5 (■), or anti-CD56 mAb plus complement (●). Results are representative of those obtained in three separate experiments.

Comparison of Cytotoxic Activity of S-LAK and A-LAK Cells. Selection of cells induced to attach to plastic by incubation with rIL-2 (A-LAK cells) resulted in enrichment for CD3⁻ CD56⁺ cells and for cells with LGL morphology

Table 1. Cytofluorometric and Morphologic Analysis of S-LAK Cells and A-LAK Cells

	Percent positive cells			
	CD3 ⁻ CD56 ⁺	CD3 ⁺ CD56 ⁺	CD3 ⁺ CD56 ⁻	LGL
Donor 1				
S-LAK	62	4	33	63
A-LAK	97	1	1	99
Donor 2				
S-LAK	46	7	45	52
A-LAK	93	2	4	98
Donor 3				
S-LAK	32	5	62	60
A-LAK	58	1	41	97
Donor 4				
S-LAK	22	12	64	42
A-LAK	51	9	39	86
Donor 5				
S-LAK	9	13	76	31
A-LAK	20	16	62	74

(Table 1). Cytofluorometric analysis of A-LAK cell preparations revealed that distribution of the three main subsets of cells ($CD3^- CD56^+$, $CD3^+ CD56^+$, and $CD3^+ CD56^-$) varied among donors. Whereas in donors 1 and 2, A-LAK cells consisted of highly purified $CD3^- CD56^+$ cells (> 90% by FACS[®] analysis), this subset represented only 20% of the A-LAK cells from donor 5. The different phenotypic composition of A-LAK cells among donors was reproducible in separate experiments performed with independently obtained A-LAK cell preparations from these individuals.

A-LAK cells from each of the five donors displayed higher cytotoxic activity against tumor cells than did S-LAK cells (Table 2). Furthermore, when seven independently obtained A-LAK preparations were analyzed, a significant correlation between cytotoxic activity of A-LAK cells against tumor cells and the percentage of $CD3^- CD56^+$ cells in the A-LAK preparations was observed ($p < 0.05$). Preparations with the highest percentage of $CD3^- CD56^+$ cells tended to have the highest cytotoxic activity, whereas A-LAK preparations with the lowest percentage of $CD3^- CD56^+$ cells usually had the lowest cytotoxic activity (Tables 1 and 2).

A-LAK cells also displayed higher cytotoxic activity than S-LAK cells against *T. gondii*-infected cells (Table 2). However, no correlation between the percentage of $CD3^- CD56^+$ cells in A-LAK preparations and cytotoxic activity of these effector cells against *T. gondii*-infected targets was observed ($p > 0.5$).

Cold Target Inhibition Assays. Fig. 6, *a* and *b* demonstrates that when unlabeled (cold) Daudi cells were added to wells

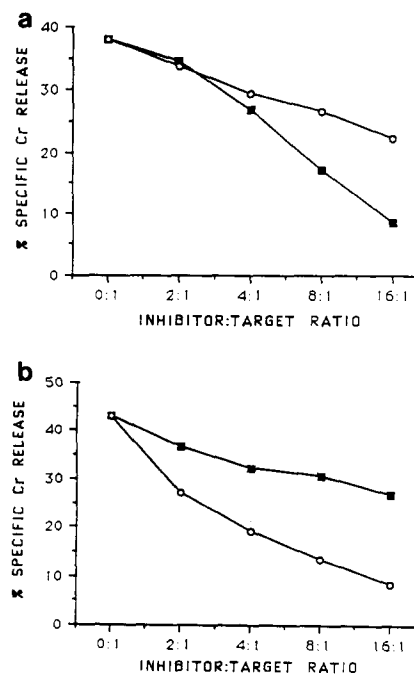


Figure 6. Effect of addition of unlabeled (cold) target cells on cytotoxic activity against either ^{51}Cr -labeled *T. gondii*-infected cells or ^{51}Cr -labeled Daudi cells. (*a*) addition of unlabeled *T. gondii*-infected cells (■) or unlabeled Daudi cells (○) to wells containing ^{51}Cr -labeled *T. gondii*-infected cells. (*b*) addition of unlabeled *T. gondii*-infected cells (■) or unlabeled Daudi cells (○) to wells containing ^{51}Cr -labeled Daudi cells. E/T ratio was 10:1 and 3:1 for experiments shown in *a* and *b*, respectively. Results are representative of those obtained in three separate experiments.

Table 2. Cytotoxic Activity of S-LAK Cells and A-LAK Cells against *T. gondii*-infected Cells, K562 Cells, and Daudi Cells

	Lytic units/ 10^7 cells		
	<i>T. gondii</i> -infected	K562	Daudi
Donor 1			
S-LAK	788	5768	3238
A-LAK	854	8858	5482
Donor 2			
S-LAK	868	4620	2250
A-LAK	1645	13838	5196
Donor 3			
S-LAK	409	4230	1474
A-LAK	761	7121	3180
Donor 4			
S-LAK	1031	1543	1064
A-LAK	1397	4043	1793
Donor 5			
S-LAK	1390	580	1339
A-LAK	4446	1426	2813

Results are expressed in lytic units per 10^7 effector cells.

containing ^{51}Cr -labeled *T. gondii*-infected cells, a significant dose-dependent inhibition of the lysis of the latter was observed ($p < 0.02$ at inhibitor/target ratios from 4:1 to 16:1). In parallel experiments, addition of unlabeled *T. gondii*-infected cells resulted in a significant dose-dependent inhibition of lysis of ^{51}Cr -labeled Daudi cells ($p < 0.008$ at all inhibitor/target ratios tested). However, both *T. gondii*-infected cells and Daudi cells were more efficient inhibitors of the lysis of their respective (homologous) ^{51}Cr -labeled target cells ($p < 0.001$ at inhibitor/target ratios from 8:1 to 16:1). Uninfected cells did not have any demonstrable inhibitory effect on the lysis of either *T. gondii*-infected cells or Daudi cells (data not shown). The results of cold target inhibition assays were not dependent on the phenotype of the effector cells since similar results were obtained with LAK cell preparations that were either highly homogeneous by FACS[®] analysis (97% $CD3^- CD56^+$) or not (50% $CD3^- CD56^+$).

Discussion

Here we report that induction of LAK activity by incubation of human PBL from seronegative donors with IL-2 results in the induction of significant cytotoxic activity against human cells infected with the obligate intracellular parasite *T. gondii*. This effect of rIL-2 may represent a mechanism of defense against this parasite. Of importance in this regard is the observation of Sharma et al. (13) who reported that in vivo ad-

ministration of rIL-2 resulted in prolonged survival of mice after a lethal challenge with *T. gondii*. Although the mechanism by which rIL-2 conferred the protection was not fully elucidated, it is possible that it was, at least in part, due to induction of LAK cells.

In the donors tested, we were unable to demonstrate lysis of *T. gondii*-infected cells by either resting NRNA cells, purified NK, or purified T cells. Of interest in this regard are the reports in which the cytotoxic activity of NK cells against extracellular tachyzoites of *T. gondii* was assessed. Dannemann et al. (12) demonstrated that human NK cells did not display significant cytotoxic activity against this form of the parasite. Although similar results were reported by Hughes et al. (11) using murine splenic NK cells from animals infected with *T. gondii*, Hauser et al. (9) and Goyal et al. (10) observed that murine splenic NK cells from infected animals were cytotoxic against extracellular tachyzoites of the parasite. Although resting PBL in the donors reported here did not have significant cytotoxic activity against *T. gondii*-infected cells, we have recently tested PBL from a seronegative donor whose resting cells were cytotoxic for these targets (Subauste, C. S., and J. S. Remington, unpublished observations). Evaluation of further cases to determine the frequency of this occurrence, and characterization of the effector cell responsible for this activity are under way.

LAK cells are a heterogeneous population of cells that are comprised of both CD3⁻ CD5⁻ cells and CD3⁺ CD5⁺ cells (31–33). Experimental evidence indicates that, when tested against tumor cells and cells infected with microorganisms such as HTLV-1, *Legionella pneumophila*, and *Mycobacterium avium-intracellulare*, LAK activity generated from human PBL originates from and is effected mainly by cells with NK phenotype (31–36). However, LAK cells with T cell phenotype have also been reported to lyse tumor cell targets (31–33). Under the experimental conditions used, our results indicate that, compared to cells with NK phenotype, cells with T cell phenotype play a minor role as precursors and effectors of the lytic activity against tumor cells. In contrast, in the case of *T. gondii*-infected cells, both cells with NK and T cell phenotypes play a significant role as precursors and effectors of cytotoxicity. Pertinent to these results is the observation of Ballas et al. (37, 38) who reported that the phenotype of precursor and effector cells in murine LAK cell preparations varied depending on the type of target cell used. Whereas the lytic activity against the tumor cell line YAC-1 originated from and was effected by cells with NK phenotype only, TNP-modified lymphoblasts could be lysed only by cells originating from and expressing T cell phenotype. It would appear that modification of the cell membrane after treatment with TNP or infection with *T. gondii* renders the cell particularly susceptible to lysis by LAK cells with T cell phenotype.

Recently, it has become possible to generate highly cytotoxic LAK cells from purified NK cell cultures (21–24). Vujanovic et al. (21) noted that incubation with IL-2 resulted in attachment of NK cells to plastic and applied this observation to establish populations of LAK (A-LAK) cells which

are highly cytotoxic against tumor cells. It is interesting to note that although generation of A-LAK cell preparations results in enrichment for cells with the CD3⁻ CD5⁺ phenotype, our studies as well as those from other investigators (22, 39) show that these populations of cells are at times still heterogeneous by FACS[®] analyses. We observed that A-LAK cells had higher cytotoxic activity than S-LAK cells against *T. gondii*-infected cells and tumor cells although no significant correlation between the percentage of CD3⁻ CD5⁺ cells in the A-LAK preparations and cytotoxicity against *T. gondii*-infected cells was observed. A-LAK cells not only have a change in their phenotypic composition compared to S-LAK, but they have also been reported to have higher expression of adhesion molecules (40, 41). It has been proposed that higher expression of these molecules might be associated with superior cytotoxic activity of A-LAK cells (41).

In a previous series of studies on LAK cells from our laboratory, Dannemann et al. (12) reported that human LAK cells displayed weak cytotoxicity against extracellular tachyzoites of *T. gondii* and that this activity was enhanced by prior incubation of the parasite with serum containing anti-*T. gondii* antibodies. Cytotoxicity of LAK cells against isolated *T. gondii* cysts could not be demonstrated (12). Since *T. gondii* is predominantly situated intracellularly, the significance of cytotoxic activity against extracellular tachyzoites remains unclear, although it may play a role when parasites are released from infected cells.

Other investigators have reported cytotoxic activity of LAK cells against nonviral intracellular pathogens such as *Rickettsia prowazekii*, *Rickettsia typhi*, *L. pneumophila*, *M. avium-intracellulare*, and *Leishmania major* (35, 36, 42–43). However, Zychlinsky et al. (44) were unable to demonstrate lysis of murine macrophages infected with *L. pneumophila*, *Listeria monocytogenes*, *M. avium*, *T. gondii*, or *Trypanosoma cruzi* by highly purified murine splenic LAK cells (A-LAK cells).

The nature of the site(s) that LAK cells recognize on target cells remains undefined. Our results may be analogous to the observation of Grimm et al. (45) who reported that LAK cells recognize cells with “altered self”. These authors demonstrated that whereas normal human PBL were not susceptible to lysis by LAK cells, TNP-modified PBL were lysed. The results of our cold target inhibition experiments suggest that LAK cells are heterogeneous in their capacity to recognize and lyse different target cells. Our results suggest that there is a subpopulation of LAK cells that recognizes both types of target cells (*T. gondii*-infected and tumor cells), whereas other subsets preferentially or exclusively recognize only one of these targets. Similar evidence that suggests that LAK cells display patterns of preferential target cell lysis was reported by Froelich et al. (34). Furthermore, heterogeneity in the mechanisms of recognition of target cells has also been suggested by others (46).

Wissemann et al. (47) reported that soluble factors produced by human leukocytes stimulated with either mitogen or *R. prowazekii* antigen (in the case of immune leukocytes) are cytolytic against cells infected with this intracellular pathogen but not against uninfected cells. The results of our experi-

ments performed with supernatants from LAK cell preparations suggest that soluble cytotoxic factors alone do not mediate to a significant extent the cytotoxic activity of LAK cells in short term cytotoxicity assays. However, we cannot rule out the role of cytotoxic molecules secreted by LAK cells after these cells contact *T. gondii*-infected cells.

The cytotoxic activity of LAK cells against *T. gondii*-infected cells may prove to be a mechanism by which LAK cells protect against infection with this parasite. Destruction of infected cells would not only deprive the parasites of their required intracellular habitat, but also would expose them to activated phagocytic cells and/or to anti-*T. gondii* antibodies resulting in the destruction of these organisms (27, 48). Cytokines produced by LAK cells (39) may also result in protection through induction of killing or inhibition of the intracellular multiplication of the parasites.

Current antimicrobial regimens for the treatment of toxoplasmosis, although effective, result in a significant incidence of side effects that frequently require their discontinuation in immunocompromised hosts (49). The development of new effective alternative therapeutic modalities is therefore critical. Immunotherapy using adoptive transfer of LAK cells, usually in conjunction with IL-2, has been proven effective in animal models of metastatic tumors and viral infection and in patients with some forms of cancer (16–20). Furthermore, adoptive transfer experiments performed in animals have demonstrated that, when compared to S-LAK cells, A-LAK cells have a superior antimetastatic effect and result in prolonged survival of the animals (25). Our results in vitro suggest that administration of LAK cells might prove of therapeutic use in immunocompromised patients with toxoplasmosis.

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