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Establishment and Characterization of an Antigen-Specific T-Cell Line from Liver Granulomas of *Schistosoma mansoni*-Infected Mice

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Granulomatous inflammations in schistosomiasis mansoni are the result of T-cell-mediated reactions to soluble egg antigens (SEA) secreted by parasite ova. To study T_{DH} effector cell function, a granuloma T-cell line was established from collagenase-digested liver granulomas of acutely infected CBA/J mice. Dispersed nonadherent granuloma cells were cultured with feeder layer cells and SEA or with feeder layer cells alone in alternate cycles for 32 weeks. The granuloma T-cell line was L3T4$^+$ Lyt-1$^+$. In vitro, the SEA-stimulated T cells showed proliferation and interleukin 2 production. One million T cells adoptively transferred SEA-specific footpad swelling, and 7.5 × 10^6 T cells adoptively transferred granulomatous hypersensitivity to injected ova or SEA-coated beads. Anti-L3T4 monoclonal antibody blocked the SEA-specific cell proliferation. Depletion of L3T4$^+$ cells abrogated, while that of Lyt-1$^+$ cells diminished the adoptive transfer of SEA-specific footpad swelling. These experiments demonstrate that the granuloma T-lymphocyte population contains T_{DH}-type effector cells. Establishment of an SEA-specific granuloma T-cell line will allow the study of the effector functions of the hitherto uncharacterized intralesional granuloma T lymphocyte.

Schistosomiasis mansoni is a tropical helminthic infection characterized by granulomatous pathophysiology (31). Parasite eggs disseminate in the tissues and secrete soluble antigens that sensitize the host and evoke a granulomatous inflammatory response (4). The granulomas consist of lymphocytes, macrophages, giant cells, epithelioid cells, and eosinophils (24).

Previous studies established that T lymphocytes play an important role in the development of the schistosome egg-induced granuloma. Immunosuppressive drugs (11), antilymphocyte serum (10), monoclonal anti-L3T4 antibody (23), and neonatal thymectomy (9) strongly inhibit granuloma formation. Moreover, athymic nude mice are deficient in mounting a granulomatous response (1, 27). Lyt-1$^+$ splenic T_{DH} lymphocytes from acutely infected animals transferred granulomatous delayed-type hypersensitivity (DTH) to normal recipients (33).

Examination of liver granulomas after collagenase dispersal showed that T lymphocytes constitute 10 to 15% of the inflammatory granuloma cells (6). In cryostat sections of granulomatous livers, indirect immunofluorescence revealed that T cells are evenly dispersed throughout the granuloma (32). Based on the above evidence, it is presumed that subpopulations of T lymphocytes initiate, maintain, and regulate the size of the granuloma. Yet nothing is known of the phenotypic profile or functional characteristics of the granuloma T_{DH} cell. To gain insight into the character and functions of the granuloma T lymphocyte, a T-cell line has been established from the vigorous granulomas of acutely infected mice, and the cells were examined for phenotypic characteristics as well as T_{DH} effector function.

MATERIALS AND METHODS

Animals and infection. Female CBA^k mice (Jackson Laboratory, Bar Harbor, Maine), 7 to 8 weeks old, were infected by subcutaneous injection of 25 cercariae of the Puerto Rican strain of *Schistosoma mansoni*.

Egg isolation and SEA preparation. Eggs were isolated from 200 cercaria-infected mice (8). For soluble egg antigen (SEA) preparation, eggs were homogenized in Dulbecco phosphate-buffered saline (4).

Culture medium. RPMI 1640 was supplemented with 2 mM sodium pyruvate, 19 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, penicillin (100 U/ml), streptomycin (100 μg/ml), 2 mM L-glutamine (all from M. A. Bioproducts, Walkersville, Md.), 5 × 10^{-5} M 2-mercaptoethanol (Eastman Organic Chemicals, Rochester, N.Y.), and 10% heat-inactivated fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.). All cultures were maintained at 37°C in a 5% CO_2–air atmosphere.

Preparation of feeder layer. Spleens from normal CBA^k mice were gently minced in RPMI. Erythrocytes were lysed in a hypotonic solution of Tris-buffered ammonium chloride for 5 min at room temperature. Cells were washed, suspended in RPMI, and irradiated over ice (2,000 rads; ^{137}Cs source, type GC-40 irradiator; Atomic Energy of Canada Ltd., Ottawa).

Establishment of the granuloma T-cell line. Eight-week-infected mice were sacrificed and the granulomatous livers were perfused with heparinized RPMI (10 U of grade I heparin from porcine intestinal mucosa per ml; Sigma Chemical Co., St. Louis, Mo.). Livers were homogenized in RPMI in a Waring blendor for 10 to 15 s at low speed. Granulomas were collected by 1 × *g* sedimentation, washed, and then digested in culture medium containing 1% collagenase (type I from *Clostridium histolyticum*; Sigma) for 50 min at 37°C in a shaking water bath. Softened granulomas were disrupted by repeated suction and expulsion through a 5-ml syringe. Dispersed cells were extensively washed in RPMI, counted, and suspended at 5 × 10^7 cells/ml in prewarmed medium (RPMI 1640, 25 mM HEPES buffer, and 5% heat-inactivated fetal calf serum). Cells were then incubated on a nylon wool column at 37°C for 45 min. The eluted nonadherent popula-

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tion contained 12% T lymphocytes and 70% eosinophils. Cells were cultured at 5×10^6/ml with 5 μg of SEA per ml.

Maintenance of granuloma T-cell line. T cells were cultured at 2×10^5/ml with 10^7 feeder layer cells per ml and SEA (5 to 10 μg/ml) for 4 days. Subsequently, T cells were washed and cultured at 5×10^5/ml with 10^7 feeder layer cells per ml without SEA for 7 to 10 days. Cultures were maintained in 25- or 75-cm^2 polystyrene tissue culture flasks (Corning Glass Works, Corning, N.Y.).

Phenotypic characterization. Phenotypes were characterized by immunofluorescence at 4°C in the presence of 0.04% sodium azide (Sigma). Thy-1 expression was evaluated with a monoclonal anti-Thy-1.2 fluorescein isothiocyanate conjugate (Miles Laboratories, Naperville, Ill.). For I-A antigen detection, a monoclonal mouse immunoglobulin G2b (IgG2b) anti-I-A^k (10-2.16; ATCC TIB93; American Type Culture Collection, Rockville, Md.) was used, followed by an affinity-purified F(ab)'2 fragment, fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Cappel Laboratories, Division of Cooper Biomedical, Malvern, Pa.). For Lyt-1, a monoclonal rat IgG2a (53-7.313; ATCC TIB104); for Lyt-2, a monoclonal rat IgG2a (53-6.72; ATCC TIB105); and for L3T4, a monoclonal rat IgG2b (GK1.5; ATCC TIB207) was used. The secondary antibody used with the Lyt-1, Lyt-2, and L3T4 monoclonal antibodies was an affinity-purified F(ab)'2 fragment, FITC-conjugated goat anti-rat IgG (Cappel). This conjugate was adsorbed with mouse splenocytes to ensure nonreactivity with mouse IgG. Cells were incubated with the first antibody for 1 h, washed, and then incubated for 30 min with the conjugate. At least 200 cells were counted with a Nikon Optiphot microscope (Nikon, Garden City, N.Y.) at 40× magnification.

Proliferation assay. Twenty-five thousand T cells and 5×10^5 irradiated syngeneic splenocytes were cultured in quadruplicate wells with SEA (10 μg/ml), keyhole limpet hemocyanin (KLH; Mann Research Laboratories, New York) (10 μg/ml), and mouse thyroglobulin (MTG) (10 μg/ml) or without antigen in a total volume of 75 μl in round-bottomed 96-well plates (Corning). On day 4, 1 μCi of tritiated thymidine ([^{3}H]TdR; 1 mCi/ml specific activity; New England Nuclear Corp., Boston, Mass.) was added to each well. One day later, the cells were harvested and added to aqueous counting solution (Amersham Corp., Arlington Heights, Ill.) for scintillation counting on an LKB Wallac beta counter (LKB, Gaithersburg, Md.).

IL-2 assay. T cells were cultured at 2×10^5/ml with 1×10^7 feeder layer cells per ml with or without SEA (5 to 10 μg/ml). After 4 days, the culture supernatant was collected and stored at −70°C. At a later date, the supernatants were assayed for interleukin 2 (IL-2) activity with the IL-2-dependent cell line CTLL-20 (16). Serial twofold dilutions of each supernatant or IL-2 standard were made; 100 μl of each dilution was added to round-bottomed microwells that contained 10^4 CTLL-20 cells in a final volume of 200 μl (quadruplicate cultures). After 18 h, 1 μCi of [^{3}H]TdR was added to each well, and the cells were harvested 6 h later. IL-2 activity was determined by comparison of each sample with a standard IL-2 preparation obtained from Lewis rat splenocytes which had been stimulated for 24 h with 5 μg of concanavalin A (ConA) per ml. The culture supernatant was absorbed with Sephadex G-10 to remove ConA.

Transfer of dermal DTH. One million T cells and 30 μg of SEA, 30 μg of KLH, or saline were injected in 30 μl into the right hind footpad of normal CBA mice. Saline (30 μl) was injected into the contralateral footpad. After 24 h, footpad swelling was measured by a micrometer. Net swelling equaled experimental footpad − contralateral footpad swelling.

Transfer of granulomatous DTH. Seven and a half million T cells and 2,000 schistosome eggs, SEA-coated beads, or control uncoated beads were injected intravenously into normal recipients in 500 μl. After 3 days lungs were removed, fixed in phosphate-buffered Formalin (Fisher, Springfield, N.J.), sectioned, and stained with hematoxylin-eosin, and the lesions were measured. Twenty lesions were measured per animal.

Beads. Cyanogen bromide-activated Sepharose 4B beads (Pharmacia Inc., Piscataway, N.J.) were sized to 60-μm mean diameter prior to coupling. SEA was covalently coupled as recommended by the manufacturer.

Depletions. The cytotoxicity medium used throughout the depletion procedure contained RPMI 1640, 25 mM HEPES buffer, and 0.3% bovine serum albumin (Cedarlane Laboratories, Division of Accurate Chemical and Scientific Corp., Westbury, N.Y.), which reduced background cytotoxicity. The following antibodies were used. For Thy-1.2, an IgM monoclonal (HO-13-4, ATCC TIB99); for Lyt-1.1, an IgG2a monoclonal (Cedarlane); and for Lyt-2.1, an IgM monoclonal (3.155.2, ATCC TIB211). For depletion of L3T4$^+$ and I-A$^+$ cells, the antibodies used for fluorescence were employed. T cells were incubated at 10^7/ml with the appropriate antibody at 4°C for 1 h, washed twice, and then incubated for another hour at 37°C with rabbit Low-Tox-M complement (diluted 1:10 in cytotoxicity medium; Cedarlane). Following depletions, the cells were washed twice, and viability was checked by dye exclusion.

Calculations and statistics. For proliferation assays, the change in counts per minute (Δcpm) was calculated as mean cpm in stimulated wells minus mean cpm of control unstimulated wells. The standard error of the Δcpm was determined by pooling the variances of control and stimulated means. Significance of differences between experimental and control groups was determined by the unpaired Student t test; $P < 0.05$ was considered significant.

RESULTS

Establishment and maintenance of the granuloma T-cell line. In murine schistosomiasis mansoni, the granulomatous response to parasite eggs is maximal between 8 and 10 weeks postinfection (3). Therefore, the T-cell line was established from vigorous liver granulomas of 8-week-infected mice which were presumed to contain the maximum number of highly active T_{DH} effector cells.

A previous granuloma T-cell line was established and was functional in vitro and in vivo. Initially the cells were functional in SEA-specific proliferation, endogenous IL-2 production, and the transfer of granuloma formation. However, after 6 weeks of repeated culture in the presence of exogenous IL-2, the line lost its functional ability. (S. Ragheb, R. C. Mathew, and D. L. Boros, Fed. Proc. **44**:1695, 1985). To avoid this problem, the newly established line was maintained through alternate cycles of antigen stimulation and rest. IL-2 was only used to expand the cell line for in vivo studies. This protocol maintained the SEA specificity of the cells. The T-cell line has been maintained in culture for up to 32 weeks (corresponding to approximately 20 passages) without loss of function.

Phenotypic characterization of the granuloma T-cell line. The nylon wool-nonadherent cell fraction used to initiate the cell line contained 12% T lymphocytes and 70% eosinophils. The T cells had an L3T4:Lyt-2 ratio of 3:1 and were I-A

TABLE 1. Phenotypic characterization of the granuloma T-cell line[a]

Membrane marker	% Fluorescent cells at cell line age:	
	5 wk	9 wk
Thy-1.2	83	94
Lyt-1.1	46	67
Lyt-2.1	2	3
L3T4	78	93
I-A^k	7	60

[a] After SEA stimulation of the cell line, blast cells were separated from dead feeder cells by Ficoll-Paque density centrifugation. Blast cells were phenotyped by immunofluorescence. Shown are the phenotypic profiles of the cell line after 5 and 9 weeks in culture.

negative. They underwent blastogenic transformation after SEA stimulation, and they transferred granulomatous hypersensitivity to normal recipients. At the time of the first phenotypic examination of the line (3 weeks in culture), eosinophils were absent from the culture. The phenotypic profile of the cell line after 5 and 9 weeks of culture is shown in Table 1. The longer the cells remained in culture, the more enriched they became for Thy-1^+ cells which had a helper/effector phenotype (L3T4^+, Lyt-1^+). The ratio of L3T4^+:Lyt-2^+ cells increased to 30:1. Expression of all surface markers except I-A was stable over time. I-A expression continued to increase until 60 to 65% of the T cells were positive. Subsequently the cell line was examined every 4 weeks. The phenotypic profile observed at 9 weeks remained stable.

Functional characterization of the granuloma T-cell line. (i) SEA-specific proliferation and IL-2 production. Having established the T-cell nature of the cell line, we wished to check its ability to specifically proliferate in response to an SEA stimulus (Fig. 1). While there was good proliferation following SEA stimulation, there was no proliferation in response to MTG. In one experiment, there was some cross-reaction with KLH. The cell line was so active that even 5×10^3 T cells showed good proliferation (stimulation index of 30) in response to SEA stimulation.

Next, we examined whether SEA stimulation resulted in IL-2 production. As shown in Table 2, after 5 weeks in culture, SEA-stimulated T cells produced IL-2 (4.4 U/ml),

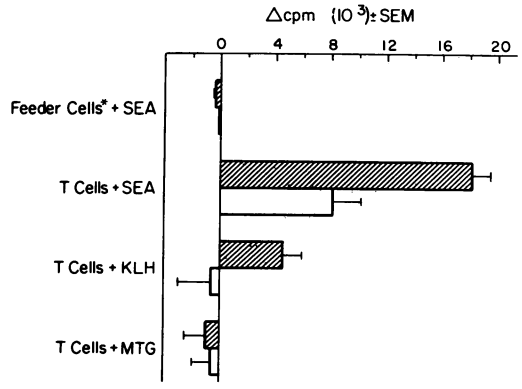

FIG. 1. Antigen-specific proliferation of granuloma T-cell line. Twenty-five thousand T cells and 5×10^5 irradiated feeder cells were cultured in quadruplicate wells with SEA (10 μg/ml), KLH (10 μg/ml), MTG (10 μg/ml), or no antigen. Cells were pulsed with [^{3}H]TdR on day 4 and harvested on day 5. *, 5×10^5 irradiated feeder cells were cultured as described above without T cells. Hatched bars, experiment 1 (5-week culture); open bars, experiment 2 (10-week culture).

whereas unstimulated cells did not spontaneously release IL-2 (<0.1 U/ml). After 10 weeks in culture, SEA-induced IL-2 production increased to 12.1 U/ml. Irradiated feeder cells cultured under the same conditions in the absence of T cells did not make any IL-2.

(ii) Passive transfer of dermal and granulomatous DTH. For in vivo function of the granuloma T-cell line, the ability of T cells to passively transfer dermal and granulomatous DTH was tested. For the elicitation of the former, 10^6 T cells were injected into the footpad of six normal recipients. Passive transfer of dermal DTH was antigen specific because the cell line could adoptively transfer SEA but not KLH sensitivity. The mean net swelling was 0.343 ± 0.028 cm (standard error of the mean) for recipients of T cells and SEA and −0.009 ± 0.025 cm for recipients of T cells and KLH. The control group of mice injected with T cells and saline did not show appreciable swelling (0 ± 0.033 cm). Feeder cells alone were unable to transfer dermal DTH to normal recipients.

For the transfer of schistosome egg-specific granulomatous sensitivity, we first established that 7.5×10^6 T cells was the minimum number required for a successful transfer. Therefore, this number of T cells was injected intravenously with eggs into normal mice to generate pulmonary granulomas. A group of mice received eggs alone to control for any primary sensitization. The granuloma T-cell line transferred granulomatous hypersensitivity to the schistosome egg; i.e., the lung granuloma area in recipients of T cells and eggs was significantly larger than that in recipients of eggs alone (Fig. 2). Feeder cells injected under the same conditions were ineffective. To test the specificity of the transfer, T cells were injected with SEA-coated beads or control uncoated beads (Fig. 2). The cellular reaction to the control beads was minimal. The mean granuloma area around SEA-coated beads was significantly larger than that around the control beads.

All functional activities of the cell line were tested at 4 to 5 weeks, 10 to 12 weeks, and 20 weeks after establishment of the culture. At other times, antigen-induced proliferation assays were done to ensure that the T-cell line was functional and retained antigen specificity.

Confirmation of effector function by blockade and depletion. The helper/effector T-cell profile of the granuloma T-cell line was also confirmed by functional assays. Two approaches were used: blockade of SEA-induced proliferation and specific depletion of T-cell subsets prior to adoptive transfer of DTH.

For the first approach, we tested the ability of antibodies directed against Lyt-1, Lyt-2, L3T4, and I-A^k to block SEA-specific proliferation (Table 3). The most profound

TABLE 2. SEA-induced IL-2 production by granuloma T-cell line

Sample	Mean [^{3}H]TdR incorporation[a] (cpm) ± SEM	IL-2 production (U/ml)
IL-2 standard[b]	112,680 ± 1,579	20.0
Control T-cell line supernatant[c]	1,422 ± 199	<0.1
T-cell line supernatant[d]	30,642 ± 1,557	4.4
T-cell line supernatant[e]	107,300 ± 479	12.1

[a] Incorporation by CTLL-20 cells. Counts shown in each case are for the undiluted sample.

[b] Rat splenocytes were stimulated with ConA for 24 h, the supernatant was collected, and the ConA was removed with Sephadex G-10.

[c] Control supernatant: T cells were cultured without SEA.

[d] Five-week culture.

[e] Ten-week culture.

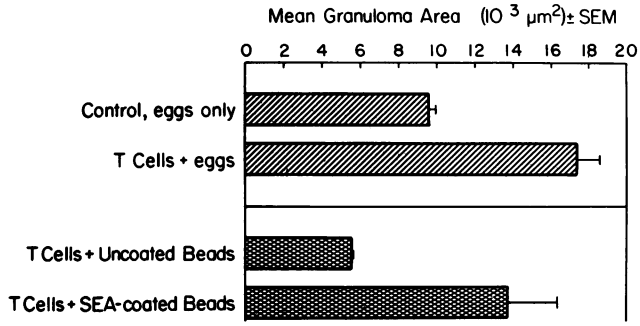

FIG. 2. SEA-specific transfer of granulomatous DTH by granuloma T-cell line. Seven and a half million T cells were injected with 2,000 eggs or 3,000 beads in a volume of 500 μl into the tail vein of normal syngeneic recipients. Lungs were removed after 3 days, fixed, sectioned, and stained with hematoxylin and eosin. Granuloma diameters were measured and used to calculate granuloma area; 20 lesions were measured per animal. This figure presents the pooled results for two experiments done after 5 and 10 weeks in culture. In each experiment there were four to five mice per group.

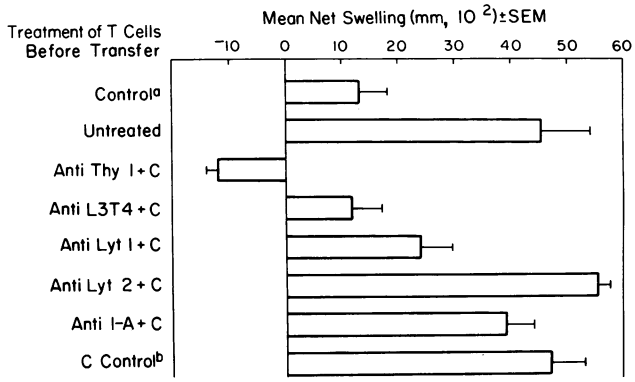

FIG. 3. Depletions of T-cell subsets prior to transfer of dermal DTH by granuloma T-cell line. One million T cells were depleted of certain T-cell subsets by treatment with specific antibody and complement. After depletion, the remaining viable cells were injected with 30 μg of SEA or saline into the right hind footpad of syngeneic normal recipients in a final volume of 30 μl. Saline was injected into the contralateral footpad. At 24 h posttransfer, footpad swelling was measured. This figure presents the pooled results for two experiments done after 5 and 10 weeks in culture. In each experiment there were five to six mice per group. Control[a], T cells and saline; Control[b], complement control. C, Complement.

effect was caused by anti-L3T4. At higher concentrations, it suppressed T-cell proliferation below the background (no antigen added) level. Even at a dilution of 1:64 it still effectively blocked SEA-specific proliferation. Anti-I-A was also quite effective at blocking proliferation. Anti-Lyt-1 had no effect. At a 1:4 dilution, anti-Lyt-2 somewhat blocked proliferation of the cell line. At higher dilutions it had no effect.

TABLE 3. Antibody blockade of SEA-induced proliferation of granuloma T-cell line

Antigen and dilution of antibody	Mean [^{3}H]TdR incorporation (cpm) ± SEM	
	Expt 1	Expt 2
No antigen	1,165 ± 221	1,142 ± 162
SEA	28,850 ± 3,308	4,911 ± 154
KLH	4,856 ± 1,415	ND[a]
SEA + anti-Lyt-1		
1:4	31,498 ± 4,349	5,225 ± 63
1:8	ND	7,020 ± 542
1:16	33,741 ± 1,907	7,655 ± 942
1:64	30,453 ± 1,906	ND
SEA + anti-Lyt-2		
1:4	16,070 ± 1,125	3,485 ± 301
1:8	ND	4,905 ± 168
1:16	25,880 ± 1,016	4,829 ± 90
1:64	26,197 ± 3,075	ND
SEA + anti-L3T4		
1:4	518 ± 77	305 ± 59
1:8	ND	344 ± 109
1:16	1,473 ± 126	366 ± 88
1:64	6,223 ± 414	ND
SEA + anti-I-A^k		
1:4	6,641 ± 917	ND
1:8	ND	ND
1:16	10,049 ± 1,338	ND
1:64	15,046 ± 1,387	ND

[a] T cells (2.5×10^4) and 5×10^5 irradiated feeder cells were cultured with SEA (10 μg/ml), KLH (10 μg/ml), or no antigen in a total volume of 75 μl in quadruplicate cultures. Serial dilutions were made of each antibody tested, and 25 μl was added per well at the start of the culture. The blocking antibodies were present throughout the culture. The cells were pulsed on day 4 and harvested on day 5. The antibodies used in these experiments were the same ones used for phenotyping (as described in Materials and Methods). ND, Not done.

In the second approach, granuloma T cells were depleted of Thy-1$^+$, Lyt-1$^+$, Lyt-2$^+$, L3T4$^+$, or I-A$^+$ cells by treatment with specific antibody and complement before adoptive transfer of dermal DTH (Fig. 3). Anti-Thy-1 and anti-L3T4 depletions completely abrogated the transfer of DTH by the cell line. Lyt-1 depletion significantly diminished but did not abrogate the transfer. Depletions of Lyt-2$^+$ and I-A$^+$ T cells had no effect.

DISCUSSION

It was previously shown that Lyt-1$^+$ splenic cells adoptively transferred granulomatous DTH, produced migration-inhibitory factor, and were functional in the generation of in vitro egg granulomas (7, 12, 13, 33). Moreover, an SEA-specific L3T4$^+$ splenic T-cell clone was shown to specifically augment in vitro granuloma formation around SEA-coupled beads (21). Infected mice treated with anti-L3T4 monoclonal antibody developed smaller granulomas and showed impaired IL-2 production (23). The accumulated data strongly implicated peripheral L3T4$^+$, Lyt-1$^+$ T lymphocytes as the T_{DH} cells that initiate and maintain the schistosome egg-induced granuloma.

In the present study we report the successful establishment of a long-lived T-cell line from the vigorous granulomas of acutely infected mice. The data provide information on the phenotypic and functional characteristics of the granuloma T lymphocyte. By immunofluorescence, the great majority of T cells displayed L3T4 and Lyt-1 markers. However, the presence in culture over many weeks of a low number of Lyt-2$^+$ cells is noteworthy. The ratio of L3T4$^+$ to Lyt-2$^+$ cells remained constant. It is assumed that the increase in the number of I-A$^+$ T cells is not attributable to increased membrane expression but rather to passive adsorption of the antigen shed by the copious number of feeder cells. The proliferative response of the T-cell line was SEA

specific. However, in some proliferation assays cross-reaction with KLH was observed. This may have occurred because the crude SEA preparation, which is composed of many glycoproteins and some polysaccharides (26), may have contained antigens that had common epitopes with KLH and a 38,000-M_r schistosomulum antigen (17). The granuloma T-cell line was functionally active in vitro as shown also by antigen-induced IL-2 production. The longer the line was maintained in culture, the better its endogenous IL-2 production became following SEA stimulation. This was not surprising since with time the cell line became enriched for L3T4$^+$ and presumably for SEA-specific T cells. The granuloma T-cell line retained its effector function even after prolonged culturing, as demonstrated by the transfer of dermal and granulomatous DTH.

The effector function of the cell line was further confirmed by antibody blockade of SEA-induced proliferation and depletions of T-cell subsets prior to in vivo transfer of DTH. Both methods led to the conclusion that the granuloma T_{DH} cell is L3T4$^+$ and/or L3T4$^+$ Lyt-1$^+$. Anti-I-A monoclonal antibody blocked the SEA-induced T-cell line proliferation. However, this does not necessarily indicate that the T cells are I-A$^+$ since depletion of I-A$^+$ cells did not diminish the passive transfer of SEA-induced footpad swelling. Rather, it is assumed that anti-I-A antibodies blocked antigen presentation to the granuloma T cells. A similar blockade of antigen presentation by granuloma macrophages to splenic and lymph node cells of infected mice has already been demonstrated (14). At higher concentrations, anti-Lyt-2 monoclonal antibody blocked the proliferation of the T-cell line, indicating that this subset also underwent proliferation. However, depletion of Lyt-2$^+$ cells did not diminish the ability of the cell line to adoptively transfer dermal DTH. Therefore, the small percentage of Lyt-2$^+$ cells within the cell line were not active as T_{DH} cells in vivo, nor did they seem to exert suppressor activity.

For granuloma formation to occur, nominal antigens must be presented in the context of Ia or DR antigens present on the antigen-presenting cell membrane. Antigen presentation, along with the IL-1 signal, induces T helper/effector cell proliferation, inflammatory lymphokine secretion, and the recruitment of inflammatory cells that constitute the granuloma (2, 18). Indeed, schistosome granuloma macrophages have been shown to present egg antigens in conjunction with I-A/I-E determinants and to secrete IL-1 monokine (14, 15). Isolated liver granulomas were also shown to secrete in culture migration-inhibitory factor and eosinophil stimulation promoter-active lymphokines (5, 19), a function attributable to intralesional T cells which constitute 10 to 15% of the total granuloma cell population. Thus, the accumulated direct and indirect evidence indicated that the schistosome egg-induced granuloma is initiated and maintained by T_{DH} lymphocytes. The phenotypic and functional characterization of a lymphocytic line established from granulomas provides strong direct evidence that granuloma lymphocytes contain T_{DH}-type helper/effector cells that expand under antigenic stimulus, produce IL-2 lymphokine, and generate dermal, as well as granulomatous, inflammations. Phenotypically and functionally, this granuloma T-cell line behaves like other T effector cell lines or clones that were established from mice or rats with schistosomiasis (21), leprosy (20), leishmaniasis (22, 28), trypanosomiasis (25), or experimental allergic encephalomyelitis (29, 30). It still remains to be shown that the granuloma T cells produce inflammatory lymphokines.

The granuloma T-cell line includes synchronous, SEA-specific cells that retain their functional capacity in long-term culture. Hence, it allows more detailed studies of the inflammatory role of the granuloma T_{DH} cell. Studies are under way to examine the dynamics of IL-2 receptor expression and IL-2 production by granuloma T cells following the antigenic stimulus and the interrelationship between IL-2 and inflammatory lymphokine production.

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