Destruction of the Multicellular Parasite *Schistosoma mansoni* by T Lymphocytes

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ABSTRACT The role of cytotoxic T lymphocytes in host defenses against infectious agents is unknown as these cells have not previously been demonstrated to kill microoganisms directly. We studied the cytotoxicity of T lymphocytes purified from peripheral blood mononuclear cells of healthy subjects for the multicellular schistosomula of *Schistosoma mansoni*. Unstimulated and phytohemagglutinin (PHA)-stimulated T cells were cultured with schistosomula at a 5,000:1 effector/target (E:T) ratio for 18 h at 37°C. Unstimulated T cells killed 2.1±0.6% of schistosomula as judged by dye uptake and did not change their infectivity for mice. In contrast, PHA-stimulated T cells killed 41.3±3.1% of schistosomula by dye uptake and 56.7±7.7% of these organisms could not mature to adult worms in vivo. Killing was associated with and dependent on increased binding of PHA-stimulated T lymphocytes to schistosomula. Significant schistosomula killing first was noted after 2 h of exposure of T cells to PHA and peaked at 24 h; enhanced killing by PHA-stimulated cells was observed at an E:T ratio of 500:1 and was maximal at 5,000:1. Exposure of T lymphocytes to oxidative mitogens, soluble antigens, and alloantigens also resulted in enhanced killing of schistosomula. These studies show that T lymphocytes activated by a variety of stimuli develop the capacity to kill schistosomula of *Schistosoma mansoni*. Direct killing of infectious agents by cytotoxic T cells may contribute to host resistance to infections.

INTRODUCTION

Human T lymphocytes spontaneously lyse certain tumor lines (1–4), thymus cells (3), embryonic fibroblasts (5), and virus-infected target cells (6–8). This natural killer (NK)[1]-cell-like activity can be augmented by a number of stimuli such as allogeneic cells (9–10), lectins (11–13), oxidative mitogens (14, 15), bacterial lipopolysaccharide (16), interferon (17–19), and soluble antigens (20–21). Recent evidence suggests that cells expressing nonspecific cytotoxicity after such activating exposures differ in a number of substantive ways from NK cells including their cellular derivation (22) and target specificity (23). Despite the demonstration of cytotoxicity of T lymphocytes for a variety of unicellular targets, direct killing of infectious agents has not been documented.

We studied the capacity of T lymphocytes to kill schistosomula of *Schistosoma mansoni*. These large multicellular larvae are destroyed by granulocytes (24–26) and mononuclear phagocytes (28–30), cell lines ascribed effector function in host resistance to schistosomiasis (31–33). Our studies show that human T lymphocytes activated by lectins and oxidative mitogens, tuberculin PPD, streptokinase-streptodornase, schistosome worm antigenic preparation, and alloantigens kill schistosomula. This is consonant with a direct role for cytotoxic lymphocytes in host defenses against infectious agents.

METHODS

Human subjects. Healthy volunteers from Cleveland, OH donated blood for all studies except those evaluating responses to schistosome soluble worm antigenic preparation (SWAP). Egyptians with chronic

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[1] *Abbreviations used in this paper*: BSA, bovine serum albumin; FCS, fetal calf serum; GO, galactose oxidase; HBSS, Hanks' balanced salt solution; MLR, mixed lymphocyte reaction; PHS, pooled human serum; NK, natural killer; PAG, peanut agglutinin; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; PPD, tuberculin purified protein derivative; SKSD, streptokinase-streptodornase; SPA, *Staphylococcus aureus* protein A; SWAP, soluble worm antigenic preparation.

schistosomiasis mansoni were used as blood donors for the latter studies.

Cell preparations. Peripheral blood mononuclear cells (PBMC) were prepared by Ficoll-Hypaque sedimentation of heparinized blood. They were depleted of adherent cells by sequential incubation in plastic Petri dishes and 600-mg acid-washed nylon wool columns. The resulting T lymphocyte-enriched population consisted of >85% sheep erythrocyte-rosetting cells, <1% B lymphocytes, and <0.5% monocytes (cells with nonspecific cytoplasmic esterase activity).

Separation of the T lymphocyte population by surface receptors for the Fc portion of immunoglobulin G (IgG) was accomplished by preparative rosetting with ox erythrocytes (BRBC, Wilfer Laboratories, Stillwater, MN) sensitized with rabbit anti-BRBC IgG (N. L. Cappel Laboratories Inc., Cochranville, PA), followed by sedimentation over Ficoll-Hypaque (16). The interface T lymphocytes contained <1.0% rosetting cells. BRBC were lysed with buffered 0.83% NH_4Cl; the pellet contained >88% rosetting cells.

T lymphocytes also were fractionated by reactivity with monoclonal antibodies followed by indirect rosetting or complement-mediated cytolysis. T lymphocytes at 2.5×10^7/ml in Hanks' balanced salt solution (HBSS) with 0.2% bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, MO) were incubated with a 1/40 dilution of monoclonal antibodies OKT4, OKT8, or OKM1 (Ortho Pharmaceutical, Raritan, NJ) for 30 min at 4°C. The sensitized T lymphocytes were washed twice. BRBC coated with *Staphylococcus aureus* protein A (SPA, Pharmacia Fine Chemicals, Uppsala, Sweden) were prepared by coupling with chromic chloride (34). Packed SPA-BRBC were mixed with three parts T lymphocytes previously sensitized with monoclonal antibodies. The cells were pelleted at 200 *g* for 10 min at 4°C and incubated for 30 min at 4°C. The pellet was resuspended gently, underlayered with Ficoll-Hypaque, and sedimented at 400 *g* for 30 min at room temperature. The interface T lymphocytes were <1.0% rosetting while the pellet contained >90.0% rosetting cells. BRBC were lysed with 0.83% NH_4Cl.

For complement-mediated cytolysis, the washed, antibody-sensitized T lymphocytes were resuspended at 2.5×10^7/ml in RPMI 1640 and combined 3:1 with fresh rabbit complement (Low Tox H, Cederlane Laboratories, Hornsby, Ontario, Canada). The mixture was incubated at 20°C for 60 min then washed three times in cold RPMI 1640. Purity of cell populations was assayed by indirect immunofluorescence using a fluorescein-conjugated $F(ab)'_2$ fragment of rabbit anti-mouse IgG (N. L. Cappel Laboratories).

T lymphocyte activation. T lymphocytes were activated by exposure to mitogens, soluble antigens, or alloantigens before addition of schistosomula. Concentration of mitogens and antigens selected were those that produced maximum DNA synthesis in cultured lymphocytes. T cells were cultured at 10^6/ml in RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS; KC Biologicals) in 16-mm tissue culture plate wells. PHA-M (1/100 dilution, Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) or concanavalin A (Con A, 10 μg/ml, Pharmacia Fine Chemicals) was added to the wells and the plates were incubated 24 h at 37°C. Activation of T cells with the oxidative mitogens required preincubation of T cells with neuraminidase (50 U/ml, *Clostridia perfringens* Fraction VI, Sigma Chemical Co.) or galactose oxidase (GO, 1.4 U/ml, Sigma Chemical Co.) or both for 30 min at 37°C. The cells were washed and cultured for 24 h in RPMI 1640 with 10% FCS. Peanut agglutinin (PAG, 125 μg/ml, Sigma Chemical Co.) was added to T cells that had been pretreated with neuraminidase.

To study activation by soluble antigens, T cells were cultured at 2×10^6/ml in RPMI 1640 with 10% heat-inactivated pooled human serum (PHS) for 5 d in 12 × 75-mm plastic tubes (Falcon Labware, Div. Becton Dickinson & Co., Oxnard, CA) with tuberculin purified protein derivative (PPD) at 100 μg/ml (gift of Lederle, Pearl River, NY) or streptokinase-streptodornase (SKSD) 200 U streptokinase/ml (Lederle). T cells then were washed, counted, and 1 ml at 10^6/ml in RPMI 1640 plus 10% FCS was added to each of 16-mm plastic wells. For activation with SWAP, T cells were cultured with SWAP 100 μg/ml (purified as described in reference 35) in RPMI 1640 plus 10% FCS for 48 h at 37°C.

Mixed lymphocyte reactions (MLR) were effected by 6-d bulk cultures of allogeneic PBMC irradiated with 3,000 rads as stimulators and T cells as responders, each at a cell density of 3.0×10^6/ml in RPMI 1640, plus 10% AB serum. Nonadherent cells were purified from these cell mixtures, washed, counted, and 1 ml at 10^6/ml in RPMI 1640 plus 10% FCS was added to each of 16-mm plastic wells.

Assays of schistosomula killing. Schistosomula of *S. mansoni* were prepared by allowing cercaria from a Puerto Rican stain of the parasite to penetrate mouse skin. 10^6 preactivated T cells were cultured with 200 schistosomula in RPMI 1640 supplemented with 10% FCS in each of 16-mm Linbro plastic wells. Triplicate wells were used for each variable. After 24 h at 37°C, the contents of wells were transferred to Sedgwick-Rafter counting chambers and methylene blue dye was added. Schistosomula that were permeable to the dye were scored as dead. 50 schistosomula were evaluated per well within 5 min of addition of dye. The basal

loss of viability of schistosomula cultured in medium without cells (1–7%) was subtracted from the killing in wells containing effector cells.

Infectivity of schistosomula for mice was determined by injecting the organisms into CF-1 mice (Carsworth Farms, NY). 8–10 mice were used per variable. After 8 wk, the animals were sacrificed, and adult worms were recovered from the portal circulation and counted.

[^3H]thymidine incorporation. T cells were cultured in microtiter plate wells (Falcon Labware) at 10^5/0.1 ml RPMI 1640 plus 10% PHS, gentamicin 5 μg/ml, penicillin G 50 U/ml and L-glutamine 2 mM (Flow Laboratories, Inc.). Mitogens or antigens were added at the appropriate concentrations to each of quadruplicate wells. 18 h before the termination of the 3-d cultures, [*methyl*-^{3}H]thymidine, 1.0 μCi (5.0 Ci/mmol, Radiochemical Centre, Amersham, England) was added per well. The cells were harvested onto glass filter paper and washed with a MASH microharvester (Microbiological Associates, Rockville, MD). The filter paper discs then were placed in ACS scintillant (Amersham) and ^{3}H content was determined by liquid scintillation spectroscopy. [^{3}H]thymidine incorporation was expressed as counts per minute.

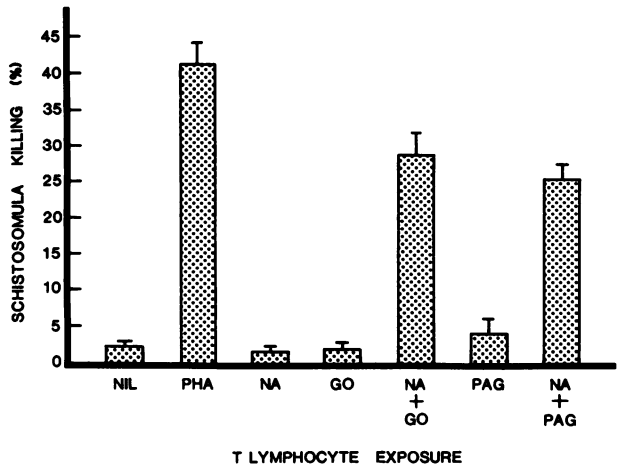

FIGURE 1 Schistosomula killing by T lymphocytes activated by lectins and oxidative mitogens assessed by dye uptake. T cells were cultured with PHA or PAG continuously and briefly exposed to neuraminidase and/or galactose oxidase. 24 h after addition of 200 schistosomula to 10^6 T cells, killing was determined by dye uptake. Data are shown as mean schistosomula killing by T cells (parasite death in medium alone has been subtracted) ±SEM. 15 experiments were performed with PHA-activated T cells, 7 with N-GO, and 2 with N-PAG activation. In each instance, exposure to mitogenic stimuli produced significant increments in schistosomula killing ($P < 0.001$, Student's t test).

RESULTS

Killing of schistosomula by mitogen-activated T lymphocytes. Preincubation of T cells with PHA produced a 19.4-fold increase in schistosomula killing as assessed by methylene blue dye uptake (Fig. 1). Viable organisms were motile, retained a yellowish cast, and excluded the dye (Fig. 2, left panel), whereas, dead organisms were nonmotile, surrounded by T lymphocytes, and permeable to the dye (Fig. 2, right panel). Quantitative studies showed that following PHA stimulation 94% of dead schistosomula had at least five T lymphocytes adhering to them (Table I). Mitogen-stimulation did not lead to increased killing of schistosomula with less than five attached cells. Live schistosomula invariably had less than five adhering cells. In additional studies, supernates of PHA-stimulated lymphocytes were not directly toxic for schistosomula. Thus, increased binding of PHA-stimulated T cells to schistosomula was required for increased killing. We studied the effects of other mitogens and lectins on T lymphocyte killing of the parasite. Exposure of T cells to neuraminidase plus galactose oxidase produced maximal mitogenesis whereas exposure to either enzyme alone had less effect (Table II). Schistosomula killing by T cells preincubated with these oxidative mitogens paralleled mitogenesis (Fig. 1); neuraminidase (N)-GO treatment produced 13.4-fold increased schistosomula killing whereas the individual enzymes had no effect. Thus, nonlectin mitogenic signals also activated T cells for increased effector function. We also studied peanut agglutinin, a galactosyl-directed lectin, which required neuraminidase treatment of T cells to exert maximal mitogenesis (Table II). Similarly, PAG produced an 11.9-fold increment in schistosomula killing by neuraminidase-treated T lymphocytes (Fig. 1). Exposure of schistosomula to mitogens without cells for 18 h did not affect their viability. Similarly, pretreatment of schistosomula with PHA or N-GO had no effect on their susceptibility to killing during subsequent culture with either untreated or PHA-activated T lymphocytes.

We next studied conditions of T cell activation by mitogens. When T cells were cultured with PHA for 24 h and washed they demonstrated 49.5±4.9% as much killing of schistosomula as when PHA was present throughout culture (Table III). To further examine reversibility of lectin-induced T cell schistosomula killing, T cells were exposed to Con A for 24 h and washed and cultured in α-methyl mannoside (Table III). In this instance, the effect of the lectin on T cell killing of the larvae was completely reversible.

The kinetics of augmentation of T cell killing by PHA are shown in Fig. 3. A significant increase in

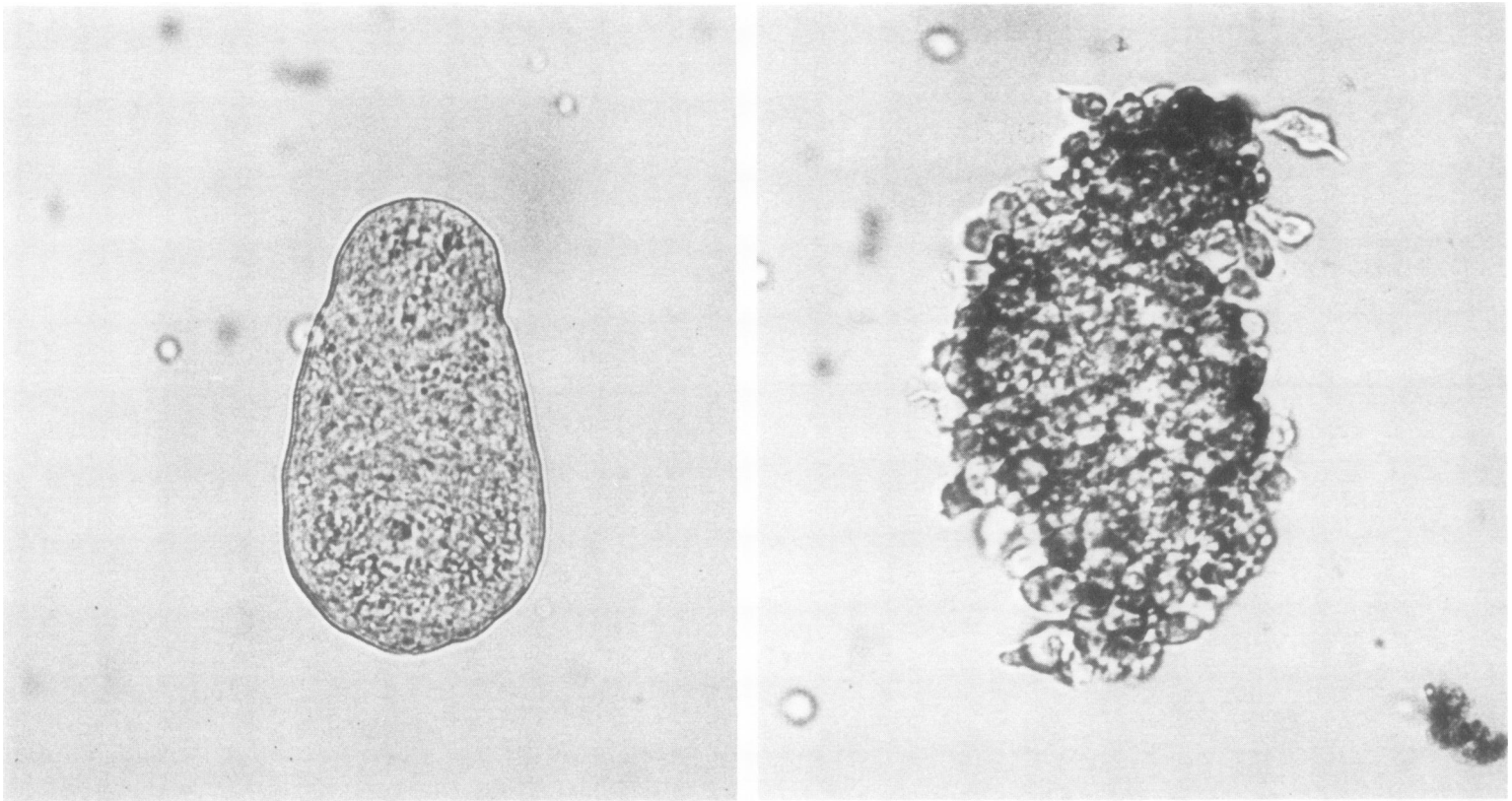

FIGURE 2 Morphologic appearance of schistosomula exposed to unstimulated and PHA-stimulated lymphocytes for 24 h. Left panel. Schistosomula cultured with unstimulated T cells. The organism was impermeable to the dye and motile and would be scored as viable. Right panel. Schistosomula cultured with PHA-stimulated T cells. The organism, which appeared disrupted, was encased by cells, granular and nonhomogeneous in appearance, and permeable to the dye. Magnification ×400.

TABLE I

Effect of PHA on T Lymphocyte Binding to and Killing of Schistosomula

	T lymphocyte exposure*			
	Nil		PHA	
	Dead schistosomula	Dead larvae with ≧ 5 adhering cells	Dead schistosomula	Dead larvae with ≧ 5 adhering cells
	% *mean±SEM*	%	% *mean±SEM*	%
Exp. 1	8.0±0.3	0	54.0±0.3	88
2	8.9±0.6	0	55.2±5.4	95
3	7.9±0.4	0	66.8±6.6	100
4	6.2±1.3	11	50.2±4.4	92

* 200 schistosomula were added to wells containing 10^6 T cells preincubated for 24 h in RPMI 1640 plus 10% FCS with or without PHA. After an additional 24 h, schistosomula death was assessed by dye uptake and the adherence of cells to dead schistosomula scored. Loss of viability of schistosomula in medium without effector cells was 5.3±1.9% in these experiments.

schistosomula killing first was noted by 2 h of culture ($P < 0.01$). Killing at 4 h was 66% of maximal. Following N-GO treatment of T cells, schistosomula killing was 15% of maximal at 4 h and peaked at 24 h (not shown).

PHA-stimulated T cells demonstrated significantly increased schistosomula killing at an effector cell to target ratio of 500:1 ($P < 0.02$, Fig. 4). Optimal killing occurred at a ratio of 5,000:1, which was used in most experiments.

In three separate experiments, schistosomula killing by PHA-stimulated T cells was assayed simultaneously by dye uptake and infectivity (Table IV). A significant decrease in maturation to adult worms was noted in schistosomula cultured with PHA-stimulated as compared to unstimulated T cells ($P < 0.05$, paired t statistic). The in vitro technique of dye uptake consistently underestimated loss of infectivity of schistosomula exposed for 24 h to PHA-activated T cells. Additional groups of mice were infected with schistosomula which had been exposed to T cells treated with neuraminidase and incubated with peanut agglutinin or treated with galactose oxidase. Adult worm recovery was 49.6±6.6 in mice infected with schistosomula exposed to unstimulated T cells, 31.2±2.4 with N-PAG treated T cells ($P < 0.05$) and 31.9±4.7 for N-GO treated cells (not significant).

T cell subpopulations responsible for augmented schistosomula killing following PHA-treatment. T lymphocytes were fractionated to determine the sub-

TABLE II

[^3H]Thymidine Incorporation of T Lymphocytes Stimulated with Lectins and Oxidative Mitogens

	Experiment		
Exposures	1	2	3
	[^{3}H]thymidine incorporation, *mean cpm±SD*		
Nil	1,484±388	1,818±444	544±149
PHA	76,188±11,282	116,732±9,716	ND*
Neuraminidase	1,191±53	1,368±99	1,135±68
Galactose oxidase	5,536±399	10,520±1,746	12,341±2,289
N-GO	35,071±3,370	101,018±6,517	90,166±7,597
Peanut agglutinin	1,329±184	2,108±688	ND
N-PAG	14,064±1,284	13,302±1,928	ND

* Not done.

TABLE III

Reversibility of Lectin-Mediated Activation of T Cells to Kill Schistosomula by Washing or Addition of α-Methyl Mannoside before Determination of Schistosomula Killing

Experiment	First 24 h: / Next 24 h:	T lymphocyte exposure* Nil / Nil	PHA / Nil	PHA / PHA	Con A α-methyl mannoside / Con A	Con A / Con A
		Percent dead schistosomula, *mean±SD*				
1		0.7±1.1	30.0±6.0	47.0±3.6	1.3±1.1	31.0±4.6
2		2.3±0.6	14.7±1.5	27.3±3.1	2.7±3.1	27.3±6.7
3		5.7±1.5	21.7±1.5	41.0±4.4	ND†	ND
4		0.3±1.0	20.3±7.1	50.0±5.6	ND	ND

* T lymphocytes were cultured in 12 × 75-mm plastic tubes for 24 h with the appropriate lectins. They then were washed five times; cells cultured with con A were washed with 0.1 mM α-methyl mannoside (Sigma Chemical Co.). The T cells were counted and 10^6 added to wells with schistosomula and the appropriate additions for 24 h.

† Not done.

sets responsible for increased schistosomula killing by PHA-activated effector cells. Incubation of schistosomula with unstimulated T, $OKT8^+$, and $OKM1^+$ cells resulted in 2.7±0.6, 1.3±0.6, and 1.0±1.0% dye uptake, respectively. Following incubation of fractionated cells with PHA for 24 h, killing by the $OKT8^+$ population exceeded that of all other subsets (Table V). The effector potential of PHA-activated T cell subsets, in fact, could be ordered as $OKT8^+ > T\gamma^- > T > OKT4^+ > T\gamma^+ = OKM1^+$. In contrast to increased schistosomula killing by lectin-stimulated T lymphocytes, pokeweed mitogen stimulation of a B lymphocyte-enriched population prepared by depletion of plastic adherent and sheep erythrocyte rosetting cells from PBMC did not augment schistosomula killing significantly.

Schistosomula killing by antigen-activated T lymphocytes. We also examined larval killing by T lymphocytes stimulated by PPD, SKSD, SWAP and alloantigens. Culture of T cells from healthy subjects with PPD or SKSD produced 7.0-fold and 5.0-fold increases in schistosomula killing (Fig. 5). Schistosome

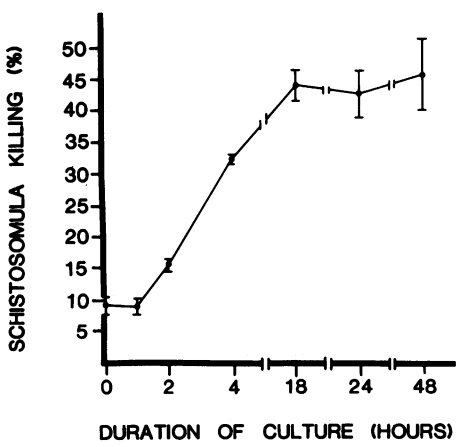

FIGURE 3 Kinetics of activation of T cell schistosomula killing by PHA assessed by dye uptake. PHA was added to 10^6 T cells and 200 schistosomula at t_0. Schistosomula killing was determined after the appropriate time intervals as dye uptake. Data are presented as the mean±SD of triplicate determinations. Loss of viability of schistosomula cultured in medium alone was 9.3±2.1% at 48 h.

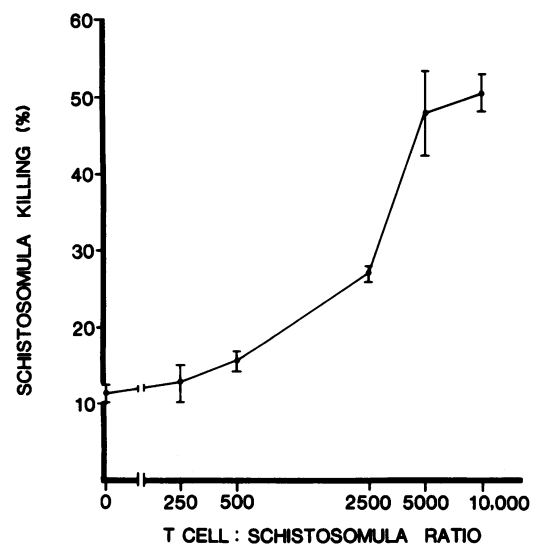

FIGURE 4 Effect of T cell/schistosomula ratio on parasite killing determined by dye uptake. 200 schistosomula were added to various numbers of PHA-stimulated T cells. Data are shown as mean±SEM of two experiments.

TABLE IV

*Schistosomula Killing by PHA-stimulated T Lymphocytes Determined In Vitro by Dye Uptake and In Vivo by Infectivity for Mice**

Experiment	Dye uptake	Adult worm recovery		
		Unstimulated T lymphocytes	PHA-stimulated T lymphocytes	Decrease
	% Dead Schistosomula (mean±SEM)	Adult worms recovered per mouse (mean±SEM)		
1	23.7±4.9	38.9±12.2	10.9±3.4	72%
2	34.0±1.0	25.8±2.9	12.7±3.7	51%
3	34.5±0.7	49.6±6.6	26.1±3.7	47%
Mean	30.7±3.5	—	—	56.7±7.7%

* After incubation of 100 schistosomula with 10^6 T lymphocytes±PHA for 24 h, schistosomula killing was assessed by dye uptake. The contents of individual wells also were injected intraperitoneally into CF-1 mice. 8–10 mice were injected per variable. Adult worms were recovered from the portal veins after 8 wk and counted.

worm antigen did not affect killing by T cells from healthy subjects in Cleveland or Cairo (not shown). However, SWAP produced a significant 5.5-fold increase in schistosomula killing in 11 of 31 Egyptian subjects with chronic *Schistosomiasis mansoni*.

Stimulation of T cells with alloantigens in bulk MLR cultures produced 8.9-fold more killing than did autologous control cultures (Fig. 5).

DISCUSSION

These studies establish that human T lymphocytes activated by mitogens, soluble antigens, and alloantigens express nonspecific cytotoxicity for the larval stage of the multicellular helminth *Schistosoma mansoni*. Schistosomula killing by T cells was defined by dye uptake and corroborated by infectivity for mice. This is the first demonstration of the capacity of human cytotoxic T cells to kill an infectious agent directly. Cytotoxic T cells may have an indirect role in viral immunity via lysis of virus-infected host cells (6–8). T lymphocytes also are capable of destroying *Shigella* opsonized with hyperimmune rabbit antibody (36). In the current study, however, direct cytotoxicity for a multicellular parasite occurred without opsonization of the target organism.

Following alloimmunization, murine cytotoxic T lymphocytes adhered to but did not morphologically damage lung stage schistosomula (37). This specific cytotoxicity required that cytotoxic T lymphocytes be immunized with cells bearing H-2 determinants of the mouse strain in which schistosomula matured to lung

TABLE V

Schistosomula Killing by PHA-stimulated T Cell Subpopulations Determined by Dye Uptake

Experiment	T	T Lymphocyte Subpopulations			
		$OKT8^+$	$OKT8^-/OKT4^+$	$T\gamma^-$	$T\gamma^+/OKM1^+$
		Percent dead schistosomula (mean±SEM)			
1*	40.7±1.8	63.3±3.0‡	37.0±1.0	48.7±2.4	29.0±2.1
2	27.3±1.2	31.0±2.1‡	22.5±0.5	ND	21.7±0.9

* In Exp. 1, T cells were fractionated by rosetting techniques into $OKT8^+$, $OKT8^-$, $T\gamma^+$, and $T\gamma^-$ subpopulations. In Exp. 2, complement-dependent cytolysis was used to prepare $OKT8^+$, $OKT4^+$, and $OKM1^+$ populations by depletion of the reciprocal subsets. The purity of the T cell populations in Exp. 1 and 2, respectively, was: $OKT8^+$: 88%, 86% $OKT8^+$; $OKT8^-/OKT4^+$: 88%, 67% $OKT4^+$, 6%, 4% $OKT8^+$; OKM1: 75% OKM1. The T cell populations were cultured with PHA for 18 h before addition of schistosomula.

‡ Exceeds killing by $OKT8^-/OKT4^+$ ($P < 0.01$), $T\gamma^+$ ($P < 0.01$), and OKM1 ($P < 0.02$) subpopulations.

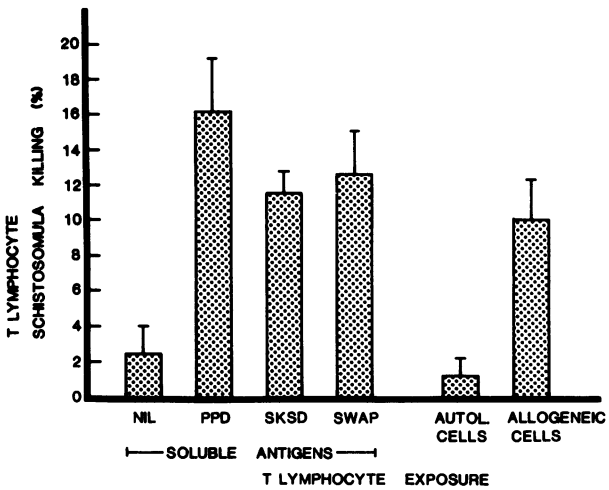

FIGURE 5 Schistosomula killing by T cells stimulated by soluble antigens and alloantigens assessed by dye uptake. T lymphocytes were incubated with PPD, SKSD, SWAP, or irradiated allogeneic PBMC. Schistosomula killing was determined as dye uptake after 24-h incubation with unstimulated or activated T cells. The data are expressed as mean schistosomula killing by T cells (parasite death in medium alone subtracted) ±SEM for six studies with PPD, eight with SKSD, and six allogeneic pairs of cells, all performed in healthy subjects in Cleveland. SWAP-induced T cell killing is presented as mean±SEM for 11 subjects from Cairo.

stage. Thus, it presumably depended on acquisition by schistosomula of H-2 antigens in vivo. Lectin-stimulated human T lymphocytes showed increased adherence to and also killing of fresh schistosomula. The basis for the greater lethality of interaction may be related to species, stimulus, or parasite stage, all of which differed from the mouse studies. In contrast to unicellular targets, damaged metazoan parasites do not undergo rapid cytolysis; however, morphologic damage assessed by dye uptake after exposure of schistosomula to activated human T cells, in fact, underestimated the loss of infectivity for mice, which is the most rigorous index of damage to schistosomula.

Schistosomula killing by PHA-stimulated T lymphocytes was a rapid event, first apparent by 2 h of culture of T lymphocytes, schistosomula, and PHA, and maximal at 18 h. The kinetics of stimulation of schistosomula killing resembled those of PHA-induced augmentation of the spontaneous cytotoxicity of T lymphocytes for the myeloblastoid line K562, which was apparent at 2 h and maximal by 24 h.[2] It was partially reversed by washing PHA-stimulated effector cells before adding targets. However, schistosomula killing by con A-stimulated T cells was abrogated by washing and culture in α-methyl mannoside. The incomplete reversibility of PHA-augmented cytotoxicity could result from bridging of effector cells and targets by PHA on the T cell surface or different activity of this lectin. In either case, the absence of augmented susceptibility of PHA-treated schistosomula to killing by unstimulated T cells suggests that lectin bridging is not sufficient for increased killing. Moreover, T cells activated by soluble antigens and alloantigens also showed increased cytotoxicity for schistosomula.

In the current studies, $OKT8^+$ cells exhibited the most efficient cytotoxicity for schistosomula after stimulation with PHA. This is in keeping with two recent findings. First, lectin-dependent cytotoxicity for tumor targets can be effected through specific cytotoxic T cells (15, 38, 39), which are $OKT8^+$ $T\gamma^-$ in human systems (40, 41). Second, augmented natural killer cell-like activity is expressed through cells other than $OKM1^+$ $T\gamma^+$, which demonstrate NK activity in the unstimulated state (23, 40). Lectin-dependent cytotoxicity of T cells for schistosomula also was expressed in the $OKT4^+$, $OKM1^+$, and $T\gamma^+$ subsets, but to a lesser extent. Thus, schistosomula killing was expressed variably in all T lymphocyte subsets after stimulation by PHA and was not restricted to classical $OKT8^+$ cytotoxic lymphocytes.

The mechanism of schistosomula or tumor killing by augmented, NK-like activity is unknown. Hydrogen peroxide (42), eosinophil major basic protein (43), and arginase (44) have been proposed as mediators by which granulocytes and activated macrophages destroy schistosomula. Activated T lymphocytes do not produce measurable quantities of arginase.[3] It seems unlikely that depletion of other essential nutrients or release of toxic metabolic products by activated T cells contributed to cytotoxicity because killing required direct apposition of effector cells and targets and supernates of activated cells were nontoxic for schistosomula. Rather, these observations suggest that if mediators released by activated T cells such as lymphotoxins (45, 46) are important in killing this multicellular organism, their release must be triggered by contact with the target or their activity must require high concentrations only attained locally.

Schistosomula killing by activated T lymphocytes peaks at the same effector to target ratio as the other major effector systems: granulocytes plus antibody or complement and mononuclear phagocytes (31, 44). In vivo studies in experimental models also support a role for T lymphocytes in host resistance to schistosomiasis; thus, antithymocyte globulin-treated mice (48) and congenitally athymic mice (49) fail to acquire resis-

[2] Schacter, B. Z. Personal communication.

[3] Ellner, J. J. Manuscript in preparation.

tance to this parasite. However, interpretation of the in vivo data is complicated by the multiple functions ascribed to the T lymphocyte in the murine infection, which include macrophage activation, granuloma formation, and the development of eosinophilia (27, 49). Thus, the precise contribution of T cell damage to *S. mansoni* to host resistance cannot be ascertained. However, our study establishes for the first time that T lymphocytes have the capability of such direct involvement in the destruction of a multicellular parasite.

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REFERENCES

1. Takasugi, M., M. R. Mickey, and P. I. Terasaki. 1973. Reactivity of lymphocytes from normal persons on cultured tumor cells. *Cancer Res.* **33:** 2898–2902.
2. Jondal, M., and H. Pross. 1975. Surface markers in human B and T lymphocytes. VI. Cytotoxicity against cell lines as a functional marker for lymphocyte subpopulations. *Int. J. Cancer* **15:** 596–605.
3. Ono, A., D. B. Amos, and H. S. Koren. 1977. Selective cellular natural killing against human leukemic T cells and thymus. *Nature (Lond.).* **266:** 546–548.
4. Rosenberg, E. B., J. L. McCoy, S. S. Green, F. C. Donnelly, D. F. Siwarski, P. H. Levine, and R. B. Herberman. 1974. Destruction of human lymphoid tissue-culture cell lines by human peripheral lymphocytes in ^{51}Cr-release cellular cytotoxicity assays. *J. Natl. Cancer Inst.* **52:** 345–352.
5. Timonen, T., and E. Saksela. 1977. Human natural cell-mediated cytotoxicity against fetal fibroblasts. *Cell. Immunol.* **33:** 340–352.
6. Labowskie, R. J., R. Edelman. R. Rustigan, and J. A. Bellanti. 1974. Studies of cell-mediated immunity to measles virus by *in vitro* lymphocyte-mediated cytotoxicity. *J. Infect. Dis.* **129:** 233–239.
7. Andersson, T., V. Steiskal, and B. Harfast. 1975. An *in vitro* method for study of human lymphocyte cytotoxicity against mumps-virus-infected target cells. *J. Immunol.* **114:** 237–243.
8. Santoli, D., G. Trinchieri, and F. S. Lief. 1978. Cell-mediated cytotoxicity against virus-infected target cells in humans. I. Characterization of the effector lymphocyte. *J. Immunol.* **121:** 526–531.
9. Callewaert, D. M., J. J. Lightbody, J. Kaplan, J. Jaroszewski, W. D. Peterson, Jr., and J. C. Rosenberg. 1978. Cytotoxicity of human peripheral lymphocytes in cell-mediated lympholysis: antibody-dependent cell-mediated lympholysis and natural cytotoxicity assays after mixed lymphocyte culture. *J. Immunol.* **121:** 81–85.
10. Seeley, J. K., and S. H. Golub. 1978. Studies on cytotoxicity generated in human mixed lymphocyte cultures. I. Time course and target spectrum of several distinct concomitant cytotoxic activities. *J. Immunol.* **120:** 1415–1422.
11. Holm, G., P. Perlmann, and B. Werner. 1964. Phytohaemagglutinin-induced cytotoxic action of normal lymphoid cells in cells in tissue culture. *Nature (Lond.).* **203:** 841–843.
12. Nelson, D. S., B. M. Bundy, H. E. Pitchon, R. M. Blaese, and W. Strober. 1976. The effector cell in human peripheral blood mediating mitogen-induced cellular cytotoxicity and antibody-dependent cellular cytotoxicity. *J. Immunol.* **117:** 1472–1481.
13. Asherson, G. L., J. Ferluga, and G. Janossy. 1973. Nonspecific cytotoxicity by T-cells activated with plant mitogens *in vitro* and the requirement for plant agents during the killing reaction. *Clin. Exp. Immunol.* **15:** 573–589.
14. Novogrodsky, A. 1975. Induction of lymphocyte cytotoxicity by modification of the effector or target cells with periodate or with neuraminidase and galactose oxidase. *J. Immunol.* **114:** 1089–1093.
15. Bradley, T. P., and B. Bonavida. 1981. Mechanisms of cell-mediated cytotoxicity at the single cell level. III. Evidence that cytotoxic T-lymphocytes lyse both antigen-specific and nonspecific targets pretreated with lectins or periodate. *J. Immunol.* **126:** 208–213.
16. Schacter, B. Z., M. E. Kleinhenz, K. Edmonds, and J. J. Ellner. 1981. Spontaneous cytotoxicity of human peripheral blood mononuclear cells for the lymphoblastoid cell line CCRF-CEM: augmentation by bacterial lipopolysaccharide. *Clin. Exp. Immunol.* **46:** 640–648.
17. Santoli, D., G. Trinchieri, and H. Koprowski. 1978. Cell-mediated cytotoxicity against virus-infected target cells in humans. II. Interferon induction and activation of natural killer cells. *J. Immunol.* **121:** 532–538.
18. Zarling, J. M., L. Eskra, E. C. Borden, J. Horoszewicz, and W. A. Carter. 1979. Activation of human natural killer cells cytotoxic for human leukemia cells by purified interferons. *J. Immunol.* **123:** 63–70.
19. Herberman, R. B., J. R. Ortaldo, and G. D. Bonnard. 1979. Augmentation by interferon of human natural and antibody-dependent cell-mediated cytotoxicity. *Nature (Lond.).* **277:** 221–223.
20. Butterworth, A. E. 1973. Nonspecific cytotoxic effects of antigen-transformed lymphocytes. Kinetics, cell-requirement and the role of recruitment. *Cell. Immunol.* **7:** 357–369.
21. Tartof, D., I. J. Check, A. Matutsis, R. H. Hunter, and F. W. Fitch. 1980. Studies on stimulation of cell-mediated cytotoxicity by skin test antigens. I. Candida antigen stimulation of cell-mediated cytotoxicity *in vitro* correlated with the skin test response to Candida antigen *in vivo*. *J. Immunol.* **125:** 2790–2796.
22. Seeley, J. K., G. Masucci, A. Poros, E. Klein, and S. H. Golub. 1979. Studies of cytotoxicity generated in human mixed lymphocyte cultures. II. Anti-K562 effectors are distinct from allospecific CTL and can be generated from NK-depleted T-cells. *J. Immunol.* **123:** 1303–1311.
23. Masucci, M. G., E. Klein, and S. Argov. 1980. Disap-

pearance of the NK-effect after explantation of lymphocytes and generation of similar nonspecific cytotoxicity correlated to the level of blastogenesis in activated cultures. *J. Immunol.* **124:** 2458–2463.
24. Butterworth, A. E., R. F. Sturrock, V. Houba, A. A. F. Mahmoud, A. Sher, and P. H. Rees. 1975. Eosinophils as mediators of antibody-dependent damage to schistosomula. *Nature (Lond.).* **256:** 727–729.
25. Dean, D. A., R. Wistar, and P. Chen. 1975. Immune response of guinea pigs to *Schistosoma mansoni*. I. *In vitro* effects of antibody and neutrophils, eosinophils and macrophages on schistosomula. *Am. J. Trop. Med. Hyg.* **24:** 74–82.
26. Anwar, A. R. E., S. R. Smithers, and A. B. Kay. 1979. Killing of schistosomula of *Schistosoma mansoni* coated with antibody and/or complement by human leukocytes *in vitro*: requirement for complement in preferential killing by eosinophils. *J. Immunol.* **122:** 628–637.
27. Bout, D. T., M. Joseph, J. R. David, and A. R. Capron. 1981. *In vitro* killing of *S. mansoni* schistosomula by lymphokine-activated mouse macrophages. *J. Immunol.* **127:** 1–5.
28. Joseph, M., A. Capron, A. E. Butterworth, R. F. Sturrock, and V. Houba. 1978. Cytotoxicity of human and baboon mononuclear phagocytes against schistosomula *in vitro*: induction by immune complexes containing IgE and *Schistosoma mansoni* antigens. *Clin. Exp. Immunol.* **33:** 48–56.
29. Mahmoud, A. A. F., P. A. S. Peters, R. H. Civil, and J. S. Remington. 1979. *In vitro* killing of schistosomula of *Schistosoma mansoni* by BCG and *C. parvum* activated macrophages. *J. Immunol.* **122:** 1655–1657.
30. Ellner, J. J., and A. A. F. Mahmoud. 1979. Killing of schistosomula of *Schistosoma mansoni* by normal human monocytes. *J. Immunol.* **123:** 949–951.
31. Mahmoud, A. A. F., K. S. Warren, and P. A. Peters. 1975. A role for the eosinophil in acquired resistance to *Schistosoma mansoni* infection as determined by antieosinophil serum. *J. Exp. Med.* **142:** 805–813.
32. Civil, R. H., K. S. Warren, and A. A. F. Mahmoud. 1978. Conditions for Bacille Calmette-Guérin-induced resistance to infection with *Schistosoma mansoni* in mice. *J. Infect. Dis.* **132:** 550–555.
33. Olds, G. R., and A. A. F. Mahmoud. 1980. Role of host granulomatous response in murine schistosomiasis mansoni: eosinophil-mediated destruction of eggs. *J. Clin. Invest.* **66:** 1191–1199.
34. Ginsburg, W. W., F. D. Finkelman, and P. E. Lipsky. 1978. Circulating and mitogen-induced immunoglobulin-secreting cells in human peripheral blood: evaluation by a modified reverse hemolytic plaque assay. *J. Immunol.* **120:** 33–39.
35. Colley, D. G., J. A. Cook, G. L. Freeman, Jr., R. K. Bartholomew, and P. Jordan. 1977. Immune responses during human schistosomiasis mansoni. I. *In vitro* blastogenic responses to heterogenous antigen preparations from schistosome eggs, worms and cercariae. *Int. Arch. Allergy Appl. Immunol.* **53:** 420–433.
36. Lowell, G. H., R. P. MacDermott, P. L. Summers, A. A. Reeder, M. J. Bertovich, and S. B. Formal. 1980. Antibody-dependent cell mediated antibacterial activity: K lymphocytes, monocytes, and granulocytes are effective against *Shigella*. *J. Immunol.* **125:** 2778–2784.
37. Brown, A. P., S. J. Burakoff, and A. Sher. 1980. Specificity of alloreactive T-lymphocytes that adhere to lung stage schistosomula of *Schistosoma mansoni*. *J. Immunol.* **124:** 2516–2518.
38. Beran, M. J., and M. Cohn. 1975. Cytotoxic effects of antigen- and mitogen-induced T-cells on various targets. *J. Immunol.* **114:** 559–565.
39. Rubens, R. P., and C. S. Henney. 1977. Studies on the mechanism of lymphocyte-mediated cytolysis. VIII. The use of Con A to delineate a distinctive killer T-cell population. *J. Immunol.* **118:** 180–186.
40. Zarling, J. M., and P. C. Kung. 1980. Monoclonal antibodies which distinguish between human NK cells and cytotoxic T-lymphocytes. *Nature (Lond.).* **288:** 394–396.
41. Shaw, S., W. J. Pichler, and D. L. Nelson. 1979. Fc receptors on human T-lymphocytes. III. Characterization of subpopulations involved in cell-mediated lympholysis and antibody-dependent cellular cytotoxicity. *J. Immunol.* **122:** 599–604.
42. Kazura, J. W., M. M. Fanning, J. L. Blumer, and A. A. F. Mahmoud. 1981. Role of cell-generated hydrogen peroxide in granulocyte-mediated killing of schistosomula of *Schistosoma mansoni in vitro*. *J. Clin. Invest.* **67:** 93–102.
43. Butterworth, A. E., D. L. Wassom, G. J. Gleich, D. A. Loegering, and J. R. David. 1979. Damage to schistosomula of *Schistosoma mansoni* induced directly by eosinophil major basic protein. *J. Immunol.* **122:** 221–229.
44. Olds, G. R., J. J. Ellner, L. A. Kearse, Jr., J. W. Kazura, and A. A. F. Mahmoud. 1980. Role of arginase in killing of schistosomula of *Schistosoma mansoni*. *J. Exp. Med.* **151:** 1551–1562.
45. Hiserodt, J. C., and G. A. Granger. 1976. *In vitro* lymphocyte cytotoxicity. II. Unstable lymphotoxin (β-LT) secreted and inactivated by mitogen-stimulated human lymphocytes. *Cell Immunol.* **26:** 211–216.
46. Hiserodt, J. C., G. J. Tiangco, and G. A. Granger. 1979. The LT system in experimental animals. I. Rapid release of high level of lymphotoxic (LT) activity from murine lymphocytes during the interaction with lectin-treated allogenic or xenogenic target cells *in vitro*. *J. Immunol.* **123:** 311–316.
47. Phillips, S. M., J. J. Diconza, J. A. Gold, and W. A. Reid. 1977. Schistosomiasis in the congenitally athymic (nude) mouse. Thymic dependency of eosinophilia, granuloma formation and morbidity. *J. Immunol.* **118:** 594–599.
48. Civil, R. M., and A. A. F. Mahmoud. 1977. A role for thymocytes in BCG-induced resistance to *Schistosoma mansoni* infection. *Fed. Proc.* **36:** 1057.
49. Sher, A., S. Hieny, S. L. James, and R. Asofsky. 1982. Mechanisms of protective immunity against *Schistosoma mansoni* infection in mice vaccinated with irradiated cercariae. II. Analysis of immunity in hosts deficient in T-lymphocytes, B-lymphocytes or complement. *J. Immunol.* **128:** 1880–1884.