Human CD4⁺ and CD8⁺ T Lymphocytes Are Both Cytotoxic to *Toxoplasma gondii*-Infected Cells

JOSE G. MONTOYA,^{1,2,3} KAREN E. LOWE,⁴ CAROL CLAYBERGER,⁵ DEWEY MOODY,⁶ DUC DO,¹
JACK S. REMINGTON,^{1,2} SOHEL TALIB,⁴ AND CARLOS S. SUBAUSTE^{1,2*}

Department of Immunology and Infectious Diseases, Research Institute, Palo Alto Medical Foundation, Palo Alto, California 94301¹; Division of Infectious Diseases, Department of Medicine,² and Department of Cardiothoracic Surgery,⁵ Stanford University School of Medicine, Stanford, California 94305; Veterans Affairs Palo Alto Health Care System, Palo Alto, California 94304³; and Department of Molecular Biology⁴
and Department of Infectious Diseases,⁶ Applied Immune Sciences, Inc.,
Santa Clara, California 95054

Received 6 June 1995/Returned for modification 3 July 1995/Accepted 27 October 1995

Studies to determine if *Toxoplasma gondii*-specific human T cells lyse parasite-infected cells have yielded conflicting results. Furthermore, attempts to obtain human cytotoxic CD8⁺ T lymphocytes have been difficult because of the lack of a reproducible system for their generation. By using paraformaldehyde-fixed, *T. gondii*-infected peripheral blood mononuclear cells as antigen-presenting cells, we developed a method whereby *T. gondii*-specific T-cell lines can be reproducibly generated. Six *T. gondii*-specific T-cell lines were generated from an individual chronically infected with *T. gondii*. Cytofluorometric analysis of these lines revealed >99% CD3⁺, 85 to 95% CD3⁺ αβ T-cell-receptor-positive (TCR⁺), 5 to 9% CD3⁺ γδ TCR⁺, 50 to 70% CD4⁺, and 20 to 40% CD8⁺ cells when cells were examined during the first 3 weeks of stimulation and >99% CD3⁺, >99% CD3⁺ αβ TCR⁺, <1% CD3⁺ γδ TCR⁺, 20 to 40% CD4⁺, and 60 to 80% CD8⁺ cells when cells were examined between 5 and 11 weeks. Both CD4⁺ and CD8⁺ T cells had remarkable cytotoxic activity against *T. gondii*-infected target cells (30 to 50% specific Cr release at an effector-to-target ratio of 30:1) but not against uninfected target cells (<10% at an effector-to-target ratio of 30:1). Cytotoxic activity by the whole T-cell lines was not *T. gondii* strain specific. Whole T-cell lines were cytotoxic for target cells infected with the C56 and ME49 strains and the RH strain (which was used to infect peripheral blood mononuclear cells). *T. gondii*-specific T-cell lines displayed the predominant expression of Vβ7 TCR. The CDR3 regions of the Vβ7 TCRs of these T-cell lines showed a striking degree of sequence identity (oligoclonality). T-cell lines obtained by the method reported here can be used to characterize functional activity of T-lymphocyte subsets in humans infected with *T. gondii*.

The major mechanism of resistance to *Toxoplasma gondii* in murine models of the infection has been shown to be mediated by T cells (5, 25). The association between toxoplasmosis and conditions that affect T-cell-mediated immunity (e.g., those in patients with lymphomas or AIDS) provides indirect evidence for the role of these cells in resistance to this parasite in humans (8, 30). Adoptive transfer studies performed in a murine model of *T. gondii* infection indicated that CD8⁺ T cells were primarily responsible for resistance, although significant protection was also conferred by CD4⁺ T lymphocytes (25).

It has been proposed that in addition to their production of cytokines, T cells may confer protection against *T. gondii* by lysis of parasite-infected cells (6, 24, 32). Studies aimed at determining if *T. gondii*-specific human T cells lyse parasite-infected cells have yielded what appear to be conflicting results (4, 32). Because of the paucity of data on human CD8⁺ T cells that are cytolytic for *T. gondii*-infected target cells, we attempted to produce such cell lines to allow us to further explore the potential role of CD8⁺ T cells in humans in resistance to *T. gondii*. By using fixed *T. gondii*-infected cells as antigen-presenting cells, we developed a method whereby *T. gondii*-specific T-cell lines could be reproducibly generated. Since the T-cell receptor (TCR) is involved in T-cell activation

and triggering of effector functions (2, 11), we studied the repertoires of TCR Vβ expression of the *T. gondii*-specific T-cell lines which were generated.

MATERIALS AND METHODS

Isolation of PBMC. Peripheral blood mononuclear cells (PBMC) were obtained from a healthy asymptomatic donor with serologic evidence of chronic *T. gondii* infection. *T. gondii* serology was performed as previously described (12, 16). The Sabin-Feldman dye test titer was 1:128, and the immunoglobulin M enzyme-linked immunosorbent assay was negative. The donor is known to have been seropositive for *T. gondii* antibodies for more than 3 years. This study was approved by the Institutional Review Board. Signed informed consent was obtained. PBMC were isolated from heparinized venous blood by centrifugation on Ficoll-Hypaque gradients (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.) as previously described (23). Cells were suspended in complete medium (CM) consisting of RPMI 1640 medium (Gibco Laboratories, Grand Island, N.Y.) supplemented with 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 10% heat-inactivated fetal bovine serum (HyClone Laboratories, Inc., Logan, Utah).

Preparation of stimulator and feeder cells. Autologous PBMC were infected with tachyzoites of the RH strain of *T. gondii* as previously described (23). Rates of infection ranged from 20 to 30%. Infected cells were then fixed for 45 min with 1% paraformaldehyde (J. T. Baker Inc., Phillipsburg, N.J.) in phosphate-buffered saline and then washed extensively prior to addition to responder cells. Paraformaldehyde-fixed infected PBMC were used as stimulator cells. Autologous, uninfected, γ-irradiated (3,000 R) PBMC were used as feeder cells.

Generation of T-cell lines. To generate *T. gondii*-specific T-cell lines, freshly isolated PBMC were cultured in 24-well plates (Flow Laboratories, McLean, Va.) at a concentration of 2.5×10^6 cells per ml in CM and stimulated weekly with 2.5×10^5 stimulator cells per ml and 5×10^5 γ-irradiated feeder cells per ml. Recombinant interleukin 2 (a gift from Chiron Corporation, Emeryville, Calif.) at a concentration of 30 IU/ml was added after 14 days of in vitro stimulation. Thereafter, recombinant interleukin 2 was added to the cell lines

* Corresponding author. Mailing address: Research Institute, Palo Alto Medical Foundation, 860 Bryant St., Palo Alto, CA 94301. Phone: (415) 326-8120. Fax: (415) 329-9853.

every 3 to 4 days. In addition, T-cell lines were generated by stimulation with autologous feeder cells, phytohemagglutinin (PHA) P (2 µg/ml) (Sigma Chemical Co., St. Louis, Mo.), and recombinant interleukin 2 (30 IU/ml) and used as controls.

CD4⁺ and CD8⁺ T cells were purified from the T-cell lines by depletion of CD8⁺ or CD4⁺ cells with MicroCELLector flasks (Applied Immune Sciences, Inc., Santa Clara, Calif.). The procedures followed were those recommended by the manufacturer (7, 29). The nonadherent cell population was recovered, and the purification process was repeated with a second MicroCELLector CD8 flask. This resulted in populations of T cells that were >98% CD3⁺ CD4⁺ by cytofluorometric analysis. To purify CD8⁺ T cells, MicroCELLector CD4 T-25 cell culture flasks were used. This resulted in populations of T cells that were >99% CD3⁺ CD8⁺ by cytofluorometric analysis. The method used for the purification of CD4⁺ and CD8⁺ T cells was completed within 3 to 4 h, and the cytotoxic assays were performed that same day.

Cytotoxicity assays. Target cells consisted of either autologous PHA-induced blasts obtained as previously described (23) or Daudi cells (American Type Culture Collection, Rockville, Md.). Tachyzoites of the RH, C56, and ME49 strains of *T. gondii* were obtained as previously described (23, 26), exposed to UV light (23), and then used to infect the PHA-induced blasts. Cytochrome preparations were made from these cell preparations, and the percentage of infected cells was assessed by light microscopy (18). Rates of infection ranged from 25 to 60%. Cytotoxicity assays were performed as previously described (23, 24).

Cytofluorometric analysis. Cells were incubated with the following monoclonal antibodies (Becton Dickinson & Co., San Jose, Calif.): fluorescein isothiocyanate-conjugated anti-CD3, phycoerythrin (PE)-conjugated anti-CD3, PE-conjugated anti-CD4, PE-conjugated anti-CD8, PE-conjugated anti-CD56, PE-conjugated anti-γδ TCR, and fluorescein isothiocyanate-conjugated anti-αβ TCR. Propidium iodine (0.5 µg/ml) (Sigma Chemical Co.) was added immediately prior to analysis of the cells with a FACScan cytofluorometer (Becton Dickinson & Co.). Background fluorescence was assessed with irrelevant isotype control monoclonal antibodies.

TCR Vβ analysis. The TCR Vβ repertoires of resting T cells (unstimulated), *T. gondii*-stimulated T-cell lines, and T-cell lines stimulated nonspecifically with PHA were determined by the method of Choi et al. (3). Briefly, total RNA was isolated from ≥2 × 10⁶ cells per sample with RNazol B (acid guanidinium thiocyanate phenol) from TEL-TEST (Friendswood, Tex.) by following the manufacturer's instructions. First-strand cDNA was prepared from 4 µg of total RNA in a 20-µl reaction mixture with oligo(dT)₁₂₋₁₈ primer and Moloney murine leukemia virus RNase H⁻ reverse transcriptase according to the GIBCO-BRL SuperScript protocol (Life Technologies, Gaithersburg, Md.). The cDNA was amplified with *Taq* DNA polymerase in a 50-µl PCR mixture for each of the 24 known TCR Vβ gene families by using a specific 5' Vβ sense primer and the same 3' Cβ antisense primer. The amplified Vβ products ranged in size from about 170 to 370 bp. Each PCR tube contained the 5' Cα and 3' Cα primers for a 604-bp internal-standard PCR product. Quantitation was carried out by established methods (3). For each cDNA sample, the percent Vβ relative expression of each of the 24 Vβ gene families was calculated by the following formula:

$$\% V\beta(n) = \frac{[\{V\beta(n) \text{ densitometric signal} \} \times 100] / [C\alpha(n) \text{ densitometric signal}]}{\sum_{n=1}^{24} [V\beta(n) / C\alpha(n)]}$$

Cloning and complementarity determining region 3 (CDR3) sequence analysis. PCR was performed separately with cDNA from the unstimulated (day 0) and *T. gondii*-stimulated (week 8) T cells, with only the 5' Vβ7 and 3' Cβ primers being used. The amplified Vβ7 products were ligated into the pCR II vector for the transformation of TA Cloning One Shot competent *Escherichia coli* cells (Invitrogen, San Diego, Calif.). Transformed colonies were picked at random for plasmid miniprep purification (QIAwell-8 Plus; QIAGEN, Chatsworth, Calif.) and double-stranded dideoxynucleotide sequencing. Sequences from 1 µg of double-stranded plasmid DNA were amplified with T7 and SP6 promoter primers (Promega, Madison, Wis.) in 20-µl *Taq* DyeDeoxy Terminator Cycle reaction mixtures (Applied Biosystems, Foster City, Calif.). PCR sequencing was performed in a Perkin-Elmer Cetus model 9600 thermal cycler with the recommended program (96°C for 15 s, 50°C for 5 s, and 60°C for 4 min for 25 cycles with a 4°C hold). Samples were analyzed with an Applied Biosystems 373A DNA sequencer.

RESULTS

Following in vitro stimulation of PBMC with autologous cells infected with *T. gondii* RH, six T-cell lines were obtained. After 5 to 11 weeks of stimulation, cells from these lines were >99% CD3⁺, >99% CD3⁺ αβ TCR⁺, <1% CD3⁺ γδ TCR⁺, 20 to 40% CD4⁺, and 60 to 80% CD8⁺. When the cells were examined during the first 3 weeks of stimulation, cells from the

FIG. 1. Cytotoxic activity of a T-cell line against autologous PHA-induced blasts that were uninfected (open circles) and infected with *T. gondii* RH (solid circles) and against Daudi cells (open squares). The infection rate was 52%.

lines were >99% CD3⁺, 85 to 95% CD3⁺ αβ TCR⁺, 5 to 9% CD3⁺ γδ TCR⁺, 50 to 70% CD4⁺, and 20 to 40% CD8⁺.

The cytotoxic activity of these T-cell lines against uninfected, autologous, PHA-induced blasts; *T. gondii*-infected, autologous, PHA-induced blasts; and Daudi cells was studied from week 5 to week 11 of in vitro culture. Whereas T cells displayed minimal (<10% specific Cr release at an effector-to-target ratio [ETR] of 30:1) cytotoxic activity against autologous, uninfected, PHA-induced blasts and Daudi cells, they exhibited remarkable cytotoxic activity against *T. gondii*-infected, PHA-induced blasts (30 to 50% at an ETR of 30:1) (Fig. 1). Infection of Daudi cells with *T. gondii* did not increase their susceptibility to lysis by T cells (data not shown). The functional activity of all the T-cell lines generated was reproducible in multiple experiments.

To determine the phenotype of the T cells responsible for the lytic activity against *T. gondii*-infected targets, purified populations of both CD4⁺ (>98% CD3⁺ CD4⁺) and CD8⁺ (>99% CD3⁺ CD8⁺) T cells were obtained from five of the T-cell lines generated. Significant cytotoxic activity against autologous, *T. gondii*-infected, PHA-induced blasts was observed with both CD4⁺ and CD8⁺ T cells, but such activity was not observed when uninfected, autologous, PHA-induced blasts were used as targets (Fig. 2). Cytotoxicity with the CD8⁺ T cells appeared to be greater (50% at an ETR of 30:1) than that

FIG. 2. Cytotoxic activity of unseparated T cells (circles), purified CD4⁺ T cells (triangles), and purified CD8⁺ T cells (squares) against uninfected (open symbols) and *T. gondii*-infected, PHA-induced blasts (solid symbols). The infection rate was 50%.

FIG. 3. Cytotoxic activity of a T-cell line against uninfected, autologous, PHA-induced blasts (open circles) and against autologous, PHA-induced blasts infected with tachyzoites from the RH (solid circles), C56 (solid squares), and ME49 (solid triangles) strains of *T. gondii*. The infection rates were 41, 35, and 25%, respectively.

with the CD4⁺ T cells (27% at an ETR of 30:1) or the unseparated T cells (29% at an ETR of 30:1).

To determine whether the cytotoxic activity of the T-cell lines was limited to the specific strain of *T. gondii* used to infect

the PBMC used as antigen-presenting cells, autologous PHA-induced blasts were infected with tachyzoites of the RH, C56, and ME49 strains and used as targets in ⁵¹Cr release assays. The T-cell lines were obtained after in vitro stimulation with PBMC infected with tachyzoites of the RH strain. T cells lysed *T. gondii*-infected, PHA-induced blasts regardless the *T. gondii* strain used to infect the target cells (Fig. 3).

To characterize the effect of *T. gondii* stimulation on the TCR, we examined the TCR Vβ repertoire of the T cells before and after exposure to *T. gondii*. The relative percent Vβ expression in resting (unstimulated) T cells was heterogeneous, with no specific predominance or deletions of any of the 24 Vβ gene families (Fig. 4A). Repeated stimulation with *T. gondii*-infected, autologous PBMC resulted in a progressive increase in Vβ7, which became the predominant Vβ TCR family by the third week of in vitro stimulation. Figure 4B shows the TCR Vβ repertoire of T cells after 8 weeks of in vitro stimulation with *T. gondii*-infected, autologous PBMC. No such increase in Vβ7 relative expression was observed in PHA-stimulated T cells (data not shown).

To further examine whether the predominant Vβ7 gene expression was due to an oligoclonal expansion of T cells, the Vβ7 CDR3 sequences of the unstimulated and *T. gondii*-stim-

FIG. 4. Reverse transcription-PCR analysis of the TCR Vβ repertoire of resting (unstimulated) T cells (A) or a T-cell line after 8 weeks of in vitro stimulation with autologous, *T. gondii*-infected PBMC (B).

Vβ	N-Dβ-N	Jβ
7.1 TGC GCC AGC AGC C A S S	CAA GAC GTA ACC AAT E D V T N	2.1 GAG CAG TTC TTC E Q F F
TGC GCC AGC AGC C A S S	CTA GCG GGA GAG GAT AAT L A G E D N	GAG CAG TTC TTC E Q F F
TGT GCC AGC AGC C A S S	TCG CAG GGT AGC GGG AAG GCG S Q G S G K A	GAG AGA CCC AGT E R P S
7.1 TGT GCC AGC AGC C A S S	CAA GGA GAC Q G D	2.2 GGC GGG GAG CTG G G E L
TGC GCC AGC AGC C A S S	CAA GGG GAG GAC TTC GCG AAC Q G E D F A N	ACC GGG GAG CTG T G E L
TGT GCC AGC AGA C A S R	GAA TCG AAC E S N	ACC GGG GAG CTG T G E L
7.1 TGC GCC AGC AGC C A S S	CAA GCG AGG TTT AGC Q A R F S	2.3 ACA GAT ACG CAG T D T Q
TGC GCC AGC AGC C A S S	CAG GCT AAA GTG GGA CTA GCT TCG ACT AGC Q A K V G L A S T S	ACA GAT ACG CAG T D T Q
7.1 TGC GCC AGC AGC C A S S	GCA GGG ACG ACA GAC A G T T D	2.5 CAA GAG ACC CAG Q E T Q
7.1 TGC GCC AGC AGC C A S S	CAA GAT CTA GAG TCT AGC GGG AGA CAC TCC Q N L E S S G R H S	2.7 TAC GAG CAG TAC Y E Q Y
TGC GCC AGC AGC C A S S	CTC TCG ACT AGC GGG AGC L S T S G S	TAC GAG CAG TAC Y E Q Y
TGC GCC AGC AGC C A S S	TTG TAC CGG L Y R	GAG CAG TAC E Q Y
TGC GCC AGC AGC C A S S	CTT TCA AGC GGC GGG ACC ACC L S S G G T T	TAC GAG CAG TAC Y E Q Y

FIG. 5. Vβ7 TCR CDR3 sequences of unstimulated T-cell clones.

ulated T cells were analyzed. Figure 5 shows the CDR3 (N-Dβ-N) region of several independently selected TCR Vβ7 cDNA clones of unstimulated T cells. Each of the 13 clones has a unique CDR3 sequence. The lengths of the CDR3 range from 9 to 30 nucleotides. The expression of TCR Vβ7 by these T cells was polyclonal on the basis of the highly diverse CDR3 sequences and lengths. Figure 6 indicates the high degree of oligoclonality observed among the TCR Vβ7 cDNA clones of the *T. gondii*-stimulated T cells. The same CDR3 sequence was displayed by 30 of 32 randomly selected clones.

DISCUSSION

Studies of *T. gondii* infection in animal models have demonstrated the critical role of T cells (5, 25) and, in particular, of CD8⁺ T cells (25) in resistance to this parasite. Some of these studies performed in murine models in which CD8⁺ T cells play the major role in protection against *T. gondii* revealed that CD8⁺ T cells were cytotoxic for *T. gondii*-infected cells (6, 24). These observations provided impetus for studies directed at examining the in vitro functional activity of subsets of human *T. gondii*-specific T cells and, in particular, at determining if such CD8⁺ T cells are cytotoxic against *T. gondii*-infected cells.

In the present study, we demonstrate that both human CD4⁺ and CD8⁺ T cells are cytotoxic against *T. gondii*-infected cells. The system of in vitro stimulation that we used to generate *T. gondii*-specific T-cell lines was chosen to mimic as closely as possible the natural process of antigen presentation for this intracellular parasite. To remove concern for the pres-

ence of live organisms, which may result in the destruction of the T cells, infected antigen-presenting cells were fixed with paraformaldehyde. The reasons why different laboratories have obtained discrepant results in regard to the cytotoxic activity of human *T. gondii*-specific T cells remain to be determined. It is likely that as was previously proposed by Curiel et al. (4), the presence of viable parasites may prevent generation of cytotoxic T-cell lines through either infection of T cells or an as-yet-undefined mechanism.

The cytotoxicity of our T-cell lines against *T. gondii*-infected cells was not specific for the strain of the parasite. These T-cell lines, which had been generated by incubation with cells infected with *T. gondii* RH, were able to lyse cells infected with the C56 and ME49 strains of the parasite. Although the lysis of target cells infected with the ME49 strain appeared to be lower than that of the RH- and C56-infected target cells, this may be explained by the lower rates of infection obtained with the ME49 strain. Lysis of target cells infected with different strains of *T. gondii* was observed despite evidence for antigenic variation among *T. gondii* strains (28). Thus, these data suggest that the three different strains that we used share a conserved epitope(s) that is recognized by *T. gondii*-specific T cells. A similar lack of strain specificity is suggested by the reports of other investigators (4, 14, 15).

We observed that in vitro stimulation with *T. gondii*-infected PBMC affected the relative TCR Vβ gene expression in T cells from a healthy donor chronically infected with *T. gondii*. The oligoclonality of the TCR Vβ7 CDR3 suggests that the preferential expansion of these T cells was stimulated by TCR Vβ7 recognition of a *T. gondii* antigen. Analysis of the TCR Vβ repertoire and major histocompatibility complex in clinical samples from patients with *T. gondii* infection may provide further insight into the T-cell response to this parasite.

In vitro stimulation of our T-cell lines with autologous PBMC infected with tachyzoites of *T. gondii* resulted in a progressive increase over time in the percentage of CD8⁺ T cells present in our T-cell lines. It is interesting to note that this in vitro phenomenon may have an in vivo correlate, since as was mentioned above, humans with acute symptomatic *T. gondii* infection exhibit an increase in CD8⁺ T cells in their peripheral blood (10, 20).

Changes in the percentages of γδ T cells in the T-cell-line preparations were observed during the course of in vitro culture. Compared with the percentage of γδ T cells present in peripheral blood T cells from the donor (1% γδ TCR⁺), the percentage of this subset of T cells from lines studied during the first 3 weeks of in vitro culture showed a remarkable increase. In this regard, we recently demonstrated that incubation of human T cells with cells with intracellular *T. gondii*

Vβ7.1	N-Dβ2.1-N	Jβ2.1
(30 of 32 clones)		
TGC GCC AGC AGC C A S S	CTC GGG ACT AGC GGG AGA GCC TAC L G T S G R A Y	AAT GAG CAG TTC TTC N E Q F F
(1 of 32 clones)		
TGC GCC AGC AGC C A S S	CTC GGG ACT AGC GGG GGA GCC TAC L G T S G G A Y	AAT GAG CAG TTC TTC N E Q F F
(1 of 32 clones; nonfunctional)		
TGC GCC AGC AGC C A S S	CCT CGG GAC TAG CGG GAG AGC CTA P R D AMBER (STOP)	CAA TGA GCA GTT

FIG. 6. Vβ7 TCR CDR3 sequences of *T. gondii*-stimulated T-cell clones.

results in preferential activation and expansion of human $\gamma\delta$ T cells (22). It is interesting to note that despite the initial expansion of $\gamma\delta$ T cells observed in our T-cell lines following in vitro incubation with *T. gondii*-infected cells, further cycles of in vitro stimulation resulted in a decline of the $\gamma\delta$ T-cell populations. A transient increase in $\gamma\delta$ T cells has also been reported to occur in the peripheral blood of patients with acute toxoplasmosis (17).

It has been proposed that lysis of *T. gondii*-infected cells may be a mechanism by which T cells confer protection against this organism (6, 24, 32). Whether lysis of *T. gondii*-infected cells results in the death of the intracellular parasite remains to be determined. In studies with cells infected with *Leishmania mexicana* subsp. *mexicana*, death of the parasite did not result from the lysis of the cells by T cells (21). However, even if lysis of *T. gondii*-infected cells does not result in the killing of the intracellular parasites, release of *T. gondii* tachyzoites would expose them to activated phagocytic cells and/or anti-*T. gondii* antibodies and complement, which would result in the killing of the parasite by phagocytosis, complement-dependent lysis, and/or opsonization (1, 16, 24). In addition, cytotoxic T lymphocytes (CTL) may exert a protective effect against *T. gondii* by the production of such cytokines as gamma interferon, which would then result in the killing or inhibition of multiplication of the parasite within macrophages (9). We cannot, however, dismiss the possibility that lysis of *T. gondii*-infected cells may be detrimental to the host. Yang et al. have proposed that lysis of *T. gondii*-infected melanoma cells by CD4⁺ T cells may play a role in the pathogenesis of toxoplasmic retinochoroiditis (31).

Demonstration of the presence of human CTL against *T. gondii*-infected cells may be of relevance to the pathogenesis of toxoplasmic encephalitis in AIDS patients. It has been reported that patients infected with human immunodeficiency virus type 1 (HIV-1) develop a progressive decrease in HIV-1-specific CTL activity (13) and in CTL activity against other intracellular pathogens, such as influenza and Epstein-Barr viruses (19), that are associated with the progression of HIV infection. Thus, these defects in CTL activity may lead to an increased susceptibility to opportunistic infections, increased HIV-1 replication, and the clinical transition from an asymptomatic state to AIDS (27). It remains to be determined if loss of CTL against *T. gondii*-infected cells is responsible, at least in part, for the recrudescence of latent *T. gondii* infection and the resulting development of toxoplasmic encephalitis in AIDS patients.

ACKNOWLEDGMENTS

This work was supported in part by grants AI04717 and AI30230 from the National Institutes of Health and by a MacArthur Foundation grant in molecular parasitology. C. S. Subauste is the recipient of grants from the American Foundation for AIDS Research and the University of California Universitywide AIDS Research Program.

We thank Gerard O'Donoghue and Ramila Philip for helpful discussions. We are grateful to Chiron Corp. for supplying us with recombinant interleukin 2.

REFERENCES

- Anderson, S. E. J., and J. S. Remington. 1974. Effect of normal and activated human macrophages on *Toxoplasma gondii*. J. Exp. Med. **139**:1154-1174.
- Bonifacio, J., M. Mercepe, J. Sussman, R. Klausner, and J. Ashwell. 1989. The T-cell antigen receptor: a complex signal transducing molecule, p. 87-104. In T. Hamaoka (ed.), Immune system and cancer. Science Society Press, Tokyo.
- Choi, Y., B. Kotzin, L. Herron, J. Callahan, P. Marrack, and L. Kappler. 1989. Interaction of Staphylococcus aureus toxin 'superantigens' with human T cells. Proc. Natl. Acad. Sci. USA **86**:8941-8945.
- Curiel, T., E. Krug, M. Purner, P. Poignard, and R. Berens. 1993. Cloned human CD4⁺ cytotoxic T lymphocytes specific for *Toxoplasma gondii* lyse tachyzoite-infected target cells. J. Immunol. **151**:2024-2031.
- Gazzinelli, R. T., F. T. Hakim, S. Hieny, G. M. Shearer, and A. Sher. 1991. Synergistic role of CD4⁺ and CD8⁺ T lymphocytes in IFN- γ production and protective immunity induced by an attenuated *Toxoplasma gondii* vaccine. J. Immunol. **146**:286-292.
- Hakim, F. T., R. T. Gazzinelli, E. Denkers, S. Hieny, G. M. Shearer, and A. Sher. 1991. CD8⁺ T cells from mice vaccinated against *Toxoplasma gondii* are cytotoxic for parasite-infected or antigen-pulsed host cells. J. Immunol. **147**:2310-2316.
- Ho, M., J. Armstrong, D. McMahon, G. Pazin, X.-L. Huang, C. Rinaldo, T. Whiteside, C. Tripoli, G. Levine, D. Moody, T. Okarma, E. Elder, P. Gupta, N. Tauxe, D. Torpey, and R. Heberman. 1993. A phase I study of adoptive transfer of autologous CD8⁺ T lymphocytes in patients with acquired immunodeficiency syndrome (AIDS)-related complex or AIDS. Blood **81**:2093-2101.
- Israelski, D. M., and J. S. Remington. 1993. Toxoplasmosis in patients with cancer. Clin. Infect. Dis. **17**:S423-435.
- Khan, I. A., K. A. Smith, and L. H. Kasper. 1990. Induction of antigen-specific human cytotoxic T cells by *Toxoplasma gondii*. J. Clin. Invest. **85**:1879-1886.
- Luft, B. J., G. Kansas, E. G. Engleman, and J. S. Remington. 1984. Functional and quantitative alterations in T lymphocyte subpopulations in acute toxoplasmosis. J. Infect. Dis. **150**:761-767.
- Morley, B., K. Chin, M. Newton, and A. Weiss. 1988. The lysine residue in the membrane-spanning domain of the β chain is necessary for cell surface expression of the T cell antigen receptor. J. Exp. Med. **168**:1971-1978.
- Naot, Y., and J. S. Remington. 1980. An enzyme-linked immunosorbent assay for detection of IgM antibodies to *Toxoplasma gondii*: use for diagnosis of acute acquired toxoplasmosis. J. Infect. Dis. **142**:757-766.
- Pantaleo, G., A. De Maria, S. Koenig, L. Butini, B. Moss, M. Baseler, H. Lane, and A. Fauci. 1990. CD8⁺ T lymphocytes of patients with AIDS maintain normal broad cytolytic function despite the loss of human immunodeficiency virus-specific cytotoxicity. Proc. Natl. Acad. Sci. USA **87**:4818-4822.
- Purner, M., E. Krug, P. Nash, D. Cook, R. Berens, and T. Curiel. 1995. Cross-reactivity of human *Toxoplasma*-specific T cells: implications for development of a potential immunotherapeutic or vaccine. J. Infect. Dis. **171**:984-991.
- Saavedra, R., and P. Herion. 1991. Human T-cell clones against *Toxoplasma gondii*: production of interferon- γ , interleukin-2, and strain cross-reactivity. Parasitol. Res. **77**:379-385.
- Sabin, A. B., and H. A. Feldman. 1948. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (toxoplasma). Science **108**:660-663.
- Scalise, F., R. Gerli, G. Castellucci, F. Spinazzi, G. Fabbietti, S. Crupi, L. Sensi, R. Britta, R. Vaccaro, and A. Bertotto. 1992. Lymphocytes bearing the $\gamma\delta$ T-cell receptor in acute toxoplasmosis. Immunology **76**:668-670.
- Sharma, S. D., J. R. Catterall, and J. S. Remington. 1986. Parasitocidal activity of macrophages against *Toxoplasma*. Methods Enzymol. **132**:626-637.
- Shearer, G., Z. Salahuddin, P. Markham, L. Joseph, S. Payne, P. Kriebel, D. Bernstein, W. Biddison, M. Sarngadharan, and R. Gallo. 1985. Prospective study of cytotoxic T lymphocyte responses to influenza and antibodies to human T lymphotropic virus-III in homosexual men. J. Clin. Invest. **76**:1699-1704.
- Sklenar, I., T. C. Jones, S. Alkan, and P. Erb. 1986. Association of symptomatic human infection with *Toxoplasma gondii* with imbalance of monocytes and antigen-specific T cell subsets. J. Pediatr. **108**:1-12.
- Smith, L., M. Rodrigues, and D. Russell. 1991. The interaction between CD8⁺ cytotoxic T cells and *Leishmania*-infected macrophages. J. Exp. Med. **174**:499-505.
- Subauste, C. S., J. Chung, D. Do, A. Koniari, C. Hunter, J. Montoya, S. Porcelli, and J. Remington. 1995. Preferential activation and expansion of human peripheral blood $\gamma\delta$ T cells in response to *Toxoplasma gondii* in-vitro and their cytokine production and cytotoxic activity against *T. gondii*-infected cells. J. Clin. Invest. **96**:610-619.
- Subauste, C. S., L. Dawson, and J. S. Remington. 1992. Human lymphokine-activated killer cells are cytotoxic against cells infected with *Toxoplasma gondii*. J. Exp. Med. **176**:1511-1519.
- Subauste, C. S., A. H. Koniari, and J. S. Remington. 1991. Murine CD8⁺ cytotoxic T lymphocytes lyse *Toxoplasma gondii*-infected cells. J. Immunol. **147**:3955-3959.
- Suzuki, Y., and J. S. Remington. 1988. Dual regulation of resistance against *Toxoplasma gondii* infection by Lyt-2⁺ and Lyt-1⁺, L3T4⁺ T cells in mice. J. Immunol. **140**:3943-3946.
- Suzuki, Y., and J. S. Remington. 1989. A method for obtaining large number of trophozoites of avirulent strains of *Toxoplasma gondii* using an antibody to interferon-gamma. J. Parasitol. **75**:174-176.
- Via, C., H. Morse III, and G. Shearer. 1990. HIV and the immune system. Altered immunoregulation and autoimmune aspects of HIV infection: relevant murine models. Immunol. Today **11**:250-255.

28. **Ware, P. L., and L. H. Kasper.** 1987. Strain-specific antigens of *Toxoplasma gondii*. *Infect. Immun.* **55**:778–783.
29. **Whiteside, T., E. Elder, D. Moody, J. Armstrong, M. Ho, C. Rinaldo, X.-L. Huang, D. Torpey, P. Gupta, D. McMahon, T. Okarma, and R. Heberman.** 1993. Generation and characterization of ex-vivo propagated autologous CD8⁺ cells used for adoptive immunotherapy of patients infected with human immunodeficiency virus. *Blood* **81**:2085–2092.
30. **Wong, S., and J. S. Remington.** 1994. Toxoplasmosis in the setting of AIDS, p. 223–257. *In* S. Broder, T. Merigan, Jr., and D. Bolognesi (ed.), *Textbook of AIDS medicine*. Williams and Wilkins, Baltimore.
31. **Yang, T.-H., F. Aosai, K. Norose, M. Ueda, and A. Yano.** 1995. Enhanced cytotoxicity of IFN- γ producing CD4⁺ cytotoxic T lymphocytes specific for *T. gondii*-infected human melanoma cells. *J. Immunol.* **154**:290–298.
32. **Yano, A., F. Aosai, M. Ohta, H. Hasekura, K. Sugane, and S. Hayashi.** 1989. Antigen presentation by *Toxoplasma gondii*-infected cells to CD4⁺ proliferative T cells and CD8⁺ cytotoxic cells. *J. Parasitol.* **75**:411–416.

Editor: S. H. E. Kaufmann